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Contents

9689A: Photonics in Dermatology and Plastic Surgery .............. 3
9689B: Therapeutics and Diagnostics in Urology .................. 15
9689C: Optical Imaging, Therapeutics, and Advanced Technology in Head and Neck Surgery and Otolaryngology .......... 21
9689D: Diagnostic and Therapeutic Applications of Light in Cardiology .............................................. 28
9689E: Diagnosis and Treatment of Diseases in the Breast and Reproductive System II .............................................. 40
9689F: Optics in Bone Surgery and Diagnostics .................. 47
9690A: Clinical and Translational Neurophotonics ................. 51
9690B: Neural Imaging and Sensing ................................... 58
9690C: Optogenetics and Optical Manipulation ................... 71
9691A: Endoscopic Microscopy XI ................................... 77
9691B: Optical Techniques in Pulmonary Medicine III ............. 86
9692: Lasers in Dentistry XXII ......................................... 93
9693: Ophthalmic Technologies XXVI ................................ 101
9694: Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXV . 120
9695: Mechanisms of Photobiomodulation Therapy XI ............ 152
9696: Molecular-Guided Surgery: Molecules, Devices, and Applications II ......................................................... 159
9697: Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine XX ................................ 148
9698: Advanced Biomedical and Clinical Diagnostic and Surgical Guidance Systems XIV ........................................... 181
9699: Optics and Biophotonics in Low-Resource Settings II ........ 194
9700: Design and Quality for Biomedical Technologies IX .......... 203
9701: Multimodal Biomedical Imaging XI ............................ 215
9702: Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XVI .......................................... 226
9703: Optical Biopsy XIV: Toward Real-Time Spectroscopic Imaging and Diagnosis ................................................ 237
9704: Biomedical Vibrational Spectroscopy 2016: Advances in Research and Industry ........................................................ 255
9705: Microfluidics, BioMEMS, and Medical Microsystems XIV . . . 265
9706: Optical Interactions with Tissue and Cells XXVII ............. 277
9707: Dynamics and Fluctuations in Biomedical Photonics XIII .... 295
9708: Photons Plus Ultrasound: Imaging and Sensing 2016 ......... 307
9709: Biophotonics and Immune Responses XI ...................... 355
9710: Optical Elastography and Tissue Biomechanics III .......... 365
9711: Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues IX .............................................. 439
9712: Multiphoton Microscopy in the Biomedical Sciences XVI .... 396
9713: Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XXIII .................... 418
9714: Single Molecule Spectroscopy and Superresolution Imaging IX ................................................................. 434
9715: Optical Diagnostics and Sensing XVI: Toward Point-of-Care Diagnostics ................................................. 446
9716: Optical Methods in Developmental Biology IV ................. 460
9717: Adaptive Optics and Wavefront Control for Biological Systems II .............................................................. 466
9718: Quantitative Phase Imaging II ...................................... 481
9719: Biophysics, Biology and Biophotonics: the Crossroads ...... 507
9720: High-Speed Biomedical Imaging and Spectroscopy: Toward Big Data Instrumentation and Management .......... 514
9721: Nanoscale Imaging, Sensing, and Actuation for Biomedical Applications XIII .................................................. 527
9722: Colloidal Nanoparticles for Biomedical Applications XI .... 536
9723: Reporters, Markers, Dyes, Nanoparticles, and Molecular Probes for Biomedical Applications VIII .......................... 550
9724: Plasmonics in Biology and Medicine XIII ...................... 560
9725: Frontiers in Biological Detection: From Nanosensors to Systems ................................................................. 571

Click on the Conference Title to be sent to that page
Assessing the colors of human skin (Keynote Presentation)
Nikiforos Kollias, Consultant (United States)
No Abstract Available

Diagnosis of female genital tract melanocytic lesions based on pigment chemistry using pump-probe laser microscopy
Francisco E. Robles, Duke Univ. (United States); Maria A. Selim, Duke Univ. School of Medicine (United States); Warren S. Warren, Duke Univ. (United States)

Melanoma of the vulva is the second most common type of malignancy afflicting that organ. This disease carries poor prognosis, and shows tendencies to recur locally and develop distant metastases through hematogenous dissemination. Further, there exists significant clinical overlap between early-stage melanomas and melanotic macules, benign lesions that are believed to develop in about 10% of the general female population. In this work we apply a novel nonlinear optical method, pump-probe microscopy, to quantitatively analyze female genitalia tract melanocytic lesions. Pump-probe microscopy provides chemical information of endogenous pigments by probing their electronic excited state dynamics, with subcellular resolution.

Using unstained biopsy sections from 31 patients, we find significant differences between melanin type and structure in tissue regions with invasive melanoma, melanoma in-situ and non-malignant melanocytic proliferations (e.g., nevi, melanocytic macules). The molecular images of non-malignant lesion have a well-organized structure, with relatively homogenous pigment chemistry, most often consistent with that of eumelanin with large aggregate size or void of metals, such as iron. On the other hand, pigment type and structure observed in melanomas in-situ and invasive melanomas is typically much more heterogeneous, with larger contributions from pheomelanin, melanins with larger metal content, and/or melamins with smaller aggregate size. Of most significance, clear differences can be observed between melanocytic macules and vulvar melanoma in-situ, which, as discussed above, can be difficult to clinically distinguish. This initial study demonstrates pump-probe microscopy's potential as an adjuvant diagnostic tool by revealing systematic chemical and morphological differences in melanin pigmentation among invasive melanoma, melanoma in-situ and non-malignant melanocytic lesions.

Noninvasive skin cancer diagnosis using multimodal optical spectroscopy
Austin J. Moy, Xu Feng, Mia K. Markey, Jason S. Reichenberg, James W. Tunnell, The Univ. of Texas at Austin (United States)

Skin cancer is the most common form of cancer in the United States and is a recognized public health issue. Diagnosis of skin cancer involves biopsy of the suspicious lesion followed by histopathology. Biopsies, which involve excision of the lesion, are invasive, at times unnecessary, and are costly procedures (~$2.8B/year in the US). An unmet critical need exists to develop a non-invasive and inexpensive screening method that can eliminate the need for unnecessary biopsies. To address this need, our group has reported on the continued development of a multimodal spectroscopy (MMS) system towards the goal of an "optical biopsy" of skin. Our approach combines Raman spectroscopy, fluorescence spectroscopy, and diffuse reflectance spectroscopy to collect comprehensive optical property information from suspicious skin lesions. We describe our present efforts to develop an updated MMS system composed of OEM components that will be smaller, less expensive, and more clinic-friendly than the previous system. Key system design choices include the selection of miniature spectrometers, a fiber-coupled broadband light source, a fiber coupled diode laser, and a revised optical probe. Selection of these components results in a ~50% reduction in system footprint, resulting in a more clinic-friendly system. We also present preliminary characterization data from the updated MMS system, showing similar performance with our revised optical probe design. Finally, we present preliminary clinical MMS data, the first from an extensive clinical study (n = 250) of the MMS system to characterize its performance in identifying skin cancers.

New imaging-based biomarkers for melanoma diagnosis using CARS microscopy (Invited Paper)
Hequn Wang, Wellman Ctr. for Photomedicine (United States); Sam Osseiran, Wellman Ctr. for Photomedicine (United States) and Harvard-MIT Health Sciences and Technology (United States); Elisabeth Roider, David E. Fisher, Cutaneous Biology Research Ctr., Massachusetts General Hospital (United States); Conor L. Evans, Massachusetts General Hospital (United States)

Recently, pheomelanin has been found to play a critical role in melanoma progression given its pro-oxidant chemical properties as well as its marked presence in pre-cancerous and malignant melanoma lesions, even in the absence of ultraviolet radiation. In addition, epidemiological evidence indicates a strong correlation between melanoma incidence and skin type, with the highest incidence occurring in individuals of the red-haired/fair-skinned phenotype. Interestingly, nevus count correlates well with melanoma incidence and skin type, except in the population most prone to developing melanoma, where nevus count strikingly drops. As such, a current hypothesis proposes that fair-skinned red-haired individuals, who are unable to stimulate production of eumelanin due to a mutation in MC1R in melanocytes, may actually harbor numerous “invisible”, pheomelanin-rich nevi that evade clinical detection, supporting the high incidence of melanoma in that population.

Here, we show for the very first time that melanocytes extracted from genetically modified MC1R-mutant, red-haired mice displayed bright perinuclear distributions of signal within the cells under coherent anti-Stokes Raman scattering (CARS) microscopy. Changes in pheomelanin production in siRNA knockdowns of cultured human melanoma cells were also sensed. We then successfully imaged pheomelanin distributions in both ex vivo and in vivo mouse ear skin. Finally, melanosomes within amelanotic melanoma patient tissue sections were found to show bright pheomelanin signals. This is the first time, to our knowledge, that pheomelanin has been found spatially localized in a human amelanotic melanoma sample. These pheomelanic CARS features may be used as potential biomarkers for melanoma detection, especially for amelanotic melanomas.
9689-5, Session 3

Non-invasive Evaluation of oxidative stress in human skin exposed to common sun filters using multiphoton microscopy

Sam Osseiran, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and Harvard-MIT Health Sciences and Technology (United States); Yusuke Saita, Elisabeth Roeder, Hequn Wang, David E. Fisher, Conor L. Evans, Cutaneous Biology Research Ctr., Massachusetts General Hospital (United States)

Skin cancer, including basal cell carcinoma, squamous cell carcinoma, and melanoma, is the most common form of cancer in North America. Paradoxically, skin cancer incidence is steadily on the rise even despite the growing use of sunscreens over the past decades. One potential explanation for this discrepancy involves the sun filters in sunscreen, which are responsible for blocking harmful ultraviolet radiation. It is proposed that these agents may produce reactive oxygen species (ROS) at the site of application, thereby generating oxidative stress in skin that gives rise to genetic mutations, which may explain the rising incidence of skin cancer.

To test this hypothesis, ex vivo human skin was treated with five common chemical sun filters (avobenzone, octocrylene, homosalate, octisalate, and oxybenzone) as well as two physical sun filters (Zinc oxide compounds), both with and without UV irradiation. To non-invasively evaluate oxidative stress, two-photon excitation fluorescence (2PEF) and fluorescence lifetime imaging microscopy (FLIM) of the skin samples were used to monitor levels of NADH and FAD, two key cofactors in cellular redox metabolism. The relative redox state of the skin was assessed based on the fluorescence intensities and lifetimes of these endogenous cofactors. While the sun filters were indeed shown to have a protective effect from UV radiation, it was observed that they also generate oxidative stress in skin, even in the absence of UV light. These results suggest that sun filter induced ROS production requires more careful study, especially in how these reactive species impact the rise of skin cancer.

9689-6, Session 3

Latest advances in confocal microscopy of skin cancers toward guiding patient care: a Mohs surgeon's review and perspective (Invited Paper)

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

About 350 publications worldwide have reported the ability of reflectance confocal microscopy (RCM) imaging to detect melanocytic skin lesions in vivo with specificity of 84-88% and sensitivity of 71-92%, and non-melanocytic skin lesions with specificity of 85-97% and sensitivity 100-92%. Lentigo maligna melanoma can be detected with sensitivity of 95% and specificity 82%. While the sensitivity is comparable to that of dermoscopy, the specificity is 2X superior, especially for lightly- and non-pigmented lesions. Dermoscopy combined with RCM imaging is proving to be both highly sensitive and highly specific. Recent studies have reported that the ratio of equivocal (i.e., would have been biopsied) lesions to detected melanomas dropped by -2X when guided by dermoscopy and RCM imaging, compared to that with dermoscopy alone. Dermoscopy combined with RCM imaging is now being implemented to guide noninvasive diagnosis (to rule out malignancy and biopsy) and to also guide treatment, with promising initial impact: thus far, about 3,000 patients have been saved from biopsies of benign lesions. These are currently under follow-up monitoring. With fluorescence confocal microscopy (FCM) mosaicing, residual basal cell carcinomas can be detected in Mohs Surgically excised fresh tissue ex vivo, with sensitivity of 94-97% and specificity 89-94%. FCM mosaicing is now being implemented for guiding Mohs surgery. To date, about 600 Mohs procedures have been performed, guided with mosaicing, and with pathology being performed in parallel to confirm the final outcome. These latest advances demonstrate the promising ability of RCM and FCM to guide patient care.

9689-7, Session 3

A machine learning method for identifying morphological patterns in reflectance confocal microscopy mosaics of melanocytic skin lesions in-vivo

Kivanc Kose, Memorial Sloan-Kettering Cancer Ctr. (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)

We present a machine learning based algorithm that can quantitatively imitate the clinicians’ qualitative and visual process of analyzing reflectance confocal microscopy (RCM) mosaics at the dermal epidermal junction (DEJ) in skin. In-vivo RCM mosaicing enables imaging of large areas (8 mm-by-8 mm) of skin. RCM enables clinicians to noninvasively examine morphological patterns at the DEJ, which is key for diagnosis of cancer, since dysplasia and malignancy originate here. We aim to differentiate between 5 diagnostically significant morphological patterns that are encountered around the DEJ. To mimic the clinicians’ visual process, we divide the mosaics into localized areas of processing, and capture the textural appearance of each area using dense scale invariant feature transformation (SIFT) features. SIFT features are composed of histograms of gradients calculated at different scales. Such features are robust against changes in scaling, orientation, and intensity. We then use a bag of SIFT features based representation where the extracted features are quantized into “words” using vector quantization and each region is then represented using the histogram of the words within it. The histogram of the regions are then classified into one of the 5 different morphological patterns using a support vector machine (SVM) based supervised classification method. Our algorithm can distinguish between meshwork, ring, clod, malignant and background patterns in benign conditions and melanomas. Preliminary results with 80 % of the image data used for training and 20% for testing shows classification with 80-67 % sensitivity and 99-78 % specificity in distinguishing these patterns.

9689-8, Session 4

If I had a magic wand....A dermatology photonics wish list (Invited Paper)

Kristen M. Kelly M.D., Univ. of California, Irvine (United States)

Challenges in dermatology that can be addressed by photonics research will be explored. Non-invasive, real-time diagnosis of skin lesions would eliminate biopsy risks, minimize patient anxiety by providing more rapid answers and allow diagnosis and treatment in a single visit. Multiple approaches have been tried, but significant limitations of current technologies make them impractical for routine clinic implementation. Photons can also be used for treatment assessment to determine if intervention is adequate or if further treatment is needed. Ideal feedback should be non-invasive, rapid and accurate. Monitoring for potential adverse effects can greatly improve treatment safety, allowing clinicians to push the limits of therapy while preventing serious complications. Light based therapies can also be improved by increasing photon penetration and selectivity for targeted cells or skin structures. Current light based treatments are in many cases limited by photon penetration. In addition, we often seek to damage a specific chromophore but are not able to distinguish between targeted disease and non-targeted normal structures such as the cells of a melanoma and normal melanocytes and port wine stain versus...
normal vasculature. Scientist and clinician collaboration can address these and other issues and greatly improve patient care.

9689-9, Session 5

Simulation of polarized light in birefringent samples

Joseph Chue-Sang, Jessica C. Ramella-Roman, Florida International Univ. (United States)

Full-field polarized light imaging provides the capability of investigating the alignment and density of birefringent tissue such as collagen abundantly found in scars, the cervix, and other sites of connective tissue. These can be indicators of disease and conditions affecting a patient. Two-dimensional polarized light Monte Carlo simulations which allow the input of an optical axis of a birefringent sample relative to a detector have been created and validated using optically anisotropic samples such as tendon yet, unlike tendon, most collagen-based tissues is significantly less directional and anisotropic. Most important is the incorporation of three-dimensional structures for polarized light to interact with in order to simulate more realistic biological environments. Here we describe the development of a new polarization sensitive Monte Carlo capable to handle birefringent materials with any spatial distribution. The new computational platform is based on tissue digitization and classification including tissue birefringence and principle axis of polarization. Validation of the system was conducted both numerically and experimentally.

9689-10, Session 5

Clinical skin imaging using color spatial frequency domain imaging

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Skin diseases are typically associated with underlying biochemical and structural changes compared with normal tissues, which alter the optical properties of the skin lesions, such as tissue absorption and scattering. Although widely used in dermatology clinics, conventional dermatoscopes don’t have the ability to selectively image tissue absorption and scattering, which may limit its diagnostic power. Here we report a novel clinical skin imaging technique called color spatial frequency domain imaging (cSFDI) which enhances contrast by rendering color spatial frequency domain (SFDI) image at high spatial frequency. Moreover, by tuning spatial frequency, we can obtain both absorption weighted and scattering weighted images.

We developed a handheld imaging system specifically for clinical skin imaging. The flexible configuration of the system allows for better access to skin lesions in hard-to-reach regions. A total of 48 lesions from 31 patients were imaged under 470nm, 530nm and 655nm illumination at a spatial frequency of 0.6mm^-1. The SFDI reflectance images at 470nm, 530nm and 655nm were assigned to blue (B), green (G) and red (R) channels to render a color SFDI image. Our results indicated that color SFDI images at f=0.6mm^-1 revealed properties that were not seen in standard color images. Structural features were enhanced and absorption features were reduced, which helped to identify the sources of the contrast. This imaging technique provides additional insights into skin lesions and may better assist clinical diagnosis.

9689-11, Session 5

Imaging of skin surface architecture with out of plane polarimetry

Joseph Chue-Sang, Jessica C. Ramella-Roman, Florida International Univ. (United States)

Knowledge of skin surface topography is of great importance when establishing environmental and age related skin damage. Furthermore an effective treatment protocol cannot be established without a quantitative measuring tool that is able to establish significant improvement in skin texture. We utilized an out-of-plane polarimeter for the characterization of skin surface profile non-invasively. The system consists of an imaging Stokes vector polarimeter where the light source and imaging apparatus are arranged at an angle equal to forty degrees with respect to the tissue normal. The light source is rotated at various azimuth angles about the tissue normal. For each position of the incident beam the principal angle of polarization is calculated. This parameter relates indirectly to surface profile and architecture. The system was used to image the forehead and hands of healthy volunteers between eighteen and sixty years of age. A clear separation appeared among different age groups, establishing out-of-plane polarimetry as a promising technique for skin topography quantification.

9689-12, Session 5

Modeling laser speckle imaging of perfusion in the skin

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Laser speckle imaging (LSI) enables visualization of relative blood flow and perfusion in the skin. It is frequently applied to monitor treatment of vascular malformations such as port wine stain birthmarks, and measure changes in perfusion due to peripheral vascular disease. We developed a computational Monte Carlo simulation of laser speckle contrast imaging to quantify how tissue optical properties, blood vessel depths and speeds, and tissue perfusion affect speckle contrast values originating from coherent excitation. The simulated tissue geometry consisted of multiple layers to simulate the skin, or incorporated an inclusion such as a vessel or tumor at different depths. Our simulation used a 30x30mm uniform flat light source to optically excite the region of interest in our sample to better mimic wide-field imaging. We used our model to simulate how dynamically scattered photons from a buried blood vessel affect speckle contrast at different lateral distances (0-1mm) away from the vessel, and how these speckle contrast changes vary with depth (0-1mm) and flow speed (0-10mm/s). We applied the model to simulate perfusion in the skin, and observed how different optical properties, such as epidermal melanin concentration (1%-50%) affected speckle contrast. We simulated perfusion during a systolic forearm occlusion and found that contrast decreased by 35% (exposure time = 10ms). Monte Carlo simulations of laser speckle contrast give us a tool to quantify what regions of the skin are probed with laser speckle imaging, and measure how the tissue optical properties and blood flow affect the resulting images.

9689-13, Session 6

Use of a smart phone based thermo camera for skin prick allergy testing: a feasibility study

Rudolf M. Verdaasdonk, Lindi Barla, Thomas Rustomeyer, John H. Klaessens, Albert J. van der Veen, Vrije Univ. Medical Ctr. (Netherlands)

Allergy testing is usually performed by exposing the skin to small quantities of potential allergens on the inner forearm and scratching the protective epidermis to increase exposure. After 15 minutes the dermatologist performs a visual check for swelling and erythema which is subjective and difficult for e.g. dark skin types. A small smart phone based thermo camera (FLIR One) was used to obtain quantitative images in a feasibility study of 17 patients. Directly after allergen exposure on the forearm, thermal images were captured at 30 seconds interval and processed to a time lapse movie over 15 minutes.
Considering the ‘subjective’ reading of the dermatologist as golden standard, in 11/17 pts (65%) the evaluation of dermatologist was confirmed by the thermo camera including 5 of 6 patients without allergic response. In 7 patients thermo showed additional spots. Of the 342 sites tested, the dermatologist detected 47 allergies of which 28 (60%) were confirmed by thermo imaging while thermo imaging showed 12 additional spots. The method can be improved with user dedicated acquisition software and better registration between normal and thermal images. The lymphatic reaction seems to shift from the original puncture site. The interpretation of the thermal images is still subjective since collecting quantitative data is difficult due to motion patient during 15 minutes. Although not yet conclusive, thermal imaging shows to be promising to improve the sensitivity and selectivity of allergy testing using a smart phone based camera.

9689-14, Session 6

Spectral-spatial classification of hyperspectral images for biomedical applications

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Hyperspectral imaging is a fast evolving imaging modality in biomedical applications. Hyperspectral imagery records full spectral resolution in every pixel of an image. This rich spectral information increases capability to distinguish different materials and structures on the imaged scene and thus opens a new perspective for biomedical diagnostics and tissue characterization. However, a large number of spectral channels also cause challenges to decomposition of the image into different spectral clusters. The most common classification techniques process each pixel independently without considering information about spatial structures like vessels, wounds and moles. Taking advantage of the spatial and spectral information simultaneously improves the classification performance. A classification map can serve as a quick overview of different regions within the sample. Further and more comprehensive analysis can thus be limited to regions identified by the initial classification. In this study a spectral-spatial classification scheme was employed to hyperspectral images of skin tissue. As the applied method is unsupervised, no a-priori tissue information is required. The algorithm is based on assessing spatial dependencies between pixels and adaptive, spectrally homogeneous neighborhoods. It ensures class continuity and provides detailed classification maps of skin regions characterized by similar optical properties. The classification procedure was tested on experimental data sets from preclinical burn model obtained with a hyperspectral push-broom imaging spectrometer (Hyspex VNIR-1600). Classification maps were generated and investigated, and optical properties of identified regions were extracted using an inverse diffusion model.

9689-15, Session 7

Fluorescence imaging of tryptophan and collagen cross-links to evaluate keratinocytes proliferation and quantitate wound size

Ying Wang, Antonio Ortega-Martinez, William A. Farinelli, Apostolos G. Doukas, Richard R. Anderson M.D., Walfre Franco, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Wound size is a key parameter in monitoring healing. Current methods to measure wound size are often subjective, time-consuming and marginally invasive. Recently, we developed a non-invasive, non-contact, fast and simple but robust fluorescence imaging method to monitor the healing of skin wounds. This method exploits the fluorescence of native molecules to tissue as functional and structural markers. The objective of the present study is to demonstrate the feasibility of using variations in the fluorescence intensity of tryptophan and cross-links of collagen to evaluate proliferation of keratinocyte cells and quantitate size of wound during healing, respectively.

Circular dermal wounds were created in ex vivo human skin and cultured in different media. Two serial fluorescence images of tryptophan and collagen cross-links were acquired every two days. Histology and immunohistology were used to validate correlation between fluorescence and epithelialization. Images of collagen cross-links show fluorescence of the exposed dermis and, hence, are a measure of wound area. Images of tryptophan show higher fluorescence intensity of proliferating keratinocytes forming new epithelium, as compared to surrounding keratinocytes not involved in epithelialization. These images are complementary since collagen cross-links report on structure while tryptophan reports on function. H&E and immunohistology show that tryptophan fluorescence correlates with newly formed epidermis. We have established a fluorescence imaging method for studying epithelialization processes during wound healing in a skin organ culture model, our approach has the potential to provide a non-invasive, non-contact, quick, objective and direct method for quantitative measurements in wound healing in vivo.

9689-16, Session 7

Noninvasive measurement of burn wound depth applying infrared thermal imaging

Mariëlle E. H. Jaspers, Ilse M. Maltha, Rode Kruis Ziekenhuis (Netherlands); John H. Klaessens, Henrica C. Vet, Rudolf M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); Paul P. Zuijlen, Rode Kruis Ziekenhuis (Netherlands)

In burn wounds early discrimination between the different depths plays an important role in the treatment strategy. The remaining vasculature in the wound determines its healing potential. Non-invasive measurement tools that can identify the vascularization are therefore considered to be of high diagnostic importance. Thermography is a non-invasive technique that can accurately measure the temperature distribution over a large skin or tissue area, the temperature is a measure of the perfusion of that area. The aim of this study was to investigate the clinimetric properties (i.e. reliability and validity) of thermography for measuring burn wound depth. In a cross-sectional study with 50 burn wounds of 35 patients, the inter-observer reliability and the validity between thermography and Laser Doppler Imaging were studied. With ROC curve analyses the 7T cut-off point for different burn wound depths were determined. The inter-observer reliability, expressed by an intra-class correlation coefficient of 0.99, was found to be excellent. In terms of validity, a 7T cut-off point of 0.96°C (sensitivity 71%; specificity 74%) could differentiate between a superficial partial-thickness and deep partial-thickness burn. A 7T cut-off point of 0.80°C (sensitivity 70%; specificity 74%) could differentiate between a deep partial-thickness and a full-thickness burn wound.

This study demonstrates that thermography is a reliable method in the assessment of burn wound depths. In addition, thermography was reasonably able to discriminate among different burn wound depths, indicating its potential use as a diagnostic tool in clinical burn practice.

9689-17, Session 7

Investigation of an angiogenesis-promoting topical treatment for diabetic wounds using multimodal microscopy

Joanne Li, Andrew J. Bower, Univ. of Illinois at Urbana-
Champaign (United States); Zane A. Arp, Claire Holland, Eric Olson, GlaxoSmithKline (United States); Eric J. Chaney, Marina Marjanovic, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Impaired skin wound healing is a significant co-morbid condition of diabetes that is caused by poor microcirculation among other factors. Hypoxia-inducible factors (HIFs) are transcription factors that mediate the effects of decreased levels of oxygen in biological environments. Inducing mild hypoxia in the tissue could promote angiogenesis, a critical step in the wound healing process in diabetic wounds. To investigate the relationship between hypoxia and diabetic wound healing, a topical treatment consisting of a HIF-activating prolyl-hydroxylase inhibitor was administered to the wounded skin of diabetic (db/db) mice. Studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed at GSK or by the ethical review process at the institution where the work was performed. The wounded area was tracked in vivo for 28 days utilizing a custom-built multimodal microscopy system. An increase in vascular density around the wounds of treated animals was observed using phase-variance optical coherence tomography (PV-OCT), in comparison to normal controls. In addition, second harmonic generation (SHG) and fluorescence lifetime imaging microscopy (FLIM) were utilized to examine the collagen regeneration and cellular metabolic activity, respectively, in the wounded skin. The utilization of these light-based methods can follow metabolic and morphologic changes in the wound healing process in ways not possible with current evaluation processes. Insights demonstrated in these studies could lead to new endpoints for evaluation of the efficacy of drugs and lead to more direct ways of detecting patient response to treatment.

9689-18, Session 7

Imaging peripheral nerve graft revascularization and myelination using angiographic and polarization-sensitive optical coherence tomography (OCT)

Ahhyun S. Nam, Isabel Chico-Calero D.V.M., Jeena M. Easow, Martin Villiger, Mark A. Randolph, Robert W. Redmond, Benjamin J. Vakoc, Wellman Ctr. for Photomedicine (United States)

Segmental peripheral nerve trauma often requires surgical graft repair. However, reinnervation of these grafts is a slow process that, in many cases, provides only partial functional recovery. Understanding the wound healing and reinnervation dynamics of these grafts is critical to improving surgical procedures and engineering better graft tissues. The rat sciatic nerve is commonly used to investigate peripheral nerve trauma and repair procedures. However, there has been relatively limited imaging of the reinnervation in this model, and, to our knowledge, no longitudinal imaging of the vascularization of nerve grafts. As such, the variations in vascularization rates across different graft procedures is not known, and their importance to long term functional outcomes is poorly understood. In this work, we demonstrate the use of OCT-based angiography to visualize the vascularization of graft repairs after transaction of the rat sciatic nerve. A customized microscope providing 2.5 cm fields of view along the nerve axis was created allowing full imaging of the 5-7 mm graft along with neighboring native nerve. To provide high-transverse resolution, a z-axis translation system was implemented to track the varying height of the nerve and thereby maintain the nerve within the microscopes’s confocal parameter. Finally, polarization-sensitive imaging was integrated into the microscope to allow longitudinal imaging of nerve birefringence as a measure of myelination status. The OCT instrument has the potential to reveal new insights in preclinical studies and may ultimately provide a means for clinical intra-surgical assessment of peripheral nerve function.

9689-19, Session 8

Advances in skin optical clearing (Invited Paper)

Valery V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation) and Institute of Precision Mechanics and Control (Russian Federation) and National Research Tomsk State Univ. (Russian Federation)

The main limitation of optical spectroscopic and imaging techniques is light scattering, which lowers the probing depth and image quality. The first optical barrier (scattering screen) for in-depth body probing is skin. Skin optical clearing (OC) by application of biocompatible optical clearing agents (OCAs) or mechanical compression can considerably reduce light scattering and significantly improve all types of optical spectroscopic and imaging modalities of many tissues hidden under skin. Skin and underlying tissues themselves have many benefits at OC. The OC represents a promising approach to increase the imaging depth for optical techniques, including linear and nonlinear spectroscopies, optical coherence tomography (OCT), Raman, terahertz imaging, and etc. The improvement of light penetration depth in tissue and blood achieved at application of such OCAs, as x-ray contrast agents, glycerol, glucose, fructose, ethylene glycol, polyethylene glycol, propylene glycol, polypropylene glycol solutions, will be demonstrated for in vitro and in vivo studies. OC can be used for determination of OCA diffusion coefficient in skin at different pathologies, such as cancer, psoriasis, diabetes. The method is based on the measurement of kinetics of skin optical reflectance at OCA application and allows one to quantify differences of OCA diffusion properties in pathology and healthy skin.

9689-20, Session 9

Optical coherence tomography based microangiography in dermatology applications (Invited Paper)

Ruikang K. Wang, Utku Baran, Woo J. Choi, Univ. of Washington (United States)

Optical coherence tomography (OCT) based microangiography (OMAG) is a new imaging technique enabling the visualization of blood flow within microcirculatory tissue beds in vivo with high resolution. In this talk, the concept and advantages of OMAG will be discussed and its potential clinical applications in the dermatology will be shown, demonstrating its usefulness in the clinical monitoring and therapeutic treatment of various skin pathologies, e.g. acne, port wine stain and wound healing.

9689-21, Session 9

High-resolution label-free vascular imaging using a commercial, clinically approved dermatological OCT scanner

Robert A. Byers, Gillian M. Tozer, Nicola J. Brown, Stephen J. Matcher, The Univ. of Sheffield (United Kingdom)

Background and Aim: Recently developed decorrelative techniques such as speckle-variance optical coherence tomography (svOCT) have demonstrated non-invasive depth-resolved imaging of the microcirculation in-vivo. However, bulk tissue motion (BTM) originating from the subject’s breathing or heartbeat remains problematic at low imaging speeds, often resulting in full frame decorrelation and a loss of vascular contrast. The aim of this study was to build upon existing svOCT techniques through utilisation of a commercially available, probe-based VivoSight OCT system running at 20 kHz Axial-scan rate.
Methods and results: Custom four-dimensional scanning strategies were developed and utilized to maximize the inter-frame correlation during image acquisition. Volumes of structural OCT data were collected from various anatomical regions and processed using the aforementioned svOCT algorithm to reveal angiographic information. Following data collection, three-dimensional image registration and novel filtering algorithms were applied to each volume in order to ensure that BTM artefacts were sufficiently suppressed. This enabled accurate visualisation of the microcirculation within the papillary dermis, to a depth of approximately 1 mm. Applications of this technique, including quantitative capillary loop density measurement, visualisation of wound healing and temperature/pressure effects are demonstrated and enhanced through wide-field mosaicing of the svOCT data.

Conclusions: Non-invasive microrcirculation imaging using an FDA 510(k) approved OCT scanner such as the VivoSight allows direct clinical utilisation of these techniques, in particular for the pathological analysis of skin diseases. This research was supported by BBSRC Doctoral Training Grant: BB/F016840/1. The authors also gratefully acknowledge the use of equipment funded by MRC grant: MR/L012669/1.

9689-22, Session 9
Three-dimensional multifunctional optical coherence tomography for skin imaging
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Optical coherence tomography (OCT) visualizes cross-sectional structure of biological tissues. Recent developments of multifunctional OCT (MF-OCT) provides multiple optical contrasts which can reveal currently unknown tissue properties. In this presentation, we demonstrate multifunctional OCT specially designed for dermatological investigation.

A Swept-source MF- OCT has been built with a wavelength scanning laser at a 1310-nm wavelength. The scanning speed is 50,000 depth-lines/s and the axial resolution is 14.5 um in tissue. Three-dimensional scanning OCT, OCT angiography, polarization uniformity tomography, and local birefringence tomography images were obtained by a single scan. They respectively contrast tissue morphology, vasculature, melanin content, and birefringent tissue.

We adopted maximum-a-posteriori (MAP) estimation theory to enable highly quantitative birefringence evaluation. Inner and outer forearm skin of five normal subjects (30 to 45 years old) were examined by the MF- OCT. The birefringence at the depth of 63 um to 314 um from the skin surface was statistically evaluated. The inner forearm skin showed significantly higher birefringence than the outer forearm (p = 0.0012, paired t-test). The skin birefringence is known to be related to the property of collagen. In addition, outer forearm would be more frequently exposed to sunlight than inner forearm. Hence, this difference in birefringence could be the result of photaging.

MF-OCT also can examine the flow and pigmentation in skin. These aspects of MF-OCT will be discussed in the conference presentation.

9689-24, Session 10
Monitoring femtosecond laser microscopic photothermolysis with multimodal microscopy
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Photothermal induction by femtosecond (fs) lasers may be a promising modality in dermatology because of its advantages of high precision due to multiphoton absorption and deeper penetration due to the use of near infrared wavelengths. Although multiphoton absorption nonlinear effects are capable of precise targeting, the femtosecond laser photothermolysis could still have effects beyond the targeted area if a sufficiently high dose of laser light is used. Such unintended effects could be minimized by real time monitoring photothermolysis during the treatment. Targeted photothermal treatment of ex vivo mouse skin dermis was performed with tightly focused fs laser beams. Images of reflectance confocal microscopy (RCM), second harmonic generation (SHG), and two-photon fluorescence (TPF) of the mouse skins were obtained with integrated multimodal microscopy before, during, and after the laser treatment. The RCM, SHG, and TPF signal intensities of the treatment areas changed after high power femtosecond laser irradiation. The intensities of the RCM and SHG signals decreased when the tissue was damaged, while the intensity of the TPF signal increased when the photothermalization was achieved.

Moreover, the TPF signal was more susceptible to the degree of the photothermalization than the RCM and SHG signals. The results suggested that multimodal microscopy is a potentially useful tool to monitor and assess the femtosecond laser treatment of the skin to achieve microscopic photothermolysis with high precision.

9689-23, Session 9
Towards the use of OCT angiography in clinical dermatology
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The visual input has been the most important information for dermatologists in clinic. Because of its invasiveness and inconvenience, the skin biopsy is usually not desirable if not necessary. Hence, the first step of visualization of the skin is key to a comprehensive examination and accurate diagnosis. The most common method of visual inspection is using a magnified glass, relying heavily on subjective assessment. Optical coherence tomography (OCT) is a revolutionary imaging technique used commonly in ophthalmology, and on the way to become clinically viable alternative in dermatology due to its capability of acquiring histopathology level details of in vivo tissue, noninvasively. In this study, we demonstrate the capabilities of OCT-based microangiography in detecting high-resolution, three-dimensional structural and microvascular features of in vivo human skin with various conditions. A swept-source OCT system that operates on a central wavelength of 1310 nm with an A-line rate of 100 kHz is used in this study. We apply optical microangiography (OMAG) technique to visualize the structural and microvascular changes in tissue. OMAG images provide detailed visualization of functional microvasculature of healthy human skin from cheek and forehead areas, abnormal skin conditions from face, chest and belly. Moreover, OMAG is capable of monitoring the progress of wound healing on human skin from arm, delivering unprecedented detail of microstructural and microvascular information during longitudinal wound healing process. The presented results promise the clinical use of OCT angiography, aiming to treat prevalent cutaneous diseases, by detecting blood perfusion, and structural changes within human skin, in vivo.
9689-25, Session 10

New insights into photodynamic therapy treatment through the use of 3D Monte Carlo radiation transfer modelling

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Photodynamic therapy (PDT) is a non-invasive treatment for non-melanoma skin cancer and dysplasia that utilizes the combination of light, a photosensitising chemical and oxygen to selectively destroy cancerous tissue. We have developed 3D Monte Carlo radiation transfer (MCRT) simulations to investigate the limitations and potential of different light sources and treatment modalities. This technique has the possibility of generating accurate information about the energy deposition and light distribution in tissue during PDT.

We use MCRT simulations to study different aspects of daylight activated PDT, which utilizes the whole absorption spectrum of the photosensitising molecule Protoporphyrin IX (PpIX), compared to conventional PDT which uses a narrow bandwidth, red LED. For a clear day, a total visible light dose of 75 J/cm² is delivered during half an hour of daylight exposure, resulting in treatment depths of approximately 2 mm. If the lesion remains in the daylight, deeper treatments of up to 3 mm can be achieved in 2.5 hours. We also explore the time-dependent contribution of different wavelengths to the treatment depth where scattering, absorption and photobleaching effects are important.

The MCRT code allows us to explore the effects arising from non-uniform spatial distributions of different optical properties, specifically the effects on the internal light distributions of complex sub-surface structures (e.g. the capillary network). The information gained from our models cannot be generated only from surface observations and measurements and is of importance for further development and understanding of PDT treatments.

9689-26, Session 10

Laser ablation of basal cell carcinomas guided by confocal microscopy

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

Laser ablation offers precise and fast removal of superficial and early nodular types of basal cell carcinomas (BCCs). Nevertheless, the lack of histological confirmation has been a limitation. Reflectance confocal microscopy (RCM) imaging combined with a contrast agent can offer cellular-level histology-like feedback to detect the presence (or absence) of residual BCCs directly on the patient. We conducted an ex vivo bench-top study to provide a set of effective ablation parameters (fluence, number of passes) to remove superficial BCCs while also controlling thermal coagulation post-ablation to allow uptake of contrast agent.

The results for an Er:YAG laser (2.9 μm and pulse duration 250μs) show that with 6 passes of 25 J/cm², thermal coagulation can be effectively controlled, to allow both the uptake of acetic acid (contrast agent) and detection of residual (or absence) BCCs. Confirmation was provided with histological examination. We also obtained similar results for a CO2 laser (10.6μm, pulse duration 720-1160 msec) with 3 passes of 6.5 J/cm².

An initial in vivo study on 38 patients shows that the uptake of contrast agent aluminum chloride) and imaging quality is similar to that observed in the ex vivo study. The detection of the presence of residual tumor or complete clearance was confirmed in 16 wounds with (additional) histology and in 22 lesions with follow-up imaging. Our results indicate that resolution is sufficient but further development and use of appropriate contrast agent are necessary to improve sensitivity and specificity. Advances in RCM technology for imaging of lateral and deep margins directly on the patient may provide less invasive, faster and less expensive image-guided approaches for treatment of BCCs.

9689-27, Session 10

Antimicrobial blue light inactivation of gram-negative pathogens in biofilms: in vitro and in vivo studies

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Background: Biofilms affect more than 80% bacterial infections in human and are usually difficult to eradicate due to their inherent drug resistance. The situation is exacerbated by the increasing emergence of multidrug-resistant bacterial strains. There is consequently a pressing need for the development of alternative approaches to tackle drug resistance in biofilm-associated infections. A novel light-based antimicrobial approach, antimicrobial blue light (aBL), has attracted increasing attention.

Methods: We first investigated the effectiveness of aBL (415 nm) inactivation of Acinetobacter baumannii (a multidrug-resistant clinical strain) and Pseudomonas aeruginosa (ATCC 19660) biofilms in 96-well microwell plates. Using a mouse model of 3rd degree thermal burn infected with A. baumannii, we further assessed the effectiveness of aBL for treating biofilm-associated infections in vivo. Bioluminescence imaging was performed to monitor in real time bacterial viability both in vitro and in vivo.

Results: In vitro study showed that, for 24-h old A. baumannii biofilms, when an exposure of 432 J/cm² aBL had been delivered, approximately 3.59-log10 inactivation was achieved ; while for 24-h old P. aeruginosa biofilms, under the same aBL exposure, approximately 3.02-log10 inactivation was achieved. In vivo study using infected mouse burns demonstrated that, for A. baumannii biofilms, approximately 360 and 540 J/cm² aBL was required to achieve 3-log10 inactivation of biofilms when aBL was delivered at 24 and 48 h after bacterial inoculation, respectively.

Conclusions: aBL is a potential alternative approach for treating biofilm-associated infections.

9689-28, Session 10

Photodynamic therapy for skin rejuvenation

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Although mechanisms are not fully understood, recently, it has been demonstrated that topical photodynamic therapy (PDT) can offer certain beneficial effects in skin rejuvenation. Our clinical study shows that low-
9689-29, Session 11

**Dermoscopy-guided reflectance confocal microscopy of skin using high-NA objective lens with integrated wide-field color camera**

David L. Dickensheets, Seth Kreitinger, Montana State Univ. (United States); Gary Peterson, Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Reflectance Confocal Microscopy, or RCM, is being increasingly used to guide diagnosis of skin lesions. Widefield dermoscopy (WFD) combined with RCM is highly sensitive (~90%) and specific (~90%) for noninvasively detecting melanocytic and non-melanocytic skin cancers. The combined WFD and RCM approach is being implemented on patients to triage lesions into benign (with no biopsy) versus suspicious (followed by biopsy and pathology). Currently, however, WFD and RCM imaging are performed with separate instruments, while using an adhesive ring attached to the skin to sequentially image the same region and co-register the images. The latest small handheld RCMs offer no provision yet for a co-registered wide-field image. This paper describes an innovative solution that integrates an ultra-miniature dermoscopy camera into the RCM objective lens, providing simultaneous wide-field color images of the skin surface and RCM images of the subsurface cellular structure. The objective lens (0.9 NA) includes a hyperhemisphere lens and an ultra-miniature CMOS color camera, commanding a 4 mm wide dermoscopic view of the skin surface. The camera obscures the central portion of the aperture of the objective lens, but the resulting annular aperture provides excellent optical sectioning and resolution. Testing on melanocytic lesions in vivo on 30 volunteers showed the feasibility of combined WFD and RCM imaging to concurrently show the skin surface in wide-field and the underlying microscopic cellular-level detail. The paper describes this unique integrated dermoscopic WFD/RCM lens, and shows representative images. The potential for dermoscopy-guided RCM for skin cancer diagnosis is discussed.

9689-30, Session 11

**Space travel thins skin as multiphoton tomography shows**

Karsten König, Univ. des Saarlandes (Germany) and JenLab GmbH (Germany)

Skin impairments belong to the most frequent health problems during space missions. The multiphoton tomograph mPTflex, which provides noninvasively and label-free high resolution optical biopsies, was employed to study skin modifications of astronauts. This flexible clinical imaging system with its optomechanical arm measured pre-flight and post-flight the epidermis and upper dermis on six different sites of the volar left forearm of three European astronauts. The results of multiphoton sectioning based on two-photon autofluorescence and SHG of the collagen network demonstrate a thinning of the epidermis, a reduction of melanin and an increased collagen level after 6month space trips.

9689-31, Session 11

**Investigation of the effect of hydration on dermal collagen in ex vivo human skin tissue using second harmonic generation microscopy**

Ravikant V. Samatham, Steven L. Jacques, Nicholas Wang, Oregon Health & Science Univ. (United States)

Effect of hydration on the dermal collagen structure in human skin was investigated using second harmonic generation microscopy. Dog ears from the Mohs micrographic surgery department were procured for the study. 10 skin samples with subject aged between 45-90 years old were used in the study. Three dimensional Multiphoton (two-photon and backward SHG) control data was acquired from the skin samples. After the control measurement, the skin tissue was either soaked in deionized water for 2 hours (Hydration) or kept at room temperature for 2 hours (Desiccation), and SHG data was acquired. The data was normalized for changes in laser power and detector gain. The collagen signal per unit volume from the dermis was calculated. Considerable variation in the collagen signal was observed between samples. So, the variation in collagen signal from sample due to change in hydration could be compared to respective control data. The desiccated skin tissue gave higher backward SHG compared to its respective control tissue, while hydration sample gave a lower backward SHG. The collagen signal decreased with increase in hydration of the dermal collagen. Hydration affected the packing of the collagen fibrils causing a change in the backward SHG signal. In this study, the use of multiphoton microscopy to study the effect of hydration on dermal structure was demonstrated in ex vivo tissue. The ability to evaluate the hydration state of the skin has potential application in various fields from studying hydration of aging skin to the evaluation of the treatment options for wound healing.

9689-32, Session 11

**Depth resolved imaging of human skin comparing a compact sub-40fs Yb fiber laser and a ~200fs Ti:Sapphire laser**

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We report on a direct comparison between Ti:Sapphire and Yb fiber lasers for depth-resolved label-free multimodal imaging of human skin. We found that the penetration depth achieved with the Yb laser was 80% greater than for the Ti-Sapphire. Third harmonic generation (THG) imaging with Yb laser excitation provides additional information about skin structure. Our results indicate the potential of fiber-based laser systems for moving into clinical use.

9689-33, Session 12

**In vivo multimodality video microscopy of human skin in the vertical plane**

Zhenguo Wu, Yunxian Tian, Jianhua Zhao, Harvey Lui, BC Cancer Agency Research Ctr. (Canada) and Photomedicine Institute, The Univ. of British Columbia (Canada); David I. McLean, Photomedicine Institute, The Univ. of British Columbia (Canada); Haishan Zeng, BC Cancer Agency Research Ctr. (Canada) and Photomedicine Institute, The Univ. of British Columbia (Canada)
3.10±1.32 um, compared to the “ground truth” segmentation provided by a depth direction. Testing on a set of 15 RCM stacks produced a mean error of clustered using spectral clustering in order to detect the textural changes in 18 bands of different directionality at different scales. We then calculate decomposition by recursively transforming the low-pass bands and obtain in texture representation. Using DT-CWT, we decompose each tile into 6 shift-invariant, and directionally selective, which makes it highly efficient (DT-CWT) to represent textural structures in each tile. DT-CWT is almost called tiles, and analyze the textural changes in between consecutive tiles human skin in-vivo. We mimic the visual process by applying complex Study of the stratum corneum (SC) in human skin is important for research in barrier structure and function, drug delivery, and determination of water permeability of skin. The optical sectioning and high resolution of reflectance confocal microscopy (RCM) allows visual examination of the layers of the SC. Here, we present an unsupervised segmentation algorithm that can automatically delineate thickness of the SC in RCM images of human skin in-vivo. We mimic the visual process by applying complex wavelet transform over non-overlapping local regions of size 16-by-16 um called tiles, and analyze the textural changes in between consecutive tiles in axial (depth) direction. We use Dual-Tree complex wavelet transform (DT-CWT) to represent textural structures in each tile. DT-CWT is almost shift-invariant, and directionally selective, which makes it highly efficient in texture representation. Using DT-CWT, we decompose each tile into 6 directional sub-bands with orientations in ±15.45, and 75 degrees and a low-pass band, which is the decimated version of the input. We apply 3 scales of decomposition by recursively transforming the low-pass bands and obtain 18 bands of different directionality at different scales. We then calculate mean and variance of each band resulting in a feature vector of 36 entries. Feature vectors obtained for each stack of tiles in axial direction are then clustered using spectral clustering in order to detect the textural changes in depth direction. Testing on a set of 15 RCM stacks produced a mean error of 3.10±1.32 um, compared to the “ground truth” segmentation provided by a clinical expert reader.

9689-34, Session 12

Unsupervised machine learning method for delineating stratum corneum in reflectance confocal microscopy stacks of human skin in vivo

Alican Bozkurt, Northeastern Univ. (United States); Kivanc Kose, Memorial Sloan-Kettering Cancer Ctr. (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)

Study of the stratum corneum (SC) in human skin is important for research in barrier structure and function, drug delivery, and determination of water permeability of skin. The optical sectioning and high resolution of reflectance confocal microscopy (RCM) allows visual examination of the layers of the SC. Here, we present an unsupervised segmentation algorithm that can automatically delineate thickness of the SC in RCM images of human skin in-vivo. We mimic the visual process by applying complex wavelet transform over non-overlapping local regions of size 16-by-16 um called tiles, and analyze the textural changes in between consecutive tiles in axial (depth) direction. We use Dual-Tree complex wavelet transform (DT-CWT) to represent textural structures in each tile. DT-CWT is almost shift-invariant, and directionally selective, which makes it highly efficient in texture representation. Using DT-CWT, we decompose each tile into 6 directional sub-bands with orientations in ±15.45, and 75 degrees and a low-pass band, which is the decimated version of the input. We apply 3 scales of decomposition by recursively transforming the low-pass bands and obtain 18 bands of different directionality at different scales. We then calculate mean and variance of each band resulting in a feature vector of 36 entries. Feature vectors obtained for each stack of tiles in axial direction are then clustered using spectral clustering in order to detect the textural changes in depth direction. Testing on a set of 15 RCM stacks produced a mean error of 3.10±1.32 um, compared to the “ground truth” segmentation provided by a clinical expert reader.

9689-35, Session 13

Cutaneous porphyrins exhibit anti-Stokes fluorescence that is detectable in acne

Yunxian Tian, Haishan Zeng, Jianhua Zhao, Zhenguo Wu, BC Cancer Agency Research Ctr. (Canada); Mohammed AI Jasser, Harvey Lui, David I. Mclean, Photomedicine Institute, The Univ. of British Columbia (Canada)

Porphyrins produced by Propionibacterium acneus represent the principal fluorophore associated with acne, and appear as orange-red luminescence under the Wood’s lamp. Assessment of acne based on Wood’s lamp (UV) or visible light illumination is limited by photon penetration depth and has limited sensitivity for earlier stage lesions. Inducing fluorescence with near infrared (NIR) excitation may provide an alternative way to assess porphyrin-related skin disorders. We discovered that under 785 nm CW laser excitation PpIX powder exhibits fluorescence emission in the shorter wavelength range of 600-715 nm with an intensity that is linearly dependent on the excitation power. We attribute this shorter wavelength emission to anti-Stokes fluorescence. Similar anti-Stokes fluorescence was also detected focally in all skin-derived samples containing porphyrins. Regular (Stokes) fluorescence was present under UV and visible light excitation on ex vivo nasal skin and sebum from uninflamed acne, but not on nose surface smears or sebum from inflamed acne. Co-registered CW laser-excited anti-Stokes fluorescence and fs laser-excited multi-photon fluorescence images of PpIX powder showed similar features. In the skin samples because of the anti-Stokes effect, the NIR-induced fluorescence was presumably specific for porphyrins since there appeared to be no anti-Stokes emission signals from other typical skin fluorophores such as lipids, keratins and collagen. Anti-Stokes fluorescence under NIR CW excitation is more sensitive and specific for porphyrin detection than UV- or visible light-excited regular fluorescence and fs laser-excited multi-photon fluorescence. This approach also has higher image contrast compared to NIR fs laser-based multi-photon fluorescence imaging. The anti-Stokes fluorescence of porphyrins within sebum could potentially be applied to detecting and targeting acne lesions for treatment via fluorescence image guidance.

9689-36, Session 13

Fluorescence excitation-emission matrix spectroscopy of vitiligo skin in vivo

Jianhua Zhao, The Univ. of British Columbia (Canada) and BC Cancer Agency Research Ctr. (Canada); Vincent Richer, Mohammed AI Jasser, Sooodabeh Zandi, Nikiforos Kollias, Sunil Kalia, The Univ. of British Columbia (Canada); Haishan Zeng, BC Cancer Agency Research Ctr. (Canada) and The Univ. of British Columbia (Canada); Harvey Lui, The Univ. of British Columbia (Canada) and BC Cancer Agency Research Ctr. (Canada)

Fluorescence signals depend on the intensity of the exciting light, the absorption properties of the constituent molecules, and the efficiency with which the absorbed photons are converted to fluorescence emission. The optical features and appearance of vitiligo have been explained primarily on the basis of reduced epidermal pigmentation, which results in abnormal white patches on the skin. The objective of this study is to explore the fluorescence properties of vitiligo and its adjacent normal skin using fluorescence excitation-emission matrix (EEM) spectroscopy. Thirty five (35) volunteers with vitiligo were acquired using a double-grating spectrophotometer with excitation and emission wavelengths of 260-450 nm and 300-700 nm respectively. As expected, the most pronounced difference between the spectra obtained from vitiligo lesions compared to normally pigmented skin was that the overall fluorescence was much higher in vitiligo; these differences increased at shorter wavelengths, thus matching the characteristic spectral absorption of epidermal melanin. When comparing the fluorescence spectra from vitiligo to normal skin we detected
three distinct spectral bands centered at 280nm, 310nm, and 335nm. The 280nm band may possibly be related to inflammation, whereas the 335 nm band may arise from collagen or keratin cross links. The source of the 310 nm band is uncertain; it is interesting to note its proximity to the 311 nm UV lamps used for vitiligo phototherapy. These differences are accounted for not only by changes in epidermal pigment content, but also by other optically active cutaneous biomolecules.

9689-37, Session 13

Measurement of diffusion of fluorescent compounds and autofluorescence in skin in vivo using a confocal instrument

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Using portable and affordable instrumentation based upon confocal imaging we have tracked the movement of fluorescent compounds through skin in near real time with high resolution and sensitivity. Being able to measure the diffusion of compounds through skin with good spatial and temporal resolution plays an important role for applications such as monitoring the penetration of pharmaceuticals applied to skin and assessing the integrity of the skin barrier. Several measurement methods exist, but they suffer from a number of problems such as being slow, expensive, non-portable and not sensitive enough. To address these issues, we adapted a technique that we previously developed for tracking fluorescent compounds in the eye to measure the autofluorescence and the diffusion of externally applied fluorescent compounds in skin in vivo. Results are presented that show the autofluorescence from different skin sites as well as the change in autofluorescence of the same skin site over the course of a week. We furthermore demonstrate the ability of the instrument to measure the diffusion speed and depth of externally applied fluorescent compounds from a number of problems such as being slow, expensive, non-portable and not sensitive enough. To address these issues, we adapted a technique that we previously developed for tracking fluorescent compounds in the eye to measure the autofluorescence and the diffusion of externally applied fluorescent compounds in skin in vivo. Results are presented that show the autofluorescence from different skin sites as well as the change in autofluorescence of the same skin site over the course of a week. We furthermore demonstrate the ability of the instrument to measure the diffusion speed and depth of externally applied fluorescent compounds both in healthy skin and after the skin barrier function has been perturbed by tape stripping or degreasing. The instrument is currently being developed further for increased sensitivity and multi-wavelength excitation. We believe that the presented instrument is suitable for a large number of applications in fields such as assessment of damage to the skin barrier, development of topical and systemic medication and tracking the diffusion of fluorescent compounds through skin constructs as well as monitoring effects of skin products.

9689-38, Session PSun

Investigation of temporal effects induced by microneedles for transdermal drug delivery with optical coherence tomography

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Transdermal drug delivery systems (TDDS) have been an attracting field in drug delivery because of its benefit over parenteral and oral administration. Recent studies illustrate that microneedles (MNs) can penetrate through stratum corneum (SC) barrier to facilitate drug delivery. However, the temporal effects on skin and realtime observation of drug delivery due to the implementation of MNs are difficult to be investigated. In this study, we used optical coherence tomography (OCT) to investigate the temporal effects on skin induced by MN for transdermal drug delivery, including the morphological and vascular changes. With OCT scanning, the penetration depth and punched area can be quantitatively evaluated. In addition, OCT was used to reconstruct micro-angiography of mouse skin and to observe the changes in micro-angiography induced by MNs. Finally, OCT is implemented for dynamic evaluation of skin recovery after using MN array for drug delivery, which can be evaluated from the speckle variance of OCT signal. The results showed that the dissolution of MNs as a function of time can be observed. The leakage of red blood cells and drug diffusion in skin also can be in vivo visualized. Therefore, OCT could be a potential tool for in vivo monitoring of effects and outcome due to MN treatments.

9689-39, Session PSun

Development of 3D printing probe for dermatologic optical coherence tomography

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Currently, dermoscopy, confocal microscopy and ultrasound imaging techniques have become common tools for clinical dermatology. Although dermoscopy and confocal microscopy can provide excellent resolutions, the imaging depths of both techniques are limited. In contrast, ultrasound imaging can probe deeper skin structure, but it is difficult to provide a high imaging resolution, making identification of the different skin layers difficult. Therefore, in this study, we demonstrated a portable, functional optical coherence tomography (OCT) system for dermatology study. To miniaturize the size of the scanning probe for the OCT system and to scan arbitrary locations of skin, we used SolidWorks software to design the mechanical components of probe and to use 3D printer to fabricate mechanical components to integrate the electrical devices and optical components. Such probe fabricated by 3D printing can provide the advantages of low cost, light weight, high flexibility. Finally, the probe was integrated with a swept-source OCT system. The developed OCT system can reconstruct 3D structural OCT images and 3D vascular patterns of skin, simultaneously. Finally, the developed system was used for the study on hair follicles and papilla of facial skin. From the results, the sizes and distribution of follicles and papilla can be identified, which can be used as the indicators for diagnosis of skin diseases. This system can be a powerful tool for diagnoses of skin diseases and for studies on cosmetics.

9689-40, Session PSun

Metal-clad waveguide characterization for contact-based light transmission into tissue

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As contemporary laser dermatology procedures, like tattoo removal and skin resurfacing, become more popular, the complications of their operation are similarly growing in prevalence. Frequent incidences of overexposure, ocular injury, and excessive thermal damage represent mounting concerns for those seeking such procedures; moreover, each of these problems is a direct consequence of the standard free-space method of laser transmission predominantly used in clinical settings. Therefore, an alternative method of light transmission is needed. Here, we demonstrate and characterize an alternative method that uses planar waveguides to deliver light into sample tissue via direct contact. To do this, glass slab substrates were clad in layers of titanium, silver, and a low refractive index polymer to constrain the light within the waveguide along its length. Active areas comprised of titanium and silver thin films allowed the totally internally reflecting light to, when in contact with the tissue sample, optically tunnel into the sample. SEM and EDS were used to characterize the metal film thickness and deposition rates
onto the glass substrates. Laser light from a Q-switched Nd:YAG source operating at 532 nm was coupled into the waveguide and transmitted into samples of pig skin, which was measured using photoacoustics along with a photodiode and integrating sphere. Transmitting light into tissue in this manner effectively resolves or circumvents the complications caused by free-space propagation methods as it reduces the operating distance to 0, which prevents hazardous back-reflections and allows for the ready incorporation of contact cooling technologies.

9689-41, Session PSun
Remote optical configuration of pigmented lesion detection and diagnosis of bone fractures
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In this paper we present a novel approach of realizing a safe, simple, and inexpensive sensor applicable to bone fractures and pigmented lesions detection. The approach is based on temporal tracking of back-reflected secondary speckle patterns generated while illuminating the affected area with a laser and applying periodic pressure to the surface via a controlled vibration. The use of such a concept was already demonstrated for non-contact monitoring of various bio-medical parameters such as heart rate, blood pulse pressure, concentration of alcohol and glucose in the blood stream and intra-ocular pressure. The presented technique is a safe and effective method of detecting bone fractures in populations at risk. When applied to pigmented lesions, the technique is superior to visual examination in avoiding many false positives and resultant unnecessary biopsies. Applying a series of different vibration frequencies at the examined tissue and analyzing the 2-D speckle pattern trajectory in response to the applied periodic pressure creates a unique signature for each and different pigmented lesion. Analyzing these signatures is the first step toward detection of malignant melanoma. In this paper we present preliminary experiments that show the validity of the developed sensor for both applications: the detection of damaged bones as well as the classification of pigmented lesions.

9689-42, Session PSun
Fabrication of multilayered optical tissue phantoms with 3D deposition for phototherapeutics
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Optical tissue phantoms have been developed for in vitro human skin experiments due to convenience of experimental use and easiness of storage. However, it is often difficult to fabricate a thin (a few hundred micrometers) layer of dermis. In the current study, to mimic human skin tissue, we fabricated multilayered optical tissue phantoms (100 µm up to several mm) consisting of epidermal and dermal layers with 3D printing technique. Gelatin plates (1.6-2.0 g/50 ml) were mixed with agar powder (1.5-2.0 g/50 ml), coffee (0.5-1.0 g/50 ml), and TiO2 powder (0.1-0.5 g/50 ml) to prepare the phantoms that represented various skin types (I-VI). A 3D printer was employed to control phantom thickness and structure. The concentration of both the coffee and the TiO2 powder determined the degree of absorption and reduced scattering coefficients for each skin type. The optical properties between 400 and 1000 nm were measured by a double-integrating sphere with inverse adding-doubling (IAD) algorithm. Mechanical properties of the fabricated phantoms were also measured by using a universal testing machine (UTM). Optical coherence tomography confirmed that the thicknesses of epidermis and dermis were measured to be 100.0±3.3 µm and 1.0±0.1 mm respectively. The color difference (ΔE) between the phantom and the human skin ranged from 2.54 to 7.1 for skin type from I to VI. The optical property measurements showed that the absorption and reduced scattering coefficients decreased with wavelength. Compared to previous studies, the overall difference in the optical properties was found to be 16.5 to 24.8 %. Young’s modulus of the phantoms increased with the amount of the mixed gelatin and was approximately 7.8 to 8.5 kPa, which was approximately 30% lower than the real tissue (11.7 kPa). The proposed optical tissue phantom can be a feasible model to indirectly evaluate human tissue responses during laser treatment of skin pigmented lesions.

9689-43, Session PSun
Application of circumferential irradiation for low-temperature laser lipolysis
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Laser lipolysis with flat fibers has frequently been associated with excessive thermal injury (burn) due to rapid temperature increase. The aim of the current study was to develop an optical diffusing fiber to induce slow thermal gradients for safe and efficient laser lipolysis. For diffusing fiber preparation (i.e., 10-mm length), 600-µm fiber was machined by CO2 laser with multiple tapering processes. For in vitro testing, two different fibers (flat and diffusing) were tested to identify thermal responses of porcine abdomen fat tissue during laser irradiation. Near-IR laser power (8, 16, and 24 W) was irradiated for 1 minute to identify the optimal treatment conditions for laser lipolysis. A thermal camera was used to monitor temperature development during the laser irradiation. During the experiment, both the flat and the diffusing fibers were used to determine the rate of fat reduction during photothermal treatments in terms of weight variations. Regardless of fiber type, the temperature in tissue increased with laser power. The diffusing fiber showed lower thermal gradient than the flat fiber. The results showed that the peak temperature of the flat fiber reached over 140 degree while the diffusing fiber generated up to 88 degree at 24 W. Due to the higher peak temperature, the flat fiber caused excessive carbonization in the fatty tissue and experienced severe deterioration 2 sec after laser irradiation. However, no thermal damage occurred at the diffusing fiber tip. Resulting from circumferential light distribution, the diffusing optical fiber covered a wider liquefied area (~1 cm long) but yielded ~15% less fat reduction, compared to the flat fiber. The proposed optical diffusing fibers can be a feasible tool for laser lipolysis in term of low temperature development and wide coverage of tissue treatment.

9689-44, Session PSun
UV photostability of insect repellents evaluated through Raman spectroscopy
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The use of insect repellents either indoors or at places with incidence of solar radiation has been common due to dengue epidemics in Brazil. The lack of studies on the photostability of these substances has motivated this study, where the main goal was to verify the photostability and photodegradation of some of the commercially insect repellents available under the simulated ultraviolet (UV) radiation, by evaluating the molecular changes using dispersive Raman spectroscopy (830 nm excitation). A laboratory-made chamber was used for irradiating the repellents, where
UV-A + UV-B radiations (UV-A: 5.5 mW/cm² and UV-B 1.5 mW/cm²) can be obtained. The chamber internal temperature did not exceed 31 °C during experiments. The compounds n, n-diethyl-m-toluamide (DEET), IR-3535, andiroba and citronella oils, used as active ingredients in insect repellents, and commercial formula containing DEET (14.5% in ethanol and isopropyl myristate) and IR-3535 (16% in carbopol gel) were continuously irradiated for 8 h. The Raman spectrum of each sample was obtained before and after UV exposure. The compounds and the commercial formula containing IR-3535 showed photo-stability when irradiated, since no changes in the peaks were found. The commercial formula containing DEET showed spectral decrease at 524, 690, 1003 and 1606 cm⁻¹, assigned to the DEET, and increase at 884 cm⁻¹, assigned to the ethanol. These results indicate that the excipient could influence the photostability of the active ingredient. The Raman spectroscopy can be suitable to monitor the photodegradation under UV irradiation rapidly and reliably.
9689-45, Session 1

Fluorescence spectroscopy incorporating a ratiometric approach for the diagnosis and classification of urothelial carcinoma

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The current most popular clinical method for the screening of urothelial carcinoma is white light cystoscopy, which has inherent disadvantages making the approach towards developing more powerful diagnostic techniques. Laser induced intrinsic fluorescence spectroscopy has been used as an adjunct to current methods for the detection of tumors. This technique allows real time results based on the changes in spectral profile between normal and tumor tissues. We conducted a pilot study based on fluorescence spectroscopy at two wavelengths 378 and 445 nm excitation for the differentiation of urothelial carcinoma. At both the excitation wavelengths, the measured fluorescence signal showed an increased intensity at longer wavelengths greater than 520 nm. In addition, the emission profile showed modulation at 580 nm which is due to the reabsorption of emitted fluorescence due to hemoglobin. Additionally, we developed a tissue characterization algorithm, Fluorescence Ratiometric (FluoRa) classifier F510/F600 and F520/F580 at 378 and 445 nm excitation wavelengths respectively. Further, the results were correlated with the pathologist’s assessment of urothelial carcinoma. This ratiometric classification algorithm yielded 81% sensitivity and 83% specificity at 378 nm and while at 445 nm excitation we achieved a sensitivity and specificity of 85% and 86% respectively. In this study we have demonstrated the potential of a simple ratiometric algorithm based on fluorescence spectroscopy could be an alternative tool to tissue biopsy. Furthermore, this technique based fiber-based fluorescence spectroscopy could be integrated into an endoscopy system for use in the operating room.

9689-47, Session 1

In-vivo investigation of the human testis by the probe-based and microscopic optical coherence tomography (OCT)

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Introduction and objective: The number of couples seeking consultation for infertility problems has steadily increased over the past decades. In about 50% male reveal fertility problems and 10% of these men show azoospermia. Because causal therapeutic options are often limited, the majority needs surgical testicular sperm extraction (TESE) to obtain sperms for assisted reproductive techniques. As far as we know, there is no imaging technique available to evaluate the testicular tissue regarding sperm containing seminal tubules. This is the reason why the chosen areas for biopsy are arbitrarily performed and the success rate of the sperm retrieval is to date only 50%. The potential of using optical coherence tomography (OCT) to image the male genital tract was recently published in an ex-vivo bovine model. Thus in a following step this study aims to investigate the feasibility of performing OCT during clinical TESE.

Material and Methods: Six patients suffering from azoospermia were examined during TESE procedure. In four of them the probe-based endoscopic NirisTM Imaging System (probe diameter 2.7 mm; central wavelength 1300 nm, lateral resolution of 25–50 µm and axial resolution of 10–20 µm) and in two patients microscopic OCT was performed. For this, an OCT camera (I/OCT, OptoMedical Technologies GmbH, Lübeck, Germany) attached to a surgical microscope (HS Hi-R 1000, Haag-Streit Surgical GmbH, Wedel, Germany) was used. It allows a variable working distance between about 20 cm and 40 cm, and operates at a central wavelength of 840 nm. The axial resolution is about 10 µm, the lateral resolution about 50 µm. Additionally, ex vivo microscopic OCT imaging was performed of two orchietomy samples from patients suffering of testicular cancer (n=2).

Results: By microscopic OCT imaging it was possible to differentiate the microarchitecture of the testis and single seminiferous tubules became visible. Obviously the penetration depth of the probe-based OCT was higher compared to the microscopic OCT, but the image resolution was distinctly reduced. As tissue of patients with azoospermia and testicular cancer were examined, the patterns of normal and elongated tubules could be demonstrated. Elongated tubules are typical for a sertoli-cell only syndrome.

Conclusions: OCT enables imaging of the testicular tissue in a micron-scale resolution. Comparison of the different systems showed that the NirisTM system lacks of detailed information and is insufficient for clinical purposes. The results of the microscopic OCT imply that application of this novel technique could be an alternative tool to tissue biopsy.

9689-46, Session 1

Cavitation bubble dynamics during thulium fiber laser lithotripsy

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Introduction: Cavitation bubble dynamics during pulsed Holmium:YAG laser delivery through an optical fiber in fluid environment for kidney stone fragmentation have been previously reported. Thulium fiber laser (TFL) is explored as alternative lithotripter. TFL parameters differ in several important ways from Holmium laser, including smaller fiber delivery, more strongly absorbed wavelength, low pulse energy/high pulse rate operation, and uniform temporal pulse structure. High speed imaging of cavitation bubbles during TFL lithotripsy was studied to determine influence of these laser parameters on bubble formation.

Methods: TFL operating at 1908 nm with 35-mJ pulse energy, 500-?s pulse duration, and 500 Hz pulse rate delivered laser energy through 105-?m-core, silica fiber. Fiber tip was submerged in saline bath, by itself, or in contact with kidney stones or Nitinol wire baskets. Cavitation bubble characteristics using bare, tapered, and ball tip fibers were studied. Imaging was performed at 105,000 fps and 10 µm spatial resolution. Cavitation bubble dynamics using Holmium:YAG laser were also performed for comparison.

Results: A train of bubbles was observed during single 500 ?s TFL pulse. Maximum bubble diameters measured 440 ± 120 ?m near bare fiber tip, with 70 ± 20 µm lifetime, and bubble stream extending 880 ± 260 µm from fiber tip (n=6). These observations are consistent with previous TFL studies reporting ablation stallout at working distances beyond ~1 mm.

Conclusions: TFL bubble formation was characterized by smaller bubble dimensions than Holmium:YAG laser possibly due to lower pulse energy operation mode and smaller fiber diameter used.

Return to Contents  +1 360 676 3290  ·  help@spie.org  15
imaging techniques might improve sperm retrieval rates during testicular sperm extraction (TESE) by optical guidance.

9689-48, Session 1

Multimodal, 3D pathology-mimicking bladder phantom for evaluation of cystoscopic technologies

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Optical coherence tomography (OCT) and blue light cystoscopy (BLC) have shown significant potential as complementary technologies to traditional white light cystoscopy (WLC) for early bladder cancer detection. Three-dimensional (3D) organ-mimicking phantoms provide realistic imaging environments for testing new technology designs, the diagnostic potential of systems, and novel image processing algorithms prior to validation in real tissue. Importantly, the phantom should mimic features of healthy and diseased tissue as they appear under WLC, BLC, and OCT, which are sensitive to tissue color and structure, fluorescent contrast, and optical scattering of subsurface layers, respectively. We present a phantom posing the hollow shape of the bladder and fabricated using a combination of 3D-printing and spray-coating with Dragon Skin (DS) (Smooth-On Inc.), a highly elastic polymer to mimic the layered structure of the bladder. Optical scattering of DS was tuned by addition of titanium dioxide, resulting in scattering coefficients sufficient to cover the human bladder range (0.49 to 2.0 mm^-1). Mucosal vasculature and tissue coloration were mimicked with elastic cord and red dye, respectively. Urethral access was provided through a small hole excised from the base of the phantom.

Inserted features of bladder pathology included altered tissue color (WLC), fluorescence emission (BLC), and variations in layered structure (OCT). The phantom surface and underlying material were assessed on the basis of elasticity, optical scattering, layer thicknesses, and qualitative image appearance. WLC, BLC, and OCT images of normal and cancerous features in the phantom qualitatively matched corresponding images from human bladders.

9689-49, Session 2

Cavitation bubble dynamics during Ho:YAG laser lithotripsy by high-speed camera

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Although laser lithotripsy is now the preferred treatment option for urolithiasis, the mechanism of laser pulse induced calculus damage is still not fully understood. This is because the process of laser pulse induced calculus damage involves quite a few physical processes and their time-scales are very short (down to sub micro second level). For laser lithotripsy, the laser pulse induced impact by energy flow can be summarized as: Photon energy in the laser pulse ? photon absorption generated heat in the water liquid and vapor (super heat water or plasma effect) ? shock wave (Bow shock, acoustic wave) ? cavitation bubble dynamics (oscillation, and center of bubble movement), super heat water effect) ? shock wave (Bow shock, acoustic wave) ? cavitation bubble generated heat in the water liquid and vapor (super heat water or plasma summarized as: Photon energy in the laser pulse ? photon absorption laser lithotripsy, the laser pulse induced impact by energy flow can be their time-scales are very short (down to sub micro second level). For this study, cavitation bubble dynamics was investigated by a high-speed camera and a needle hydrophone. A commercialized, pulsed Ho:YAG laser at 2.12 um, StoneLightTM 30, with pulse energy from 0.5J up to 3.0 J, and pulse width from 150 ns up to 800 us, was used as laser pulse source. Pulses with Burst Mode Technology (1 Hz burst of 2 pulses in 30 Hz rep rate) of energy of up to 4 J were also tested. The fibers used in the investigation are SureFlexTM fibers. Model S-LLF273/365, 273/365 um core diameter fibers. A high-speed camera with frame rate up to 1 million fps was used in this study. The results revealed the cavitation bubble dynamics (oscillation and center of bubble movement), and the 3-D shock wave pressure magnitude profile of the bubble induced by laser pulse at different energy level and pulse width. A preliminary comparison of cavitation bubble dynamics of Ho:YAG laser with Q-Switched Tm:YAG laser was also included in this study. More detailed investigation on the relationship between cavitation bubble dynamics and calculus damage (fragmentation/dusting) will be conducted as a future study.

9689-50, Session 2

Feasibility of laser-integrated high intensity focused ultrasound (HIFU) treatment for bladder tumors: in vitro study

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Previous studies have shown that photothermal therapy combined with high intensity focused ultrasound (HIFU) can provide a promising method to achieve rapid thermal coagulation during surgical procedures. The current study investigated the feasibility of the laser-integrated high intensity focused ultrasound (HIFU) application to treat bladder tumors by enhancing thermal effects and therapeutic depth in vitro. To generate thermal coagulation, a single element HIFU transducer with a central frequency of 2.0 MHz was used to transmit acoustic energy to 15 fresh porcine bladders injected with an artificial tumor (100 µl gelatin and hemoglobin solution) in vitro. Simultaneously, an 80-W 532-nm laser system was also implemented to induce thermal necrosis in the targeted tissue. The intensity of 570 W/cm² at the focus of HIFU and laser energy of 0.9 W were applied to all the samples for 40 s. The temperature rise increased up to about 1.6 or 3 folds (i.e., ΔT=32±3.8 K for laser-integrated HIFU, ΔT=20±6.5 K for HIFU only, and ΔT=7±5.6 K for laser only). The estimated lesion depth also increased by 1.3 and 2 folds during the dual-thermal treatment, in comparison with the treatment by either HIFU or laser. The results indicated that the laser-integrated HIFU treatment can be an efficient hyperthermic method for tumor coagulation.

9689-51, Session 2

Thulium fiber laser lithotripsy using small spherical distal fiber tips

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Introduction: Fibers consisting of 240-7µm-core trunk fiber with rounded, 450-7µm-diameter ball tips are available for Holmium:YAG laser lithotripsy, to reduce mechanical damage to inner lining of ureteroscope working channel during fiber insertion, and prolong ureteroscopy lifetime. This study explores an application of smaller ball tip fiber during Thulium fiber laser (TFL) lithotripsy.

Methods: A 100-7µm-core fiber with 300-7µm-diameter distal ball tip was
used. TFL was operated at 1908 nm, with 35-mJ pulse energy, 500-ns pulse duration, and 300-Hz pulse rate. Human calcium oxalate/phosphate stone samples (4.5-mm diameter) were weighed, and ablation rates measured for ball tip fibers with direct comparison to conventional bare tip fibers. High magnification images of ball tips were taken before and after each procedure to track ball tip degradation and determine number of procedures completed before need for replacement.

Results: There was no statistical difference (P>0.05) between stone ablation rates for single-use ball tip fiber (1.3 ± 0.4 mg/s) (n=10), multiple-use ball tip fiber (1.3 ± 0.5 mg/s) (n=44), and conventional single-use bare tip fibers (1.4 ± 0.2 mg/s) (n=10). Ball tip durability varied widely, but fibers averaged > 4 stone procedures before failure, as indicated by rapid decline in stone ablation rate. Mechanical damage (fracture/crack formation) at front surface of ball tip was major limiting factor in fiber lifetime.

Conclusions: The miniature ball tip fiber may provide cost-effective design for safe fiber insertion through ureteroscope working channel and into ureter without risk of damage or perforation, and without compromising stone ablation efficiency during TFL lithotripsy.

9689-52, Session 2
Application of novel optical diffuser for urethral stricture treatment
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Optical fibers have frequently been used for photothermal laser therapy due to its efficiency to deliver laser energy directly to tissue. The aim of the current study was to develop a diffusing optical fiber to achieve radially uniform light irradiation for endoscopically treating urethral stricture. The optical diffuser was fabricated by micro-machining helical patterns on the fiber surface using CO2 laser light at 5 W. Visible light emission (632 nm) and spatial emissions (including polar, azimuthal, and longitudinal emissions) of the fiber tip were evaluated to validate the performance of the fabricated diffuser. Prior to tissue tests, numerical simulation on heat distribution was developed to estimate the degree of tissue coagulation depth during interstitial coagulation. Due to a high absorption coefficient by tissue water, 1470 nm laser was used for photothermal therapy treatment of urethral stricture to obtain a more precise depth profile. For in vitro tissue tests, porcine liver tissue was irradiated with three different power levels (3, 6, and 9 W) at various irradiation times. Porcine urethral tissue was also tested with the diffuser for 10 sec at 6 W to validate the feasibility of circumferential photothermal treatment. The treated tissue was stained with hematoxylin and eosin (H&E) and then imaged with an optical transmission microscope. The spatial emission characteristics of the diffusing optical fiber presented an almost uniform power distribution along the diffuser tip (less than 10% deviation) and around its circumference (less than 5% deviation). The peak temperature in simulation model at the tissue interface between the glass-cap and the tissue was 373 K that was higher than that at the distal end. The tissue tests showed that higher power levels resulted in lower coagulation thresholds (e.g., 1 sec at 9 W vs 8 sec at 3 W). Furthermore, the coagulation depth was approximately 20% thinner than the simulation results (p<0.001). The extent of coagulation thickness in urethral tissue was measured to be 1.3±0.2 mm, which was slightly thicker (18%) than the liver testing (1.1±0.1 mm) under the same conditions (p<0.001). The proposed optical diffuser may be a feasible tool to treat the urethral stricture in a uniform manner.

9689-53, Session 3
Miniaturized rapid scanning, forward-viewing catheteroscope for optical coherence tomography
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Patients afflicted with bladder cancer undergo annual surveillance in the clinic with flexible white light cystoscopy (WLC). However, WLC lacks the sensitivity to detect all bladder tumors and provides no stage information. Optical coherence tomography (OCT) can overcome these limitations of WLC due to its ability to visualize subsurface details of the bladder wall to stage cancers and to detect tumors otherwise invisible to WLC. A major challenge, however, to realizing OCT imaging during clinical cystoscopies is developing a forward-viewing OCT catheteroscope capable of passing through the 2.4-mm working channel of a standard flexible cystoscope. Additionally, to aid in identifying new tumors, the OCT system must be fast enough to collect data over the entire surface of the bladder without significantly increasing the procedure time. We have developed the first rapid-scanning forward-viewing OCT catheteroscope that uses scanning fiber technology and is suitable for integration into flexible cystoscopes. The scanning fiber scope has a resonance frequency exceeding 2 kHz, which enables rapid volumetric data collection at a rate of 12.5 Hz. We expand on our previous design of such a scope by miniaturizing the scope package to a diameter of 129 mm and a rigid length of 19 mm, making this the smallest such package for forward-viewing, scanning OCT scopes. We validate the imaging quality of our prototype scope using phantom and ex vivo pig bladder samples. The miniaturized, rapid-scanning OCT scope is a promising tool to enable early detection and staging of bladder cancer during flexible WLC.

9689-54, Session 3
High efficiency for prostate biopsy qualification after training 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. 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. Recent studies have emphasized the essential learning process for clinicians to fully apprehend this novel imaging modality. This study aimed to define standard reading criteria for the benign/malignant classification of prostate biopsies based on FFOCT images, and test the procedure amongst doctors.

A set 25 images of prostate biopsies was used to construct an atlas with emphasized specific benign and malignant structures. After training on the atlas, pathologists and urologists were asked to blindly analyze another set of 113 FFOCT biopsy images, notify the presence/lack of malignant structures and state on the malignancy of the biopsy. Pathologists and urologists obtained very high accuracy scores, above 90%, when compared to the pathological diagnosis of the biopsies. FFOCT is as a fast and non-destructive imaging technique that provides a quick assessment of the tissue morphology and appears as a potential additional detection tool for prostate cancer screening. After training,
The system detection sensitivity was measured to be 105 dB. A semi-swept laser light source centered at approximately 1,300 nm with a full width at half maximum bandwidth of 100 nm, yielding approximately 10 ?m axial and lateral image resolution in tissue. In vivo imaging scans were projected onto the approximated bladder surface to generate a virtual 3D bladder reconstruction. Intraoperative WLC videos from rigid cystoscopies (1280 x 720 pixels) were recorded at 30 Hz followed by immediate camera calibration to correct for image distortions. Video data were fed into an automated structure-from-motion algorithm that generated a 3D point cloud followed by a 3D mesh to approximate the bladder surface. The highest quality cystoscopic images were used for screening PC, >60% of the PSA detected cancers are indolent, thereby facilitating rapid clinical translation, application to other forms of endoscopy and new opportunities for longitudinal studies of cancer recurrence.

Optical coherence tomography (OCT) is a rapidly emerging noninvasive form of imaging that uses the light scattering characteristics of tissue to provide high-resolution cross-sectional images of tissue microstructure. While this technology has been in use clinically mainly in ophthalmology, its potential clinical application in the operating room (OR) has received less attention. In renal transplantation, damage from ischemic insult to the kidney contributes to delayed graft function (DGF) that is marked by a prolonged recovery period and a reduction in the lifetime of the transplanted kidney. The proximal convoluted tubules (PCTs) of the kidney are particularly sensitive to ischemic insult and respond with swelling of the tubular walls leading eventually to cell rupture. The degree to which swelling occurs is indicative of the extent to which acute tubular necrosis (ATN) has occurred and is a measure of the ischemic induced delayed graft function. The PCTs comprise the majority of the cortex in the kidney and can be visualized more than several layers deep through the intact renal capsule using OCT. We have developed a rapid, fully automated system to measure the swelling of the PCTs and determine the degree of ATN. This system was used to analyze OCT scans acquired in the OR both prior to and following the transplant of both cadaver and living human donor kidneys. Preliminary results demonstrate a correlation between swelling of the PCTs and DGF, as evidenced by an evaluation of renal functional parameters (i.e., serum creatinine, BUN, GFR).
spectroscopic (PWS) microscopy, which can quantify intracellular nanoscale organizations (e.g., chromatin structures) that are not accessible by conventional microscopy. PWS microscopy has previously been shown to predict the risk of cancer in seven different organs (N ~ 800 patients). Herein we use PWS measurement of label-free histologically-normal prostatic epithelium to distinguish indolent from aggressive PC and predict PC risk. Our results from 38 men with low-grade PC indicated that there is a significant increase in progressors compared to non-progressors (p=0.002, effect size=110%, AUC=0.80, sensitivity=88% and specificity=72%), while the baseline clinical characteristics were not significantly different. We further improved the diagnostic power by performing nuclei-specific measurements using an automated system that separates in real-time the cell nuclei from the remaining prostate epithelium. In the long term, we envision that the PWS based prognostication can be coupled with AS without any change to the current procedure to mitigate the harms caused by overtreatment.

9689-60, Session 4
Using optical coherence tomography (OCT) to evaluate the status of human donor kidneys
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The main cause of delayed renal function following the transplant of donor kidneys is ischemic induced acute tubular necrosis (ATN). The ability to determine the degree of ATN suffered by donor kidneys prior to their transplant would enable transplant surgeons to use kidneys that might otherwise be discarded and better predict post-transplant renal function. Currently, there are no reliable tests to determine the extent of ATN of donor kidneys prior to their transplant. In ongoing clinical trials, we have been using optical coherence tomography (OCT) to non-invasively image the superficial proximal tubules of human donor kidneys prior to and following transplant, and correlate these observations with post-transplant renal function. Thus far we have studied over 40 living donor kidneys and 10 cadaver donor kidneys, and demonstrated that this imaging can be performed in a sterile and expeditious fashion in the operating room (OR). Because of many variables associated with a diverse population of donors/ recipients and transplant operation parameters, more transplant data must be collected prior to drawing definite conclusions. Nevertheless, our observations have thus far mirrored our previously published laboratory results indicating that damage to the kidney proximal tubules as indicated by tubule swelling is a good measure of post-transplant ATN and delayed graft function. We conclude that OCT is a useful procedure for analyzing human donor kidneys.

9689-61, Session 4
A method for tuning the excitation wavelength of an LED light source during fluorescence-based cystoscopy
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In clinical applications of fluorescence-guided endoscopy of the bladder (cystoscopy) it can be observed that the contrast in light from autofluorescence and from photodynamic diagnosis (PDD) varies from patient to patient. To compensate for this effect, a new method is presented for tuning the wavelength of a LED-based light source during fluorescence guided endoscopy of the bladder i.e. photodynamic diagnosis of bladder tumours. In the present embodiment, the wavelength of the LED source, developed in our laboratory, can be tuned to vary the excitation wavelength of both the sensitised fluorescence in the tumours (PDD) as well as the native fluorescence of the bladder mucosa and blood vessels. The contrast of the image observed through the CCD-camera attached to the cystoscope is thereby increased. In this way, patient to patient variations in autofluorescence and in sensitised fluorescence of tumours can be compensated for during fluorescence-guided cystoscopy in the clinic.

9689-62, Session PSun
Proximal fiber optic tip degradation during Holmium:YAG and Thulium fiber laser lithotripsy
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Introduction: The Thulium fiber laser (TFL) is studied as alternative to standard Holmium:YAG laser for lithotripsy. TFL beam originates within 18-µm-core thulium doped silica fiber, and its near-single mode beam profile allows transmission of higher laser power through smaller (50-150 µm) fibers than Holmium lithotripsy. This study examines whether improved TFL beam profile reduces proximal fiber tip damage compared to Holmium multimodal beam.

Methods: A TFL beam at 1908 nm was coupled into 105-µm-core silica fibers, with 35-mJ energy, 500-µs pulse duration, and pulse rates of 50-500 Hz. For each pulse rate, 500,000 pulses were delivered, and average power output monitored every 10,000 pulses. Magnified images of proximal fiber tips were taken before and after each trial. For comparison, 12 single-use, 270-µm-core fibers were collected after Holmium laser lithotripsy clinical procedures performed with standard settings (600 mJ, 350 µs, 6 Hz). Total energy, total number of pulses, and total irradiation time were recorded, and fibers were rated for damage.

Results: For TFL studies, output pulse energy and power were stable, and no proximal fiber damage was observed after delivery of 500,000 pulses at settings up to 35 mJ, 500 Hz, and 17.5 W average power. Confocal microscopy images of fiber tips after Holmium lithotripsy showed proximal fiber tip degradation, indicated by small ablation craters in all fibers examined.

Conclusions: The proximal fiber tip of 105-µm-core fiber transmitted average TFL power up to 17.5 W without degradation, compared to degradation of 270-µm-core fibers upon delivering 3.6 W of Holmium power.

9689-63, Session PSun
Computer simulations of laser thermal remodeling during minimally invasive transvaginal and transurethral approaches to treatment of female stress urinary incontinence
Luke A. Hardy, Chun-Hung Chang, The Univ. of North Carolina at Charlotte (United States); Erinn Myers M.D., Michael J. Kennelly M.D., Carolinas Medical Ctr. (United States); Nathaniel M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: A non-surgical method treating female stress urinary incontinence (SUI) by laser thermal remodeling of subsurface tissues in combination with surface tissue cooling, is explored. Light transport, heat transfer, and thermal damage in tissue are simulated, comparing transvaginal and transurethral approaches.
Methods: In Phase 1, Monte Carlo (MC) simulations provided spatial distribution of absorbed photons in tissue layers (vaginal mucosa, endopelvic fascia, urethral wall). Optical properties \((n, ?a, ?s, g)\) were assigned to each tissue at 1064 nm. A 5-mm-diameter laser beam and power of 5 W for 15 s was used, based on experiments. In Phase 2, MC output was converted into absorbed energy, serving as input for ANSYS simulation software of tissue temperatures. Convective heat transfer simulated probe contact cooling. Thermal properties \((k, c, p)\) were assigned to each tissue. In Phase 3, MATLAB code was used for Arrhenius integral calculations. A temperature matrix was constructed from ANSYS output, and finite sum was incorporated to approximate Arrhenius integral calculations. Tissue damage properties \((E_a, A)\) were used to compute Arrhenius sums. Results: For transvaginal approach, 37% of energy was absorbed in endopelvic fascia target layer with 0.8% deposited beyond it. Peak temperature was 71°C, treatment zone was 0.8-mm-diameter, and almost entire vaginal mucosa (2-6-mm-thick-layer) was preserved. For transurethral approach, 18% energy was absorbed in endopelvic fascia with 0.3% deposited beyond layer. Peak temperature was 80°C, treatment zone was 2.0-mm-diameter, and only 0.6 mm of urethra was preserved. Conclusions: Computer simulations suggest that minimally invasive, transvaginal approach to thermal remodeling of endopelvic fascia is most feasible.

9689-64, Session PSun

Diffusing, side-firing, and radial delivery laser balloon catheters for creating subsurface thermal lesions in tissue

Chun-Hung Chang, Nathaniel M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: Infrared lasers have been used in combination with applied cooling methods to preserve superficial skin layers during cosmetic surgery. Similarly, combined laser irradiation and tissue cooling may also allow development of minimally invasive laser therapies beyond dermatology. This study compares diffusing and side-firing laser balloon catheter designs for creation of subsurface lesions in tissue, ex vivo, using near-infrared laser and applied contact cooling.

Methods: An Ytterbium fiber laser at 1075 nm delivered energy through custom built 18 Fr (6-mm-OD) balloon catheters incorporating either 1-cm-long diffusing fiber or 90 degree side-firing fiber through central lumen. Chilled solution was flowed through separate lumen into 1-cm-diameter balloon to keep device cooled at 70°C. Porcine liver tissue samples were used as a preliminary tissue model for direct and immediate observation of thermal lesion creation.

Results: Diffusing fiber produced subsurface thermal lesions measuring 49.3 ± 10.0 mm2 and preserved 0.8 ± 0.1 mm of surface tissue. Side-firing fiber produced subsurface thermal lesions 2.4 ± 0.9 mm2 diameter and preserved 0.5 ± 0.1 mm of surface tissue. Optimal power and irradiation time measured 15 W and 100 s for diffusing fiber and 1.4 W and 20 s, for side-firing fiber, respectively.

Conclusions: Diffusing and side-firing laser balloon catheter designs provided subsurface thermal lesions in tissue. However, the divergent laser beam in both designs limited ability to preserve thicker layer of tissue surface. Further optimization of laser and cooling parameters may be necessary to create deeper thermal lesions and preserve thicker surface tissue layers.

9689-65, Session PSun

Indocyanine green (ICG)-enhanced photoacoustic imaging of bladder tumors

Van Phuc Nguyen, Pukyong National Univ. (Korea, Republic of); Suhyun Park, Samsung Advanced Institute of Technology (Korea, Republic of); Junghwan Oh, Pukyong National Univ. (Korea, Republic of); Hyun Wook Kang, Pukyong National Univ. (Korea, Republic of) and Ctr. for Marine-Integrated Biomedical Technology (Korea, Republic of)

Photoacoustic imaging (PAI) has widely been used as a nonionizing, noninvasive for monitoring treatment process. However, the bladder tumor was ambiguously visualized by PAI due to lack of light absorption. Therefore, the aim of this study was to investigate the enhancement of PA image contrast for bladder tumor with the aid of indocyanine green (ICG) as a contrast agent. For in vitro tests, ICG with different concentrations from 0 to 300 nM was mixed with gelatin and hemoglobin. The solution of 100 µl was then injected into fresh porcine bladder tissue in order to create an artificial tumor, and the prepared tissue was mapped with PAI. The ICG-injected bladder tumor was clearly observed due to the increase in absorption. The photoacoustic amplitude at 780 nm was increased up to four-fold, compared with non-injected. In vitro results also showed that 200 nM of ICG enabled PAI to clearly distinguish the position of tumor from the native tissue. Therefore, ICG can be an efficient and safe material for diagnostic of cancer.

9689-66, Session PSun

Thermal damage control in kidney tumor model during diffuser-assisted photocoagulation

Trung Hau Nguyen, Hyun Wook Kang, Kyu Kyu Hlaing, Pukyong National Univ. (Korea, Republic of)

Laser induced interstitial thermotherapy (LITT) has frequently been employed for treating various types of tumors. Numerical models have been developed to predict the extent of tissue damage as well as to optimize laser dosage for LITT. However, to constantly maintain hyperthermic temperature within the targeted tissue area is often difficult due to undesirable damage to the peripheral tissue. In this study, a proportional-integral-derivative (PID) controller-based laser was developed to control the extent of thermal damage volume in tissue during LITT. The numerical models were established to have optical and thermal properties that were temperature-dependent. The simulation was conducted with continuous wave (CW) mode, pulsed mode, and proportional-integral-derivative (PID) controlled mode to compare therapeutic performance in term of temperature development and thermal damage volume. Tissue experiments were implemented under the same conditions as the simulation models. During the experiment, a 600 µm glass-caped diffusing optical fiber was used to deliver 15W 1470 nm laser light to the targeted kidney tissue, and a thermal-couple was also applied to monitor the feedback temperature during LITT. The simulation results showed a good agreement with experimental data in term of temperature distribution as well as the extent of thermal denaturation. The peak temperature in the PID modulation mode demonstrated significant lower temperature under the same power level, compared to CW mode. Moreover, PID controller-based laser maintained the predetermined temperature of 60 degree (less than 5% deviation) as well as limited the coagulation depth in the tissue (<2 cm), comparable to the size of the kidney tumor (2-4 cm). The current numerical model demonstrated that optical diffuser-based laser treatment combined with temperature feedback control could provide a feasible method to treat kidney tumors in safely and efficiently manner.
Noncontact diffuse optical assessment of blood flow changes in head and neck free tissue transfer flaps
Chong Huang, Jeffrey P. Radabaugh, Rony K. Aouad, Yu Lin, Thomas J. Gal, Amit B. Patel, Joseph Valentino, Yu Shang, Guoqiang Yu, Univ. of Kentucky (United States)

Head and neck cancer accounts for 3 to 5% of all cancers in the United States. Primary or salvage surgeries are extensive and often lead to major head and neck defects that require complex reconstructions with local, regional, or free tissue transfer flaps. Knowledge of tissue blood flow (BF) changes after free tissue transfer may enable surgeons to predict the failure of flap thrombosis at an early stage. This study used our recently developed noncontact diffuse correlation spectroscopy to monitor dynamic BF changes in free flaps without getting in contact with the targeted tissue. Eight free flaps were elevated in patients with head and neck cancer; one of the flaps failed. Multiple BF measurements probing the transferred tissue were performed during and post the surgical operation. Postoperative BF values were normalized to the intraoperative baselines (assigning '1') for the calculation of relative BF change (rBF). The rBF changes over the seven successful flaps were 1.89 ± 0.15, 2.26 ± 0.13, and 2.43 ± 0.13 (mean ± standard error) respectively on postoperative days 2, 4, and 7. These postoperative values were significantly higher than the intraoperative baseline values (p < 0.001), indicating a gradual recovery of flap vascularity after the tissue transfer. By contrast, rBF changes observed from the unsuccessful flap were 1.34 and 1.34 respectively on postoperative days 2 and 4, indicating a less flow recovery. Measurement of BF recovery after flap anastomosis holds the potential to act early to salvage ischemic flaps.

Monitoring longitudinal changes in irradiated head and neck cancer xenografts using diffuse reflectance spectroscopy
Karthik Vishwanath, Miami Univ. (United States); Shudong Jiang, Jason R. Gunn, Kayla Marra, Jacqueline M. Andreozzi, Brian W. Pogue, Dartmouth College (United States)

Radiation therapy is often used as the preferred clinical treatment for control of localized head and neck cancer. However, during the course of treatment (6-8 weeks), feedback about functional and/or physiological changes at the diseased tissue are not frequently obtained given the onerous financial and/or logistical burdens of scheduling MRI, PET or CT scans. Diffuse optical sensing is well suited to address this problem since the instrumentation can be made low-cost and portable while still being able to non-invasively provide information about vascular oxygenation in vivo.

Here we report results from studies that employed an optical fiber-based portable diffuse reflectance spectroscopy (DRS) system to longitudinally monitor changes in tumor vasculature within two head and neck cancer cell lines (SCC-15 and FaDu) xenografted in the flanks of nude mice, in two separate experiments. Once the tumor volumes were 100mm3, 67% of animals received localized radiation therapy in five fractions (8Gy/day, for 5 days) while 33% of the animals served as controls. DRS measurements were obtained from each animal (in triplicate) on each day of treatment, and then for two weeks post-treatment. Reflectance spectra were fit using a Monte Carlo based model to extract total hemoglobin concentration and blood oxygen-saturation. The analyzed time-trends of these optical parameters differed between cell-lines. Preliminary analyses suggest that early changes (days 1-3) in measured hemoglobin concentration and blood oxygenation differed for irradiated animals exhibiting local control vs. those with disease progression. These findings indicate that DRS measurements in irradiated tumors may help in identifying early radiotherapy-response.

Progress in reflectance confocal microscopy for imaging oral tissues in vivo
Gary Peterson, Miguel A. Cordova, Snehal Patel M.D., Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

We report progress in development and feasibility testing of reflectance confocal microscopy (RCM) for imaging in the oral cavity of humans. We developed a small rigid relay telescope (120mm long x 14mm diameter) and a small water immersion objective lens (12mm diameter, NA 0.7) to a commercial handheld RCM scanner (Vivascope 3000, Caliber ID, Rochester NY). This scanner is designed for imaging skin but we adapted the front end (the objective lens and the stepper motor that axially translates it) for intra-oral use. This adaption required a new approach to address the loss of the automated stepper motor for acquisition of images in depth. A helical spring-like cap (with a coverslip to contact tissue) was designed for approximately 150 um of travel. By manually and gently pushing against tissue, we can regain the ability to acquire images in depth. The relay telescope optics is being evaluated in a clinical setting. With the capture of video and video-mosaicing, extended areas can be imaged. The feasibility of imaging oral tissues was initially investigated in volunteers. RCM imaging in buccal mucosa in vivo shows nuclear and cellular detail in the epithelium and epithelium junction, and connective tissue and blood flow in the underlying lamina propria. Similar detail, including filiform and fungiform papillae, can be seen on the tongue in vivo. Clinical testing during head and neck surgery is now in progress and patients are being imaged for both normal tissue and cancerous margins in lip and tongue mucosa.

Intraoperative detection and elimination of microscopic tumors in head and neck (Invited Paper)
Ekaterina Y. Lukianova-Hleb, Rice Univ. (United States); Yoo-Shin Kim, The Methodist Hospital Research Institute (United States); Ihar Belatsarkouski, N.N. Alexandrov National Cancer Ctr. of Belarus (Belarus); Ehab Y. Hanna, Ann M. Gillenwater, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); Brian O’Neill, The Methodist Hospital Research Institute (United States); Dmitri Lapotko, Rice Univ. (United States)

Failure of cancer surgery to intraoperatively detect and eliminate microscopic residual disease (MRD) causes lethal recurrence and metastases, whereas removal of important normal tissues causes excessive...
Fourier domain optical coherence tomography (FD-OCT) is a noninvasive imaging modality that has previously been used to image the human larynx. However, differences in anatomical geometry and short imaging range of conventional OCT limits its application in a clinical setting. In order to address this issue, we have developed a gradient-index (GRIN) lens rod-based hand-held probe in conjunction with a long imaging range 200 kHz Vertical-Cavity Surface Emitting Lasers (VCSEL) swept-source optical coherence tomography (SS-OCT) system for high speed real-time imaging of the human larynx in an office setting. This hand-held probe is designed to have a long and dynamically tunable working distance to accommodate the differences in anatomical geometry of human test subjects. A nominal working distance (~6 cm) of the probe is selected to have a lateral resolution < 100 um within a depth of focus of 6.4 mm, which covers more than half of the 12 mm imaging range of the VCSEL laser. The maximum lateral scanning range of the probe at 6 cm working distance is approximately 8.4 mm, and imaging an area of 8.5 mm by 8.5 mm is accomplished within a second. Using the above system, we will demonstrate real-time cross-sectional OCT imaging of larynx during phonation in vivo in human and ex-vivo in pig vocal folds.

9689-73, Session 2

Development of a high-speed VCSEL OCT system for real-time imaging of conscious patients larynx using a hand-held probe

Swathi Rangarajan, OCT Medical Imaging Inc. (United States); Li-Dek Chou, OCT Medical Imaging Inc. (United States) and Beckman Laser Institute and Medical Clinic (United States); Carolyn Coughlan, Giriraj Sharma, Univ. of California, Irvine (United States); Brian J. F. Wong, Univ. of California, Irvine (United States) and Beckman Laser Institute and Medical Clinic (United States); Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States) and Univ. of California, Irvine (United States); Tirunelveli S. Ramalingam, OCT Medical Imaging Inc. (United States)

Fourier domain optical coherence tomography (FD-OCT) is a noninvasive imaging modality that has previously been used to image the human larynx. However, differences in anatomical geometry and short imaging range of conventional OCT limits its application in a clinical setting. In order to address this issue, we have developed a gradient-index (GRIN) lens rod-based hand-held probe in conjunction with a long imaging range 200 kHz Vertical-Cavity Surface Emitting Lasers (VCSEL) swept-source optical coherence tomography (SS-OCT) system for high speed real-time imaging of the human larynx in an office setting. This hand-held probe is designed to have a long and dynamically tunable working distance to accommodate the differences in anatomical geometry of human test subjects. A nominal working distance (~6 cm) of the probe is selected to have a lateral resolution < 100 um within a depth of focus of 6.4 mm, which covers more than half of the 12 mm imaging range of the VCSEL laser. The maximum lateral scanning range of the probe at 6 cm working distance is approximately 8.4 mm, and imaging an area of 8.5 mm by 8.5 mm is accomplished within a second. Using the above system, we will demonstrate real-time cross-sectional OCT imaging of larynx during phonation in vivo in human and ex-vivo in pig vocal folds.

9689-74, Session 2

A study of balloon treatment for circopharyngeal dysfunction by using full range optical coherence tomography

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Patients with circopharyngeal dysfunction (CPD) often suffer from dysphagia for both solids and liquids. The symptoms of regurgitation, choking, and pharyngeal residue may cause other downstream health status complications including pneumonia, malnutrition and dehydration. Balloon dilatation treatment is commonly used for CPD. However, the required diameter and position of the balloon vary for different patients. The treatments usually depend on personal preference and experience of the operator, due to the lack of monitoring methods. We utilized a full range optical coherence tomography (OCT) imaging system to quantitatively determine the optimized diameter and position of a balloon dilatation catheter, and to evaluate the therapeutic effect of balloon dilatation for CPD.

9689-75, Session 2

Long range OCT during drug induced sleep endoscopy: preliminary investigations

Erica Su, Joseph C. Jing, Emon Heidari, Beckman Laser Institute and Medical Clinic (United States); Max Wiedmann, Univ. of California, Irvine (United States); Bryan Lemieux, Beckman Laser Institute and Medical Clinic (United States); Gurpreet S. Ahuja, Univ. of California, Irvine (United States); Zhongping Chen, Brian J. F. Wong, Beckman Laser Institute and Medical Clinic (United States)

No Abstract Available

9689-76, Session 2

Swept-source anatomic optical coherence elastography of porcine trachea

Ruofei Bu, Hillel B. Price, Sorin Mitran, Carlton Zdanski, Amy L. Oldenburg, The Univ. of North Carolina at Chapel Hill
diseases. The vocal cord could yield to a better pediatric management of laryngeal pathologies and cysts and laryngeal papillomatosis and compared them to healthy tissue. We imaged 20 patients undergoing direct laryngoscopy during which we based handheld probe suitable for pediatric laryngological imaging. The probe allows for rapid three-dimensional imaging of vocal fold lesions. The system is adapted to allow for high-resolution intra-operative imaging. The catheter is employed in the bore of a commercial, flexible bronchoscope. The system captures quantitative cross-sectional-areas (CSA) of the airway lumen, and has an imaging distance of 12mm from the catheter tip, a resolution of 25.6 µm axial and 100-400 µm transverse, and a signal-to-noise ratio of 98dB at 2mm.

To perform elastography, aOCT images were collected as a function of pressure, and the resulting CSA used to estimate airway wall compliance. The catheter was introduced into a body-valve-mask (BVM) through a diaphragm via the bronchoscope. Air pressure (0-60cmH2O) was manually applied by the BVM, and the CSA change caused by wall expansion was measured. Experiments performed on a Latex rubber tube resulted in a compliance of 0.68±0.02mm2/cmH2O(R2=0.95). Next, freshly isolated porcine trachea was used and the calculated compliance was 3.2±0.3mm2/cmH2O(R2=0.92); the linearity confirms the elastic nature of the airway, while the value is slightly smaller than that from other measurements of human upper airway (typically 4-10 mm2/cmH2O). Going forward, spatially-resolved estimates of wall compliance will be enabled by the use of fiducial markers and development of a physiologic trachea model.

Intraoperative handheld probe for 3D imaging of pediatric benign vocal fold lesions using optical coherence tomography

Fouzi Benboujja, Ecole Polytechnique de Montréal (Canada); Jordan Garcia, Harvard Medical School (United States); Kathy Beaudette, Mathias Struppler, Ecole Polytechnique de Montréal (Canada); Christopher J. Hartnick M.D., Harvard Medical School (United States); Caroline Boudoux, Ecole Polytechnique de Montréal (Canada)

Excessive and repetitive force applied on vocal fold tissue can induce benign vocal fold lesions. Children affected suffer from chronic hoarseness. In this instance, the vibratory ability of the folds, a complex layered microanatomy, becomes impaired. Histological findings have shown that lesions produce a remodeling of sup-epithelial vocal fold layers. However, our understanding of lesion features and development is still limited. Indeed, conventional imaging techniques do not allow a non-invasive assessment of sub-epithelial integrity of the vocal fold. Furthermore, it remains challenging to differentiate these sub-epithelial lesions (such as bilateral nodules, polyps and cysts) from a clinical perspective, as their outer surfaces are relatively similar. As treatment strategy differs for each lesion type, it is critical to efficiently differentiate sub-epithelial alterations involved in benign lesions.

In this study, we developed an optical coherence tomography (OCT)-based handheld probe for pediatric laryngological imaging. The probe allows for rapid three-dimensional imaging of vocal fold lesions. The system is adapted to allow for high-resolution intra-operative imaging. We imaged 20 patients undergoing direct laryngoscopy during which we looked at different benign pediatric pathologies such as bilateral nodules, cysts and laryngeal papillomatosis and compared them to healthy tissue. We qualitatively and quantitatively characterized laryngeal pathologies and demonstrated the added advantage of using 3D OCT imaging for lesion discrimination and margin assessment. OCT evaluation of the integrity of the vocal cord could yield to a better pediatric management of laryngeal diseases.

Subglottic stenosis in a rabbit model: OCT and texture analysis

Erica Su, Ashley Hamamoto, Alex Wang, Tony D. Nguyen, Jason Chen, Beckman Laser Institute and Medical Clinic (United States); Kathryn Osann, Univ. of California, Irvine (United States); Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States); Gurpreet S. Ahuja, Univ. of California, Irvine (United States); Brian J. F. Wong, Beckman Laser Institute and Medical Clinic (United States)

Airway compliance measurements using optical coherence tomography

Joseph C. Jing, Li-Dek Chou, Bryan Lemieux, Brian J. F. Wong, Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States)

Raman spectroscopy and oral exfoliative cytology: investigating misclassifications between contralateral normal and tumor sites

Aditi Sahu, Sneha Tawde, Poonam Gera, Sudhir Nair, C. Murali Krishna, Advanced Ctr. for Treatment, Research & Education in Cancer (India)

Oral cancers are associated with low disease-free survival rates, attributed mainly to delayed diagnosis and recurrence-locoregional, second primary and field tumors. Hence new approaches for early diagnosis are being extensively explored. Studies based on optical spectroscopic techniques like Raman spectroscopy (RS) have shown to classify healthy, premalignant, contralateral normal and malignant conditions, and even detect early changes like cancer-field-effects (CFE). Several misclassifications between contralateral normal with premalignant, tumor and tobacco habitues have been encountered in these studies. These have been attributed to a) tumor heterogeneity, b) presence of inflammatory foci, c) transformation zones. Due to ethical considerations, histopathological evidence for these misclassifications could not be obtained. Therefore, this study on RS of oral...
9689-82, Session 3

Simultaneous multi-scale microscopy as a potential dedicated tool for intra-operative parathyroid identification during thyroid surgery

Étienne De Montigny, Nadir Goulamhoussen, Wendy-Julie Madore, Mathias Strupler, Ecole Polytechnique de Montréal (Canada); Anastasios Maniakas M.D., Tareck Ayad M.D., Ctr. Hospitalier de l’Univ. de Montréal (Canada); Caroline Boudoux, Ecole Polytechnique de Montréal (Canada)

While thyroidectomy is considered a safe surgery, dedicated tools facilitating tissue identification during surgery could improve its outcome. The most common complication following surgery is hypocalcaemia, which results from iatrogenic removal or damage to parathyroid glands. This research project aims at developing and validating an instrument based on optical microscopy modalities to identify tissues in real time during surgery. Our approach is based on a combination of reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) to obtain multi-scale morphological contrast images. The orthogonal field of views provide information to navigate through the sample.

To allow simultaneous, synchronized video-rate imaging in both modalities, we designed and built a dual-band wavelength-swept laser which scans a 30 nm band centered at 780 nm and a 90 nm band centered at 1310 nm. We built an imaging setup integrating a custom-made objective lens and a double-clad fibre coupler optimized for confocal microscopy. It features high resolutions in RCM (2 μm lateral and 20 μm axial) in a 500 μm x 500 μm field-of-view and a larger field-of-view of 2 mm (lateral) x 5 mm (axial) with 20 μm lateral and axial resolutions in OCT. Imaging of ex vivo animal samples is demonstrated on a bench-top system. Tissues that are visually difficult to distinguish from each other in an operatively such as parathyroid gland, lymph nodes and adipose tissue are imaged to show the potential of this approach in differentiating neck tissues. We will also provide an update on our ongoing clinical pilot study on patients undergoing thyroidectomy.

9689-83, Session 3

Biochemical and molecular characterization of thyroid lesions by micro-Raman spectroscopy and gene expression analysis

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Thyroid carcinomas represent the main endocrine malignancy and their diagnosis may produce inconclusive results. Raman spectroscopy and gene expression analysis have shown excellent results on the differentiation of carcinomas. This study aimed to improve the discrimination between different thyroid pathologies by combining both analyses. A total of 35 thyroid tissues samples including normal tissue (n=10), goiter (n=10), papillary (n=10) and follicular carcinomas (n=5) were analyzed. Confocal Raman spectra were obtained by using a Rivers Diagnostic System, 785 nm laser excitation and CCD detector. The data were processed by the software Labspec5 and Origin 8.5 and statistically analyzed by Minitab® program. The gene expression analysis was performed by qRT-PCR technique and statistically analyzed by Mann-Whitney test. The confocal Raman spectroscopy allowed a maximum discrimination of 91.1% between normal and tumor tissues, 84.8% between benign and malignant pathologies and 84.6% among carcinomas analyzed. Significant differences in the gene expression were observed for TG, LGALS3, SERPINA1 and TFF3 between benign lesions and carcinomas, and SERPIN1 and TFF3 genes between papillary and follicular carcinomas. Principal component analysis was performed using PC1 and PC2 analysis in papillary carcinoma samples that showed over expression in the molecular markers when compared with normal sample, where 90% of discrimination was observed analyzing the loading plot at the Amide 1 spectra region (1655 cm⁻¹), and at the tyrosine spectra region (856 cm⁻¹). The confocal Raman spectroscopy biochemical features combined to molecular changes indicate that these techniques are promising tools to be used in the diagnosis of thyroid lesions.

9689-84, Session 3

Simultaneous fingerprint and high-wavenumber fiber-optic Raman endoscopy for in vivo diagnosis of laryngeal cancer

Kan Lin, Wei Zheng, Jianfeng Wang, C. M. Lim, Zhiwei Huang, National Univ. of Singapore (Singapore)

We report a unique simultaneous fingerprint (FP) and high-wavenumber (HW) fiber-optic confocal Raman spectroscopy for in vivo diagnosis of laryngeal cancer of the patients undergoing wide-field endoscopic imaging. The FP/HW Raman endoscopy technique was performed on 29 patients and the diagnostic algorithms based on principal components analysis (PCA) and linear discriminant analysis (LDA) together with the leave-one-patient-out validation method on in vivo FP/HW Raman spectra yielded a diagnostic sensitivity of 85% and specificity of 88% for laryngeal cancer identification. This work shows the great potential of the FP/HW Raman endoscopic technique developed for non-invasive, in vivo diagnosis of laryngeal carcinoma during routine endoscopic examination.

9689-85, Session 4

A novel mosaicking algorithm for in vivo full-field thickness mapping of the human tympanic membrane using low coherence interferometry

Paritosh Pande, Ryan L. Shelton, Guillermo L. Monroy, Ryan M. Nolan, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Tympanic membrane (TM) thickness can provide crucial information for diagnosing several middle ear pathologies. An imaging system integrating low coherence interferometry (LCI) with the standard video otoscope has been shown as a promising tool for quantitative assessment of in-vivo TM thickness. The small field-of-view (FOV) of TM surface images acquired by the combined LCI-otoscope system, however, makes the spatial registration
of the LCI imaging sites and their location on the TM difficult to achieve. It is therefore desirable to have a tool that can map the imaged points on to an anatomically accurate full-field surface image of the TM. To this end, we propose a novel automated mosaicking algorithm for generating a full-field surface image of the TM with co-registered LCI imaging sites from a sequence of multiple small FOV images and corresponding LCI data. Traditional image mosaicking techniques reported in the biomedical literature, mostly for retinal imaging, are not directly applicable to TM image mosaicking because unlike retinal images, which have several distinctive features, TM images contain large homogeneous areas lacking in sharp features. The proposed algorithm overcomes these challenges of TM image mosaicing by following a two-step approach. In the first step, a coarse registration based on the correlation of gross image features is performed. Subsequently, in the second step, the coarsely registered images are used to perform a finer intensity-based co-registration. The proposed algorithm is used to generate, for the first time, full-field thickness distribution maps of in-vivo human TMs.

9689-86, Session 4
Effect of low level laser therapy (LLLT) on ouabain induced spiral ganglion neuron damaged auditory neuropathy in gerbils
Chung-Ku Rhee M.D., Sung Huyn Bae, So-Young Chang, Phil-Sang Chung, Jae-Yun Jung, Dankook Univ. Hospital (Korea, Republic of)

Aim: to investigate effectiveness of Low level laser therapy (LLLT) in rescuing ouabain induced spiral ganglion cell damage using Mongolian gerbils. Methods: Animals were divided into 3 groups; Control, Ouabain, Ouabain + LLLT group. Auditory neuropathy was induced by topical application of ouabain (1 mmol/L, 3uL) on the round window membrane in gerbils. Transmeatal LLLT was irradiated into the right ear for 1h (200mW, 720 J) daily for 7d in Ouabain + LLLT group. Before and 7 days after ouabain application, hearing was evaluated using both ABR and distortion product otoacoustic emissions (DPOAE). Seven days after ouabain application, animals were sacrificed to evaluate the morphological changes of cochlea using cochlear section image and whole mount Immunofluorescent staining. Results: DPOAE tests were normal in all animals after ouabain topical treatment indicating intact outer hair cells. Ouabain group showed ABR threshold increase compared with control group. Ouabain+LLLT group showed significant improvement of ABR threshold compared to ouabain only group. H&E stains of mid-modiolar section of cochlear showed spiral ganglion cells, neurofilaments, and post synaptic receptor counts compared to ouabain group. Conclusions: The results demonstrated that LLLT was effective to rescue ouabain-induced spiral ganglion neuropathy.

9689-87, Session 4
Differentiation of bacterial versus viral otitis media using a combined Raman scattering spectroscopy and low coherence interferometry probe
Youbo Zhao, Ryan L. Shelton, Hachua Tu, Ryan M. Nolan, Guillermo L. Monroy, Eric J. Chaney, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Otitis media (OM) is a highly prevalent disease that can be caused by either a bacterial or viral infection. Because antibiotics are only effective against bacterial infections, blind use of antibiotics without definitive knowledge of the infectious agent, though commonly practiced, can lead to the problems of potential harmful side effects, wasteful misuse of medical resources, and the development of antimicrobial resistance. In this work, we investigate the feasibility of using a combined Raman scattering spectroscopy and low coherence interferometry (LCI) device to differentiate OM infections caused by viruses and bacteria and improve our diagnostic ability of OM. Raman spectroscopy, an established tool for molecular analysis of biological tissue, has been shown capable of identifying different bacterial species, although mostly based on fixed or dried sample cultures. LCI has been demonstrated recently as a promising tool for determining tympanic membrane (TM) thickness and the presence and thickness of middle-ear biofilm located behind the TM. We have developed a fiber-based ear insert that incorporates spatially-aligned Raman and LCI probes for point-of-care diagnosis of OM. As shown in human studies, the Raman probe provides molecular signatures of bacterial- and viral-infected OM and normal middle-ear cavities, and LCI helps to identify depth-resolved structural information as well as guide and monitor positioning of the Raman spectroscopy beam for relatively longer signal acquisition time. Differentiation of OM infections is determined by correlating in vivo Raman data collected from human subjects with the Raman features of different bacterial and viral species obtained from cultured samples.

9689-88, Session 4
A short-wave infrared otoscope for middle ear disease diagnostics
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Otitis media, a range of inflammatory conditions of the middle ear, is the second most common illness diagnosed in children. However, the diagnosis can be challenging, particularly in pediatric patients. Otitis media is commonly over-diagnosed and over-treated and has been identified as one of the primary factors in increased antibiotic resistance. We describe the development of a short-wave infrared (SWIR) otoscope for objective middle ear effusion diagnosis. The SWIR otoscope can unambiguously detect the presence of middle ear fluid based on its strong light absorption in the SWIR. This absorption causes a stark, visual contrast between the presence and absence of fluid behind the tympanic membrane. Additionally, when there is no middle ear fluid, the deeper tissue penetration of SWIR light allows the SWIR otoscope to better visualize middle ear anatomy through the tympanic membrane than is possible with visible light. We demonstrate that in healthy, adult human ears. SWIR otoscopy can image a range of middle ear anatomy, including landmarks of the entire ossicular chain, the promontory, the round window niche, and the chorda tympani. We suggest that SWIR otoscopy can provide valuable diagnostic information complementary to that provided by visible pneumotoscopy in the diagnosis of middle ear effusions, otitis media, and other maladies of the middle ear.

9689-89, Session 4
Signal and response properties indicate an optoacoustic effect underlying the intracochlear laser-optical stimulation
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Optical cochlea stimulation is under investigation as a potential alternative to conventional electric cochlea implants in treatment of sensorineural hearing loss. If direct optical stimulation of spiral ganglion neurons would be feasible, a smaller stimulation volume and, therefore, an improved frequency resolution could be achieved. However, it is unclear whether the mechanism of optical stimulation is based on direct neuronal stimulation or on optoacoustics. Animal studies on hearing vs. deafened guinea pigs already identified the optoacoustic effect as potential mechanism for intracochlear optical stimulation. In order to characterize the optoacoustic stimulus more thoroughly the acoustic signal along the beam path of a pulsed laser in water was quantified and compared to the neuronal response properties of hearing guinea pigs (n=8) stimulated with the same laser parameters. Two pulsed laser systems were used for analyzing the influence of variable pulse duration, pulse energy, pulse peak power and absorption coefficient. The results of the experiments in water and in vivo show a similar dependency of response signals on the applied laser parameters: Both datasets show an on- and offset signal at the beginning and the end of the laser pulse. Further, the resulting signal amplitude depends on the pulse peak power, the absorption coefficient as well as the temporal development of the applied laser pulse. The data indicates the maximum of the first derivative of power as the decisive factor. In conclusion, the present study strongly supports the hypothesis of optoacoustics as the underlying mechanism for optical stimulation of the cochlea.

9689-90, Session 4
Three-dimensional imaging of intracochlear tissue by scanning laser optical tomography (SLOT)

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The presented study focuses on the application of scanning laser optical tomography (SLOT) for non-destructive visualization of anatomical structures inside the human cochlea ex vivo. SLOT is a laser-based highly efficient microscopy technique, which allows for tomographic imaging of the internal structure of transparent large-scale specimens (up to 1 cm²). Thus, in the field of otology this technique is best convenient for an ex vivo study of the inner ear anatomy. For this purpose, the preparation before imaging comprises mechanically assisted decalcification, dehydration as well as optical clearing of the cochlea samples. Here, we demonstrate results of SLOT imaging visualizing hard and soft tissue structures of the human cochlea with an optical resolution in the micrometer range using absorption and auto-fluorescence as contrast mechanisms.

Furthermore, we compare our results with the method of X-ray microtomography (micro-CT, µCT) as clinical gold standard which is based only on absorption. In general, SLOT can provide the advantage of covering all contrast mechanisms known from other light microscopy techniques, such as e.g. fluorescence or scattering. For this reason, a protocol for antibody staining has been developed, which additionally enables selective mapping of cellular structures within the cochlea. Thus, we present results of SLOT imaging rodent cochleae showing specific anatomical structures such as hair cells and neurofilament via fluorescence. In conclusion, the presented study has shown that SLOT is an ideally suited tool in the field of otology for in toto visualization of the of the inner ear microstructure.

9689-91, Session 4
Combination therapy of antioxidant and LLLT in noise induced hearing loss

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Protective effect of N-acetyl-L-cysteine (L-NAC) and acetyl L-carnitine (ALCAR) to attenuate noise-induced hearing loss has been studied previously. Aim: To test effect of combining antioxidants and low level laser therapy (LLLT) to reduce impact of noise in the inner ear and improving auditory function. Methods: Seventeen male SD rats were divided into 5 groups; Noise, NAC, ALCAR, NAC+LLLT, and ALCAR+LLLT groups. Animals were exposed to 16 kHz narrow band noise, 120 dB SPL for 5 h. Antioxidant NAC (100mg/kg) and ALCAR (100mg/kg) were administered into intraperitoneal cavity, once daily for 12 days. Transmeatal LLLT was irradiated into left ear for 60 min/day for 12 days using 808 nm diode laser, with a laser output of 165 mW. Hearing test was performed by ABR testing before and immediately after the noise exposure, 6th, and 12th day. The number of hair cells were counted by confocal microscope. Results: Significant hearing improvement was noted in NAC+LLLT group compared to control and all other groups. The number of hair cells was significantly higher in NAC+LLLT group compared to other groups. The number of hair cells was not significantly higher in ALCAR+LLLT group compared to ALCAR group, but it was significantly higher compared to the control group. Conclusion: Combination therapy of NAC+LLLT was more effective to improve noise induced hearing loss compared to both antioxidants alone and ALCAR+LLLT groups.

9689-175, Session 4
Comparison of advanced optical imaging techniques with current otolaryngology diagnostics for improved middle ear assessment

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Otolaryngologists utilize a variety of diagnostic techniques to assess middle ear health. Tympanometry, audiometry, and otoacoustic emissions examine the mobility of the tympanic membrane (eardrum) and ossicles using ear canal pressure and auditory tone delivery and detection. Laser Doppler vibrometry provides non-contact vibrational measurement, and acoustic reflectometry is used to assess middle ear effusion using sonar. These technologies and techniques have advanced the field beyond the use of the standard otoscope, a simple tissue magnifier, yet the need for direct visualization of middle ear disease for superior detection, assessment, and management remains.

In this study, we evaluated the use of portable optical coherence tomography (OCT) and pneumatic low-coherence interferometry (LCI)
systems with handheld probe delivery to standard tympanometry, audiometry, otoacoustic emissions, laser Doppler vibrometry, and acoustic reflectometry. Comparison of these advanced optical imaging techniques and current diagnostics was conducted with a case study subject with a history of unilateral eardrum trauma. OCT and pneumatic LCI provide novel dynamic spatiotemporal structural data of the middle ear, such as the thickness of the eardrum and quantitative detection of underlying disease pathology, which could allow for more accurate diagnosis and more appropriate management than currently possible.

9689-176, Session 5

**Histologic evaluation of blood vessels sealed with 1470-nm diode laser: determination of adequate condition for laser vessel sealing**

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Energy-based devices have enabled more rapid and efficient methods for vessel and tissue ligation. In the present study, we performed histologic evaluation to determine the optimal condition of laser for an effective vessel sealing. The porcine arteries (5mm diameter) were compressed between two glass-slides to eliminate their luminal space and were irradiated with 1470-nm diode laser under various conditions, including laser power (5-30W), irradiation time (5 or 10 seconds), and focus mode (focus or defocus). On histologic examination, the extent of tissue change was positively correlated to duration and power of laser. In defocus mode, the irradiated vessels showed sufficient tissue denaturation for sealing effect without severe tissue destruction. The irradiation of laser for 2 minutes at 20 W was appropriate for sealing the blood vessels and high bursting pressure (> 400 mmHg) was needed to break the sealed vessels. Even if the relatively high power of laser was required, the adequate power and irradiation duration of laser can render blood vessels to be sealed effectively.

9689-177, Session 5

**Primary investigations on the potential of a novel diode pumped Er:YAG laser system for middle ear surgery**

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Flashlamp pumped Er:YAG lasers are successfully used clinically for both precise soft and hard tissue ablation. Since several years a novel diode pumped Er:YAG laser system (Pantec Engineering AG) is available, with mean laser power up to 30W and pulse repetition rate up to 1kHz. The aim of the presented study is to investigate the resulting ablation efficiency and loudness of this laser system while bone tissue ablation at various irradiation parameters.

Firstly an experimental set-up was realized with a beam focusing unit and a computer controlled stepper unit to move the sample (slices of porcine bone) with a defined velocity while irradiation at various laser parameters. A microphone was positioned in a defined distance to the ablation point and the resulting signal of the ablation process was recorded. For comparison, measurements were also performed with a flashlamp pumped Er:YAG laser system. After irradiation the resulting ablation quality and efficacy were determined using light microscopy.

The results show efficient bone ablation using the diode pumped Er:YAG laser system. Also an increase of the sound level with increasing pulse energy and with decreasing pulse duration was observed. At the same mean laser power, we detected significantly less noise for the diode pumped Er:YAG laser compared to the flashlamp pumped system, which is due to the minor pulse energy and higher pulse repetition rate of the diode pumped system.

In conclusion, these first experiments demonstrate the high potential of the diode pumped Er:YAG laser system for use in middle ear surgery.
Heartbeat OCT: a new tool for interventional imaging

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We have developed a super fast intravascular optical coherence tomography (OCT) system called Heartbeat OCT. Heartbeat OCT relies on a Fourier Domain Mode Locked (FDML) laser and a micro-motor based catheter. The system enables acquisition of a uniformly sampled data set within one cardiac cycle, triggered by the ECG, to restore 3D OCT image fidelity. Here, we present a robust and easy to operate preclinical prototype system for interventional imaging which greatly facilitates data acquisition. The new system is using a fully automatic 1.6 MHz turnkey FDML laser with increased stability. The entire system was mounted into a 1.6 x 0.8 x 0.7 m cart. It is coupled to a guidewire compatible rapid-exchange catheter that can be used for routine imaging. The system is robust, compact, and moveable. We present the design, demonstrate ex vivo imaging results and discuss in vivo applications, as well as considerations for clinical translation of the technology. The ex vivo imaging experiments were conducted with metal stents and bioresorbable vascular scaffold. The images provide clear and comprehensive visualization of the stents structure not only in cross-section but also in longitudinal rendering and 3D construction.

Accurate vectorial flow measurements in catheter-based optical coherence tomography

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Intracoronary flow measurements in the clinical setting are traditionally limited by point sampling with the use of direct techniques like Doppler wires, or indirectly with pressure transducers. Multidimensional flow profiles can provide additional insights in complex lesions, as well as in the study of the pathogenesis of atherosclerotic plaques, by studying their relation to perturbed endothelial shear stress and turbulent blood flow. Doppler OCT can determine intravascular flow profiles, but its angle sensitivity makes Doppler an inappropriate choice for the determination of complex flow profiles. To address this problem, we have previously developed intensity-based dynamic light scattering optical coherence tomography (iDLS-OCT), which can accurately determine flow using only the intensity of the OCT signal. In particular, we have reported on techniques to properly account for multiple sources of error in flow estimation using intensity-based techniques, including noise and axial gradients. We present the first comprehensive approach based on iDLS-OCT to enable quantitative, three-dimensional, 2-component vectorial flow maps using a rotating catheter probe. We present measurements in a phantom setup using both intralipid and blood. These promising results pave the way for in vivo measurements in the near future.

First clinical pilot study with intravascular polarization sensitive OCT

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Polarization sensitive (PS) OCT measures the polarization states of the light backscattered by tissue and provides measures of tissue birefringence and depolarization in addition to the structural OCT signal. Ex vivo studies have demonstrated that birefringence is increased in tissue rich in collagen and with elevated smooth muscle cell content. Preliminary data further suggests that depolarization can identify regions of macrophage infiltration, lipid, and irregularly arranged collagen fibers. These are important aspects of the mechanical integrity and vulnerability of atherosclerotic plaques. To evaluate the potential of PS-OCT in the clinical setting, we combined our custom PS-OCT system with commercially available OCT catheters (Fastview, Terumo Corporation) and performed a pilot study in 30 patients, scheduled to undergo percutaneous coronary intervention (PCI) on the grounds of stable or unstable angina. A total of 82 pullbacks in 39 vessels were performed, either in the native coronary arteries or post procedure. Comparing consecutive pullbacks of the same coronary artery, we found excellent agreement between the polarization features in the repeat pullbacks, validating the repeatability and robustness of PS-OCT in the clinical in vivo setting. In addition we observed that the birefringence and depolarization features vary significantly across lesions with identical structural OCT appearance, suggesting morphological subtypes. This first human pilot study proved the feasibility and robustness of intravascular PS-OCT. PS-OCT achieves improved tissue characterization and may help in identifying high-risk plaques, with the potential to ultimately improve risk stratification and help guiding PCI.

Mechanical modeling of cholesterol crystallization in atherosclerotic plaques base on Micro-OCT images

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Plaque rupture is the critical cause of cardiovascular thrombosis but this process is still under discussion. Recent studies show that, during crystallization, cholesterol crystals in atheromatous plaques accumulate rapidly in a limited space and may result in plaque rupture. However, the actual role of cholesterol crystals on plaque rupture remains unclear due to the lack of detailed morphological information of cholesterol crystals. In this
study, we used a Micro-optical coherence tomography (μOCT) setup with 1-2 μm spatial resolution to extract the geometry of cholesterol crystals from human atherosclerotic artery ex vivo firstly. With measured dimensions of cholesterol crystals by this μOCT system (the average length and thickness of 269±180.16 μm and 3.0±0.35 μm), we developed a two-dimensional mechanical model in which rectangular shaped cholesterol crystals distribute at different locations spatially. We predicted the stress on the thin cap induced by the expansion of cholesterol crystals by use of finite-element method. Since a large portion of plaques (58%) rupture at points of peak circumferential stress (PCS), we used PCS as the primary indicator of plaque stability with blood pressure of 14.6 kPa on the lumen. The results demonstrate that loading of the concentrated crystals especially at the cap shoulder destabilize the plaque by proportionally increasing the PCS, while evenly distributed crystals loading along the cap might impose less PCS to the plaque than the concentrated case.

9689-97, Session 1

**Endomyocardial imaging using ultrahigh resolution spectral domain optical coherence tomography (SD-OCT)**

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Life-threatening cardiac diseases such as arrhythmias and heart failure may be correlated to microstructural changes in the myocardial tissue. Most commercial real-time medical imaging modalities do not have sufficient resolution to visualize cellular level remodeling that occurs during cardiac diseases. We show that different types of cardiac tissue structures can be identified using an ultrahigh-resolution SD-OCT system with an axial resolution of 2.57μm.

The SD-OCT system used a broadband light spectrum centered at 840nm that was filtered out from the output of a low-noise supercontinuum source (NKT). The system has a measured axial resolution of 2.57μm in air, a lateral resolution of 8.8μm, and an imaging range of 1.7mm. The sensitivity was measured to be 90dB with a 6-dB fall-off range of 0.6mm.

Cross-sectional images and 3D volumes were acquired ex vivo on tissue specimens from various parts of 6 human hearts and 14 swine hearts obtained from the National Disease Research Interchange (NDRI) Tissue Bank and Columbia University’s tissue sharing program, respectively. Analysis of histological slides showed that with the increased resolution and contrast from the high-resolution system, features such as adipocytes, fibrosis, Purkinje fibers, and collagen were observed, which were otherwise not shown in our previous work using the Thorlabs Telesto system with 5.5μm axial resolution. This shows the promise of high-resolution OCT imaging for applications in assessing ventricular remodeling associated with cardiac arrhythmias and heart failure. We also observed dependence of light polarization with respect to the fiber orientation, which may help to differentiate the fiber sheets.

9689-98, Session 2

**Blood coagulation profiling in patients using optical thromboelastography (OTEG)**

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Impaired blood coagulation is often associated with increased postoperative mortality and morbidity in cardiovascular patients. The capability for blood coagulation profiling rapidly at the bedside will enable the timely detection of coagulation defects and open the opportunity for tailoring therapy to correct specific coagulation deficits. Optical Thromboelastography (OTEG), an optical approach to quantify blood coagulation status within minutes using a drop of whole blood. The goal of the current study is to evaluate the diagnostic accuracy of OTEG for rapid coagulation profiling in patients. In OTEG, temporal laser speckle intensity fluctuations from a drop of clotting blood are measured using a CMOS camera. To quantify coagulation status, the speckle intensity autocorrelation function is measured, the mean square displacement of scattering particles is extracted, and viscoelastic modulus (G), during coagulation is measured via the generalized Stokes-Einstein relation. By quantifying time-resolved changes in G, the coagulation parameters, reaction time (R), clot progression time (K), clot progression rate (Angle), and maximum clot strength (MA) are derived. In this study, the above coagulation parameters were measured using OTEG in 269 patients and compared with standard mechanical Thromboelastography (TEG). Our results showed a strong correlation between OTEG and TEG measurements for all parameters: R-time (R=0.80, p<0.001), clotting time (R=0.78, p<0.001), Angle (R=0.58, p<0.001), and MA (R=0.60, p<0.001). These results demonstrate the unique capability of OTEG for rapid quantification of blood coagulation status to potentially improve clinical capability for identifying impaired coagulation in cardiovascular patients at the point of care.

9689-99, Session 2

**Optical profiling of anticoagulation status**

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Defective blood coagulation resulting from excessive procoagulant activity often leads to thrombotic disorders such as stroke and myocardial infarction. A variety of oral and injectable anticoagulant drugs are prescribed to prevent or treat life-threatening thrombosis. However, due to bleeding complications often associated with anticoagulant treatment, routine monitoring and accurate dosing of anticoagulant therapy is imperative. We have developed Optical thromboelastography (OTEG), a non-contact approach that utilizes a drop of whole blood to measure blood coagulation status in patients. Here, we demonstrate the capability of OTEG for rapidly monitoring anticoagulation in whole blood samples. OTEG monitors coagulation status by assessing changes in blood viscosity from temporal intensity fluctuations of laser speckle patterns during clotting. In OTEG a blood drop is illuminated with coherent light and the blood viscosity is measured from the speckle intensity autocorrelation curve, g2 (τ). The metrics, clotting time (R+k), clot progression (angle) and maximum clot stiffness (MA) are then extracted. The aim of the current study was to evaluate the accuracy of OTEG in assessing anticoagulation status of common anticoagulants including heparin, argatroban and rivaroxaban status. A dose-dependent prolongation of R+k was observed in anticoagulated blood, which closely corresponded with standard-reference Thromboelastography (TEG) (r 0.87-0.99, P<0.01 for all cases). OTEG angle was unaltered by anticoagulation whereas TEG angle presented a dose-dependent diminution probably linked to clot rupture. In both OTEG and TEG, MA was unaffected by heparin, argatroban or rivaroxaban. We conclude that OTEG can accurately monitor anticoagulation status following treatment, potentially providing a powerful tool for routine monitoring of patients in the doctor’s office or in the home setting.
Brillouin spectroscopy of clotting dynamics in a model system

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Keys to successful treatment of disease include early diagnosis and timely treatment. Pathologies such as atherothrombotic vascular disease manifest through abnormal blood clotting dynamics. It is hypothesized that early clotting events may contribute to a pro-thrombotic state that exacerbates atherothrombotic vascular disease. Thus, a diagnostic tool based upon detection of early clotting dynamics could inform therapeutic treatment options. Recent rheometric studies have established that a viscoelastic analysis of the sol-gel transition, or gel point, characteristics provides significant markers of haemostasis in healthy and therapeutically modified clotting. In this work, we seek to expand and exploit these findings through the application of Brillouin spectroscopy to a model fibrin-thrombin clotting system. Brillouin spectroscopy involves inelastic coupling of light with phonons (sound waves) and enables non-contact viscoelastic characterization of samples at the microscale. Recent advances in instrumentation have allowed us to develop a Brillouin micro-spectroscopy system capable of measuring spectral shifts on the order of 100s of MHz. The use of a single mode laser at 780 nm enables measurement of the system in a spectral band that will minimize absorption in whole blood or plasma. The measurement of clotting dynamics at the microscale could provide characterization that is not possible with standard rheometric techniques and permit the study of proto-clots. Here, the clotting dynamics of the model clotting system are measured at various pathological concentrations of fibrin and thrombin.

In-vivo continuous monitoring of mixed venous oxygen saturation by photoacoustic transesophageal echocardiography

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Mixed venous oxygen saturation (SvO2), measured from pulmonary arteries, is a gold-standard measure of the dynamic balance between the oxygen supply and demand in the body. In critical care, continuous monitoring of SvO2 plays a vital role in early detection of circulatory shock and guiding goal-oriented resuscitation. In current clinical practice, SvO2 is measured by invasive pulmonary artery catheters (PAC), which are associated with a 10% risk of severe complications. To address the unmet clinical need for a non-invasive SvO2 monitor, we are developing a new technology termed photoacoustic transesophageal echocardiography (PA-TEE). PA-TEE integrates transesophageal echocardiography with photoacoustic oximetry, and enables continuous assessment of SvO2 through an esophageal probe that can be inserted into the body in a minimally invasive manner. We have constructed a clinically translatable PA-TEE prototype, which features a mobile OPO laser, a modified ultrasonography console and a dual-modality esophageal probe. Comprised of a rotatable acoustic array detector, a flexible optical fiber bundle and a light-integrating acoustic lens, the oximetric probe has an outer diameter smaller than 15 mm and will be tolerable for most patients. Through custom-made C++/Qt software, our device acquires and displays ultrasonic and photoacoustic images in real time to guide the deployment of the probe. SvO2 is calculated on-line and updated every second. PA-TEE has now been used to evaluate SvO2 in living swine. Our findings show that changing the fraction of oxygen in the inspired gas modulates SvO2 measured by PA-TEE. Statistic comparison between SvO2 measurements from PA-TEE in vivo the gold-standard laboratorial analysis on blood samples drawn from PACs will be presented.

Evaluation of combined near-IR spectroscopic (NIRS)-IVUS imaging as a means to detect lipid-rich plaque burden in human coronary autopsy specimens

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Intracoronary near-infrared spectroscopy (NIRS) can identify lipid core plaques, but lacks depth resolution. A novel catheter is in widespread clinical use that combines NIRS with intravascular ultrasound (IVUS), which provides depth-resolved structural information. A measure designated as lipid-rich plaque burden (LRPB) has been proposed as a means to interpret the combined acoustic and optical information of NIRS-IVUS. LRPB is computed as the area comprising the NIRS lipid-rich arc that intersects the IVUS-measured plaque burden. We determined the correlation in human coronary autopsy specimens between LRPB and the area of lipid-rich plaque as determined by the gold-standard of histology.

Nine artery segments from 5 human autopsy hearts were imaged with the TCV NIRS-IVUS system (InfraReDx Inc.). Arteries were then fixed and divided into 2mm blocks for histological staining. Pathological contouring of lipid-rich areas was performed on the histology sections for 45 lipid-rich blocks. Computation of LRPB was performed on transverse NIRS-IVUS frames corresponding to the histologic sections. The quantified LRPB was frequently higher than the lipid-rich plaque area determined by histology, because the region denoted by the EEL and lumen within the NIRS lipid-rich arc is not entirely comprised of lipid. Overall, a moderate correlation (R=0.74) was found between LRPB determined by NIRS-IVUS imaging and the lipid-rich plaque area determined by histology. LRPB, which can be measured in patients with NIRS-IVUS imaging, corresponds to the amount of lipid-rich plaque in a coronary artery. LRPB should be evaluated in prospective clinical trials for its ability to identify vulnerable plaques.

In-vivo validation of a multi-modal fluorescence lifetime imaging (FLIm)-IVUS catheter in swine coronary arteries

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Multispectral fluorescence lifetime imaging (FLIm) can measure biochemical tissue properties based on autofluorescence generated by a pulsed UV laser. Specifically, FLIm is able to assess the degradation of collagen and elastin matrix and the infiltration of macrophages in the fibrous cap of atherosclerotic plaque. When integrated with intravascular ultrasound (IVUS) into a multimodal catheter, FLIm provides a fast and robust solution...
to add biochemical specificity to the morphological plaque characterization provided by IVUS. We report a combined FLim and IVUS into a custom 3.7 Fr catheter and demonstrate the first in vivo application in healthy swine coronary arteries of this new catheter. The catheter is able to acquire 25,000 independent multispectral FLim point measurements co-registered with IVUS, covering a 20 mm long artery segment in 5 seconds. We are reporting progress towards the clinical translation of this technique for human use. Further in vivo studies in swine were performed to optimize signal collection while minimizing the amount of flushing solution (<50 cc/pullback); the robustness of the collected FLim data has been validated across numerous pullbacks from 3 healthy swine. Ex vivo human coronary samples were also imaged to better evaluate the ability of current catheter to characterize plaque with emphasis on thin cap fibroatheroma (TCFA); IVUS has poor accuracy for the identification of TCFA, but FLim allows for improved specificity of identification of the lipid or necrotic core.

9689-104, Session 3
Assessment of intravascular stent-associated inflammation and intraplaque hemorrhage using fully integrated high-speed intravascular OCT/NIRF imaging
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Intracoronary inflammation and intraplaque hemorrhage (IPH) play essential role in plaque rupture in coronary artery. However, the capability of the diagnosis of both lesions was limited because of the lack of the imaging contrast technique. Intravascular near-infrared fluorescence (NIRF) is a novel imaging modality targeting specific molecules inside high-risk plaques and visualizing the plaque vulnerability. Such advantages of NIRF imaging is especially effective when combined with optical coherence tomography (OCT) which provides three-dimensional structural imaging of the blood vessels. In our work, stent-associated inflammation in swine coronary artery and intraplaque hemorrhages (IPHs) in rabbit aorta was specifically targeted by fully-integrated high-speed OCT/NIRF intravascular imaging system. Indocyanine green (ICG) targeting inflammation and custom-fabricated nanoprobes targeting macrophage mannose receptors (MMRs) were used as exogenous NIRF contrast agents. As a result, increased NIRF intensities along stent struts were observed at two weeks after DES deployment inside swine coronary arteries, which is significantly higher than the NIRF signal measured right after the DES placement. In rabbit aorta, high NIRF intensities was shown at the black-dotted neovessels surrounded by heterogeneous OCT signals, suggesting the association of IPH formations and MMR positive macrophages. Both ex-vivo fluorescence imaging and histology showed agreement with the in-vivo images, which demonstrated the co-localization of NIRF signal with MMR and hemorrhagic compositions. In addition, crosstalk was suppressed by adjusting the diameter of the double clad fiber in order to improve the OCT image quality.

9689-105, Session 3
Ultrafast IVUS-OCT imaging of rabbit and swine arteries in vivo at 72 frames per second
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Fully combined intravascular ultrasound optical coherence tomography (IVUS-OCT) has raised great interest of physicians due to successful in vivo demonstrations. However, the limited imaging speed posed a significant barrier to translate this technology for clinical practices. Limited speed means more injection of contrast agents and also a higher risk of catheter-induced spasm. To address this challenge, we developed an ultrafast IVUS-OCT system. In 2014, a speed of 50 frames per second (fps) was achieved. This year, we further pushed this speed limit and 72 fps was reached. A series of technical advancements were applied to enable this speed: a more advanced IVUS pulser/receiver, electrical slip ring, graphic processing unit, solid state drive, and customized catheter design. The imaging ability of this ultra-fast system was evaluated in both rabbits and pigs in vivo. Volumetric imaging of atherosclerotic plaques in live rabbits and of a pig coronary artery was safely performed. We further demonstrated that this system can accurately differentiate between vulnerable plaque and stable plaque. By providing ultrafast imaging of arteries with high resolution and deep penetration depth simultaneously, this hybrid ultrafast IVUS-OCT technology opens new and safe opportunities to evaluate in real-time the risk of plaques, detect vulnerable plaques (as well as possible risk of plaque rupture or erosion), and optimize treatment decisions.

9689-106, Session 3
Compensation of spectral artifacts in dual-modality intravascular optical coherence tomography and near-infrared spectroscopy
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Intravascular optical coherence tomography (OCT) is a high-resolution catheter-based imaging method that provides three-dimensional microscopic images of coronary artery in vivo, facilitating coronary artery disease treatment decisions based on detailed morphology. Near-infrared spectroscopy (NIRS) has proven to be a powerful tool for identification of lipid-rich plaques inside the coronary walls. We have recently demonstrated a dual-modality intravascular imaging technology that integrates OCT and NIRS into one imaging catheter using a two-fiber arrangement and a custom-made dual-channel rotary junction. It therefore enables simultaneous acquisition of microstructural and composition information at 100 frames/second for improved diagnosis of coronary lesions. The dual-modality OCT-NIRS system employs a single wavelength-swept light source for both OCT and NIRS modalities. It subsequently uses a high-speed photoreceiver to detect the NIRS spectrum in the time domain. Although use of one light source greatly simplifies the system configuration, such light source exhibits pulse-to-pulse wavelength and intensity variation due to mechanical scanning of the wavelength. This can be in particular problematic for NIRS modality and sacrifices the reliability of the acquired spectra. In order to address this challenge, here we developed a robust data acquisition and processing method that compensates for the spectral variations of the wavelength-swept light source. The proposed method
High speed intravascular photoacoustic imaging of atherosclerotic arteries

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Cardiovascular disease is the leading cause of death in the industrialized nations. Accurate quantification of both the morphology and composition of lipid-rich vulnerable atherosclerotic plaque are essential for early detection and optimal treatment in clinics. In previous works, intravascular photoacoustic (IVPA) imaging for detection of lipid-rich plaque within coronary artery walls has been demonstrated in ex vivo, but the imaging speed is still limited. In order to increase the imaging speed, a high repetition rate laser is needed. In this work, we present a high speed integrated IVPA/US imaging system with a 500 Hz optical parametric oscillator laser at 1725 nm. A miniature catheter with 1.0 mm outer diameter was designed with a 200 µm multimode fiber and an ultrasound transducer with 45 MHz center frequency. The fiber was polished at 38 degree and enclosed in a glass capillary for total internal reflection. An optical/electrical rotary junction and pull-back mechanism was applied for rotating and linearly scanning the catheter to obtain three-dimensional imaging. Atherosclerotic rabbit abdominal aorta was imaged as two frame/second at 1725 nm. Furthermore, by wide tuning range of the laser wavelength from 1680 nm to 1770 nm, spectroscopic photoacoustic analysis of lipid-mimicking phantom and an human atherosclerotic artery was performed ex vivo. The results demonstrated that the developed IVPA/US imaging system is capable for high speed intravascular imaging for plaque detection.

Lipid detection by intravascular photoacoustic imaging with flexible catheter at 20 frames per second

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Intravascular Photoacoustic (IVPA) imaging is a promising new technology to assess lipid content of coronary atherosclerotic plaque, an important determinant of the risk associated with the plaque triggering a heart attack. Clinical translation of IVPA imaging requires real-time image acquisition, which has been a technological challenge. In this work, we demonstrate a high-speed, dual-wavelength IVPA imaging system at 1.7 µm wavelength, operating with a flexible catheter of 1.2 mm outer diameter (including outer sheath). The catheter was custom designed and fabricated, and used a 40 MHz transducer for intravascular ultrasound (IVUS) and IVPA imaging. The optical excitation is provided by a dual OPO system, pumped by CW diode-pumped Q-switched Nd:YAG lasers, with a repetition rate of 5 kHz. Each OPO can be tuned to a custom wavelength between 1690 and 1750 nm; two wavelengths only are needed to discriminate between plaque lipids and adipose tissue. The pulse energy is about 80 µJ. We tested the imaging performance of the presented system in a polyvinyl-alcohol (PVA) vessel mimicking phantom and human coronary arteries ex vivo. IVPA identified lipid deposits inside atherosclerotic plaque, while IVUS showed tissue structure. We demonstrated IVPA imaging at a speed of 20 frames per second, with 250 A-scans per frame. This is significantly faster than previous IVPA imaging systems, and will enable the translation of IVPA imaging into clinical practice.

Design and validation of the ball lens-based intravascular catheter for fluorescence lifetime imaging microscopy of atherosclerosis

Xi Chen, Wihan Kim, Michael J. Serafino, Javier A. Jo, Brian E. Applegate, Texas A&M Univ. (United States)

We report the design and validation of a ball lens-based imaging catheter and broadband UV rotary joint for fluorescence lifetime imaging microscopy of atherosclerosis. The performance of the catheter endoscope was modeled and optimized using the ray-tracing program Zemax. A 1.55-m-long multi-mode fiber was spliced with a short length of coreless fiber, and then heated and polished to fabricate the angled ball lens. The fiber endoscope was enclosed in a torque cable and protective sheath and had a diameter of 4 Fr. The catheter was affixed to a custom built lensless rotary joint which had high coupling efficiency (>90%) over a broad spectral range, accommodating both the UV (355 nm) excitation and the broad fluorescence emission (395 nm - 600 nm). The computer controlled rotary joint and translation stage for pullback imaging can routinely achieve rotation rates of 6000 rpm. The resolution is determined by lens-to-sample distance. Experiments conducted using a resolution target demonstrate a lateral resolution of 0.08 radians and 80 µm for 1 mm lens-to-sample distance. Assuming 80 µm longitudinal sampling, the system is capable of pullback velocities >8 mm/s. Experiments conducted using a fluorescein phantom and a segment of ex vivo pig coronary artery demonstrate the system performance for fluorescence lifetime imaging. This study demonstrates the novel design of a ball lens-based FLIM catheter and rotary joint to record fluorescence in a continuous helical scanning method across broad-spectral emission bands.

Localization analysis of lipid core plaques detected by a near infrared spectroscopy system as compared to histological finding: intracoronary imaging application

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A catheter-based near infrared spectroscopy (NIRS) system has been cleared by the FDA for detection of lipid core plaques (LCP) in patients undergoing coronary arteriography. NIRS detection results are summarized as a 2D probability map (Chemogram), its performance is evaluated based on ROC analysis of 2mm block Chemogram against histology. Recently an algorithm is developed to enhance Chemogram with automated detection of LCP objects (LO). The enhanced Chemogram has less/no outliers with
9689-111, Session 4

Support vector machine based classification and mapping of atherosclerotic plaques using fluorescence lifetime imaging

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The progression of atherosclerosis in coronary vessels involves distinct pathological changes in the vessel wall. These changes manifest in the formation of a variety of plaque sub-types. The ability to detect and distinguish these plaques, especially thin-cap fibroatheromas (TCFA) may be relevant for guiding percutaneous coronary intervention as well as investigating new therapeutics. In this work we demonstrate the ability of fluorescence lifetime imaging (FLIm) derived parameters (lifetime values from sub-bands 390/40 nm, 452/45 nm and 542/50 nm respectively) for generating classification maps for identifying eight different atherosclerotic plaque sub-types in ex vivo human coronary vessels. The classification was performed using a support vector machine based classifier that was built from data gathered from sixteen coronary vessels in a previous study. This classifier was validated in the current study using an independent set of FLIm data acquired from four additional coronary vessels with a new rotational FLIm system. Classification maps were compared to co-registered histological data. Results show that the classification maps allow identification of the eight different plaque sub-types despite the fact that new data was gathered with a different FLIm system. Regions with diffuse intimal thickening (n=10), fibrotic tissue (n=2) and thick-cap fibroatheroma (n=1) were correctly identified on the classification map. The ability to identify different plaque types using FLIm data alone may serve as a powerful clinical and research tool for studying atherosclerosis in animal models as well as in humans.

9689-112, Session 5

Optical mapping models of the atria enabled by OCT tissue characterization

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Optical mapping models are powerful tools for interpreting optical mapping experiments and exploring the mechanisms of arrhythmias. However, there remains a lack of such models including detailed atrial structure. We present the use of OCT images to characterize the topology and fiber orientation of the atria in optical mapping models. OCT volumes were acquired from rabbit and swine atria samples, ex vivo. The rabbit volume was comprised of multiple, separate OCT volumes registered via an image stitching algorithm to yield a field of view that covered almost the entire atria. The topology, and fiber orientation for the swine sample, were extracted from the images. The structural data were used to generate 3D models of the tissue, and the propagation of a point stimulus was simulated in the multi-scale modeling software, “Continuity 6”. The resulting voltages were coupled with modeled uniform excitation light intensity to compute the fluorescent emission intensity at each node, and light scattering was computed by a Monte Carlo approach, the “TIM-OS” algorithm, to generate the modeled optical action potential. The fiber orientation of the swine tissue contained heterogeneity that induced nonuniform conduction. The combined runtime for electrophysiology and photon scattering for one simulation was around 45 minutes, and future simulations will be set up in parallel on a cluster for speedup. Next steps will include human samples and the modeling of applied RF ablation lesions, as well as the incorporation of varying tissue types such fibrosis, fat, collagen, and myocardium as measured from OCT images.

9689-113, Session 5

OptoDyCE: automated system for high-throughput all-optical dynamic cardiac electrophysiology

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In the last two decades, >30% of the total drugs withdrawn from the market after approval were withdrawn due to cardiac toxicity. Subsequently, international regulatory agreements mandate preclinical testing of cardiac liability for any new drug. The recognition that pro-arrhythmic effects can often be the result of drug’s action on multiple ion channels, an integrative cell-level measurements are needed using recently emerging stem-cell derived human cardiomyocytes (e.g. induced pluripotent stem-cell-derived cardiomyocytes, iPSC-CMs), yet there are no high-throughput approaches for cellular cardiac electrophysiology. Optical techniques for actuation and sensing are uniquely can provide instant parallelism, enabling contactless dynamic high-throughput testing of cells and small-tissue constructs, not affordable by other means.

We consider, computationally and experimentally, the requirements for all-optical electrophysiology when applied to drug testing, then implement and validate OptoDyCE, a fully automated system for all-optical cardiac electrophysiology, built using simple low-power LEDs and optics. We validate optical actuation by delivery of optogenetic drivers in iPSC-CMs or through the modular use of dedicated light-sensitive somatic “spark” cells in conjunction with non-modified cardiomyocytes. We demonstrate the high-throughput capabilities using multicellular samples in 96-well format by combining optogenetic actuation with simultaneous fast high-resolution optical sensing of voltage or intracellular calcium. We show that this automated all-optical approach provides high-throughput means of cellular interogation, i.e. novel dynamic testing of >600 multicellular samples.
or compounds per hour, and yields high-content information about the action of a drug over time, space, and doses.

9689-114, Session 5  
**Functional cardiac imaging platform by using ultrahigh-phase-stable swept source optical coherence tomography**  
Christine P. Hendon, Yuye Ling, Columbia Univ. (United States)

Functional extensions to optical coherence tomography (OCT) provide useful imaging contrasts that are complementary to conventional OCT. Our goal is to characterize tissue types within the myocardial due to remodeling and therapy. High-speed imaging is necessary to extract mechanical properties and dynamics of fiber orientation changes in a beating heart.

Functional extensions of OCT such as polarization sensitive and optical coherence elastography (OCE) require high phase stability of the system, which is a drawback of current mechanically tuned swept source OCT systems. Here we present a high-speed functional imaging platform, which includes an ultrahigh-phase-stable swept source equipped with KTN deflector from NTT-AT. The swept source does not require mechanical movements during the wavelength sweeping; it is electrically tuned. The inter-sweep phase variance of the system was measured to be less than 300 ps at a path length difference of -2 mm.

The axial resolution of the system is 20 μm and the -10 dB fall-off depth is about 3.2 mm. The sample arm uses a two-axis MEMS mirror, which is programmable and capable of scanning arbitrary patterns at a sampling rate of 50 kHz.

Preliminary imaging results showed differences in polarization properties and image penetration in ablated and normal myocardium. In the future, we will conduct dynamic stretching experiments with strips of human myocardial tissue to characterize mechanical properties using OCE. With high speed imaging of 200 kHz and an all-fiber design, we will work towards catheter-based functional imaging.

9689-115, Session 5  
**Development of multifunctional optical coherence tomography and application to mouse myocardial infarction model in vivo**  
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Optical coherence tomography (OCT) is a useful imaging method for in vivo tissue imaging with deep penetration and high spatial resolution. However, imaging of the beating mouse heart is still challenging due to limited temporal resolution or penetration depth. Here, we demonstrate a multifunctional OCT system for a beating mouse heart, providing various types of visual information about heart pathophysiology with high spatiotemporal resolution and deep tissue imaging. Angiographic imaging and polarization-sensitive (PS) imaging were implemented with the electrocardiogram (ECG)-triggered beam scanning scheme on the high-speed OCT platform (A-line rate: 240 kHz). Depth-resolved local birefringence and the local orientation of the mouse myocardial fiber were visualized from the PS-OCT. ECG-triggered angiographic OCT (AOC) with the custom-built motion stabilization imaging window provided myocardial vasculature of a beating mouse heart. Mice underwent coronary artery ligation to derive myocardial infarction (MI) and were imaged with the multifunctional OCT system at multiple time points. AOC and PS-OCT visualize change of functionality of coronary vessels and myocardium respectively at different phases (acute and chronic) of MI in an ischemic mouse heart. Taken together, the integrated imaging of PS-OCT and AOC would play an important role in study of MI providing multi-dimensional information of the ischemic mouse heart in vivo.

9689-116, Session 5  
**Real-time optical monitoring of permanent lesion progression during RF ablation: Implications for treatment of atrial fibrillation**  
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Despite considerable advances in guidance of radiofrequency ablation (RFA) therapies for atrial fibrillation, success rates have been hampered by an inability to intraoperatively characterize the extent of permanent injury. Insufficient lesions can elusively create transient conduction blockages that eventually reconduct. Prior studies suggest significantly greater myocardial fibrosis (Mmb) concentrations in the lesion core than those in the healthy myocardium and may serve as a marker for irreversible tissue damage. In this work, we present real-time monitoring of permanent injury through spectroscopic assessment of Mmb concentrations at the catheter tip. Atrial wedges (n=6) were excised from four fresh swine hearts and submerged under pulsatile flow of warm (37ºC) phosphate buffered saline. A commercial RFA catheter inserted into a fiber optic sheath allowed for simultaneous measurement of tissue diffuse reflectance (DR) spectra (500-650nm) during application of RF energy. Optical measurements were continuously acquired before, during, and post-ablation, in addition to healthy neighboring tissue. Met-myoglobin, oxy-myoglobin, and deoxy-myoglobin concentrations were extracted from each spectrum using an inverse Monte Carlo method. Tissue injury was validated with Masson’s trichrome and hematoxylin and eosin staining. Time courses revealed a rapid increase in tissue Mmb concentrations at the onset of RFA treatment and a gradual plateauing thereafter. Extracted Mmb concentrations were significantly greater post-ablation (p<0.0001) as compared to healthy tissue and correlated well with histological assessment of severe thermal tissue destruction. On going studies are aimed at integrating these findings with prior work on near infrared spectroscopic lesion depth assessment. These results support the use of spectroscopy-facilitated guidance of RFA therapies for real-time permanent injury estimation.

9689-117, Session 5  
**OCT imaging of myocardium extending to pulmonary vein based on optical scattering coefficient**  
Zhifang Li, Fujian Normal Univ (China); Timm Dickfeld, Univ. of Maryland School of Medicine (United States); Qinggong Tang, Bohan Wang, Yu Chen, Univ. of Maryland (United States)

Atrial fibrillation (AF) is the most common cardiac arrhythmia. One of the root causes of atrial fibrillation is initiating triggers from atrial myocardium extending into the pulmonary veins (PV). Recently, radiofrequency ablation of AF has been introduced as a potentially curative alternative treatment. Local electrical signals were used to guide the ablation surgery. However, current treatment induces the recurrence rate of 50% or more in longer-term follow-up evaluation. In this study, we propose to use optical coherence tomography to enable a direct visualization of myocardium extending into the PV, which is the first step for guiding ablation of AF. The sample used in study was the sheep heart together with PV. The myocardium, fibrous tissue and the transition region in the left atrium were firstly imaged by OCT, respectively. Then, single scattering model was used for extracting the scattering coefficient of tissue. The results showed that there are obvious differences in the morphology and scattering coefficients of myocardium, fibrous tissue, and the transition region in the left atrium. In addition, there is a peak area in the scattering coefficient distribution of the transition region. This characteristic was used for extracting the structures
of myocardium extending into PV. The results are in agreement with the histological analysis. Our results indicate that OCT has a potential for radiofrequency ablation of AF.

9689-118, Session 6

Influence of distance and incident angle on light intensities in intravascular optical coherence tomography pullback runs

Shengnan Liu, Jeroen Eggermont, Ron Wolterbeek, Jouke Dijkstra, Leiden Univ. Medical Ctr. (Netherlands)

Background:
Intravascular optical coherence tomography (IVOCT) is an intravascular imaging modality which enables arterial structures to be visualized at a microstructure level. The determination of these structures is mainly on the basis of relative image intensities which is difficult to be quantified because there are many factors which can influence the intensities apart from tissue attenuation. Even for homogenous tissue light intensities will differ. Although this problem has been reported before to the best of our knowledge, no specific research has been carried out to analyze potential causes. In this study the incident light intensity is modeled to be related to both the distance between catheter and inner lumen wall as well as incident angle.

Methods and Results:
A three-level hierarchical model is constructed to validate this model to include the potential effect of different pull-back runs and/or frame numbers. The model is solved using 80107 data points from 169 images out of 9 pull-backs recorded with a St.Jude OCT system. The hierarchical linear regression takes into consideration fixed effects (distance and incident angle) because they have fixed values while pull-back number and frame number are treated as “unobservable random variables”. F-tests results for each of the fixed effects specified in the model indicate that both distance and incident angle effects contribute to the model statistically significantly with p<0.001.

Based on the results from the statistical analysis a potential compensation method is introduced which can compensate for intensity loss due to distance, incident angle or small shadow effects.

9689-119, Session 6

Common path ball lens probe for optical coherence tomography

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Background: Common path probes are highly desirable for optical coherence tomography (OCT) as they reduce system complexity and cost. In this work we report an all-fiber common path side viewing monolithic probe for coronary artery imaging.

Methods: Our common path probe was designed for spectrometer based Fourier domain OCT at 1310 nm wavelength. Light from the fiber expands in the coreless fiber region and then focused by the ball lens. Reflection from ball lens-air interface served as reference signal. The monolithic ball lens probe was assembled within a 560 µm outer diameter drive shaft which was attached to a rotary junction. The drive shaft was placed inside an outer, transparent sheath of 800 µm diameter.

Results: With a source input power of 25 mW, we could achieve sensitivity of 100.5 dB. The axial resolution of the system was found to be 15.6 µm in air and the lateral resolution (full width half maximum) was approximately 49 µm. As proof of principal, images of skin acquired using this probe demonstrated clear visualization of the stratum corneum, epidermis, and papillary dermis, along with sweat ducts.

Conclusion: In this work we have demonstrated a monolithic, ball lens common, path probe for OCT imaging. The designed ball lens probe is easy to fabricate using a laser splicer. Based on the features and capability of common path probes to provide a simpler solution for OCT, we believe that this development will be an important enhancement for certain types of catheters.

9689-120, Session 6

Light intensity matching between different intravascular optical coherence tomography systems

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Background:
Currently two commercial intravascular optical coherence tomography (IVOCT) systems are available: Illumien Optis from St. Jude Medical (SJM) and Lunawave from Terumo. Both systems store the light intensity data in a raw vendor specific polar format. However, whereas SJM uses 16-bits per pixel Terumo uses 8-bits meaning the intensity values are in different ranges. This complicates quantitative light intensity based analysis when comparing results based on data from both systems. Therefore, this work aims to find an intensity transformation function from Terumo’s 8-bit OFDI data to SJM's 16-bit range.

Methods and Results:
The data consists of 8 pullbacks, 4 acquired with each system in the same arteries of 2 different patents pre- and post-stent implantation. A total of 133 matching sections without stent struts from the two sets of pullbacks were identified based on landmarks such as side-branches and calcified regions. Since the main region of interest in the image is the tissue region only the pixels within 2mm behind the lumen border are used. In order to match the SJM data range, the Terumo data was rescaled and cumulative distribution functions (CDF) were calculated based on the histogram distributions. Comparing these two CDF’s, an intensity difference curve was determined. In order to generalize a transformation function, the normalized difference curves from 133 sections have been fitted by a 3rd power polynomial. Application of this transformation function not only improves the visual similarity of matching slices it can also aid quantitative light intensity analysis and attenuation estimation.

9689-121, Session 6

The Lumivascular technology platform: real time OCT guidance for therapeutic intra-vascular revascularization

Manish Kankaria, Arjun Desai, Avinger, Inc. (United States)

Peripheral arterial disease often encompasses non-uniform occlusive lesions with milieu of pathological compositions that could span over multiple vascular territories.

Because the current therapies have to rely on two-dimensional angiographic guidance, they often inadvertently disrupt the medial/adventitial border during the therapeutic revascularization.

The Lumivascular technology platform is OCT guided, real-time catheter based platform for therapeutic revascularization of occlusive arterial disease. Currently, it encompasses the Ocelot catheters family (FDA cleared), which enable the crossing of chronic total occlusions (CTO) in the legs and Pantheris, an atherectomy device (currently under clinical investigation) that enables intra-vascular revascularization while preserving the medial/adventitia border.
Current research has demonstrated that the adventitial disruption is highly correlated with increased inflammation and has been shown to negatively impact long-term arterial patency. Thus, the development of platform for intravascular guided revascularization catheters utilizing optical coherence tomography (OCT) have the premise to effectively minimize the trauma to the arterial wall when treating PAD.

Recent clinical data has confirmed the safety, clinical efficacy and the added benefits the Luminvascular platform, including the Connect II study (Ocelot; Final Results of the Chronic Total Occlusion Crossing With the Ocelot System II, CONNECT II, Study, Selmon et al, J ENDOVASC THER 2013;20:770–781) and the Vision study (6 month VISION Trial Outcomes will be released at TCT 2015).

In addition the Luminvascular platform could revolutionize the endovascular practice, enabling minimizing the x-ray burden and replacing angiographic fluoroscopy with the OCT guidance.

9689-95, Session 7

Assessment of atherosclerotic plaque collagen content and architecture using polarization-sensitive optical coherence tomography

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Acute myocardial infarction, caused by the rupture of vulnerable coronary plaques, is the leading cause of death worldwide. Collagen is the primary extracellular matrix macromolecule that imparts the mechanical stability to a plaque and its reduction causes plaque instability. Intracoronary polarization sensitive optical coherence tomography (PS-OCT) measures the polarization states of the backscattered light from the tissue to evaluate plaque birefringence, a material property that is elevated in proteins such as collagen with an ordered structure. Here we investigate the dependence of the PS-OCT parameters on the quantity of the plaque collagen and fiber architecture. In this study, coronary arterial segments from human cadaveric hearts were evaluated with intracoronary PS-OCT and compared with Histopathological assessment of collagen content and architecture from picrosirius-red (PSR) stained sections. PSR sections were visualized with circularly-polarized light microscopy to quantify collagen birefringence, and the additional assessment of color hue indicated fibril thickness. Due to the ordered architecture of thick collagen fibers, a positive correlation between PS-OCT retardation and quantity of thick collagen fibers (r=0.54, p=0.04), and similarly with the total collagen content (r=0.51, p=0.03) was observed. In contrast, there was no perceivable relationship between PS-OCT retardation and the presence of thin collagen fibers (r=0.08, p=0.7), suggesting that thin and disorganized collagen fiber architecture did not significantly contribute to the PS-OCT retardation. Further analysis will be performed to assess the relationship between PS-OCT retardation and collagen architecture based on immunohistochemical analysis of collagen type. These results suggest that intracoronary PS-OCT may open the opportunity to assess collagen architecture in addition total collagen content, potentially enabling an improved understanding of coronary plaque rupture.

9689-122, Session 7

Aortic endothelium detection using spectral estimation optical coherence tomography

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The evaluation of the endothelium coverage on the vessel wall is most wanted by cardiologists. Arterial endothelial cells play a crucial role in keeping low-density lipoprotein and leukocytes from entering into the intima. The damage of endothelial cells is considered as the first step of atherosclerosis development and the presence of endothelial cells is an indicator of arterial healing after stent implantation. Intravascular OCT (IVOCT) is the highest-resolution coronary imaging modality, but it is still limited by an axial resolution of 10-15 µm. This limitation in axial resolution hinders our ability to visualize cellular level details associated with coronary atherosclerosis. Spectral estimation optical coherence tomography (SE-OCT) uses modern spectral estimation techniques and may help reveal the microstructures underlying the resolution limit. In this presentation, we conduct an ex vivo study using SE-OCT to image the endothelium cells on the fresh swine aorta. We find that in OCT images with an axial resolution of 10 µm, we may gain the visibility of individual endothelium cells by applying the autoregressive spectral estimation techniques to enhance the axial resolution. We believe the SE-OCT can provide a potential to evaluate the coverage of endothelium cells using current IVOCt with a 10-µm axial resolution.

9689-123, Session 7

A pilot study using laser-based technique for non-invasive diagnostics of hypertensive conditions in transgenic mice

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Endothelial dysfunction is the primary underlying culprit linked to preeclampsia, a maternal hypertensive disorder affecting up to 7% of all pregnancies. Preeclampsia kills mums everyday and women who survive childbirth are likely to die prematurely of heart disease. Endothelial dysfunction is a common association linking preeclampsia and cardiovascular disease. We sought to define a new non-invasive test for the diagnosis of preeclampsia using established mouse models of preeclampsia. A laser-based multifunctional diagnostics system (LAKK-M) was chosen to carry out non-invasive analysis of multiple parameters. The device was used to simultaneously record the microcirculatory blood flow and oxygen saturation, as well as fluorescence levels of endogenous fluorophores. Preliminary experiments were conducted on adenosiral (Ad-) ?mediated overexpression of sFlt-1 (Ad-sFlt-1) to mimic pre-eclampsia-like symptoms in pregnant mice. The recorded data displayed the ability of the LAKK-M diagnostics device to detect significant differences in perfusion measurements between the control and Ad-sFlt-1 treatment. Such preliminary results provide a potential avenue to employ such diagnostics technology to monitor and aid in maintaining control of live animal conditions throughout the experiment and treatment.
High frequency ultrasound probes such as intravascular ultrasound (IVUS) and intracardiac echocardiography (ICE) catheters can be invaluable for guiding minimally invasive medical procedures in cardiology such as coronary stent placement and ablation. With current-generation ultrasound probes, ultrasound is generated and received electrically. The complexities involved with fabricating these electrical probes can result in high costs that limit their clinical applicability. Additionally, it can be challenging to achieve wide transmission bandwidths and adequate wideband reception sensitivity with small piezoelectric elements. Optical methods for transmitting and receiving ultrasound are emerging as alternatives to their electrical counterparts. They offer several distinguishing advantages, including the potential to generate and detect the broadband ultrasound fields (tens of MHz) required for high resolution imaging. In this study, we developed a miniature, side-looking, pulse-echo ultrasound probe for intravascular imaging, with fibre-optic transmission and reception. The axial resolution was better than 70 microns, and the imaging depth in tissue was greater than 1 cm. Ultrasonic transmission was performed by photoacoustic excitation of a carbon nanotube/polydimethylsiloxane composite material; ultrasound reception, with a fibre-optic Fabry-Perot cavity. Ex vivo tissue studies, which included healthy swine tissue and diseased human tissue, demonstrated the strong potential of this technique. To our knowledge, this is the first study to achieve an all-optical pulse-echo ultrasound probe for intravascular imaging. The potential for performing all-optical B-mode imaging (2D and 3D) with virtual arrays of transmit/receive elements, and hybrid imaging with pulse-echo ultrasound and photoacoustic sensing are discussed.

Arterial stiffness (AS) is a recognized predictor of cardiovascular risk and mortality, and a potential marker for monitoring the beneficial effects of medical treatments for arterial diseases. AS is typically measured indirectly, using low-cost optical components. Two light lines generated by blue LEDs are projected on the target, and the skin displacement is measured using a CCD sensor. The measurement of the PWV is performed by calculating the time delay of the pulse waves measured in the two points, through a suited cross-correlation technique. We will present results of PWV measurement on different volunteers patients, showing the effectiveness of the technique.

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 time scale (time constant, \( \tau \)) of temporally evolving laser speckle fluctuations. To enable coronary evaluation in vivo, an optical ILSI catheter has been developed that accomplishes omni-directional illumination and viewing of the entire coronary circumference without the need for mechanical rotation. Here, we describe the capability of ILSI for evaluating human coronary atherosclerosis in cadaveric hearts. ILSI was conducted in conjunction with optical coherence tomography (OCT) imaging in five human cadaveric hearts. The left coronary artery (LCA), left anterior descending (LAD), left circumflex artery (LCX), and right coronary artery (RCA) segments were resected and secured on custom-developed coronary holders to enable accurate co-registration between ILSI, OCT, and histopathology. Speckle time constants, \( \tau \), calculated from each ILSI section were compared with lipid and collagen content measured from quantitative Histopathological analysis of the corresponding Oil Red O and Picrosirius Red stained sections. Because the presence of low viscosity lipid elicits rapid speckle fluctuations, we observed an inverse correlation between \( \tau \) measured by ILSI and lipid content (\( R = -0.64, p < 0.05 \)). In contrast, the higher viscoelastic modulus of fibrous regions resulted in a positive correlation between \( \tau \) and collagen content (\( R = 0.54, p < 0.05 \)). These results demonstrate the feasibility of conducting ILSI evaluation of arterial mechanical properties using a miniaturized omni-directional catheter.

Arterial stiffness (AS) is a recognized predictor of cardiovascular risk and mortality, and a potential marker for monitoring the beneficial effects of medical treatments for arterial diseases. AS is typically measured indirectly, by assessing the speed of travel of pressure waves in the aorta and other arteries, which is called pulse wave velocity (PWV). PWV is generally measured using contact piezoelectric transducers, or via a complex ultrasound technique. In the project “NISTAS” (Non-invasive screening of the status of the vascular system), funded by the European Commission, we measure the PWV using a contactless laser method based on laser triangulation. The measurement principle consists in the detection of the small (around 100 \( \mu m \)) displacement of the neck skin, induced by the transit of the pressure wave in the carotid. By measuring the displacement caused by the pulse wave in two distinct points along the carotid, the PWV can be retrieved. The chosen technique for the skin displacement measurement is laser triangulation in its 2D variant, which is robust and can be implemented using low-cost optical components. Two light lines generated by blue LEDs are projected on the target, and the skin displacement is measured using a CCD sensor. The measurement of the PWV is performed by calculating the time delay of the pulse waves measured in the two points, through a suited cross-correlation technique. We will present results of PWV measurement on different volunteers patients, showing the effectiveness of the technique.

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Feasibility of linear-array based co-registered photoacoustic and high frequency multi-modal ultrasound for non-invasive carotid imaging

Carotid artery disease also called carotid artery stenosis is a leading cause of stroke and an indication for other symptoms and diseases, including...
coronary artery disease (CAD). Photonic acoustic (PA) imaging provides an integrated platform for structural and functional imaging by combining high contrast and spectroscopic based specificity of optical imaging with high spatial resolution of ultrasound (US) imaging. In this study we demonstrate the feasibility of co-registered PA and high frequency multi-modal US, based on multi-element linear-array transducer combined with multichannel collecting system, for non-invasive imaging of human carotid artery. An integrated PA and multi-modal US system was used for imaging. The system consisted of high frequency linear-array transducer (256 elements) coupled to a tuneable Nd:YAG laser (680-970 nm) with a 20 Hz pulse repetition rate and 5 ns pulse width. The frame rate was 5 Hz. The signal from the carotid artery region was acoustically coupled to the transducer probe head and successive multi-spectral PA and multi-modal US scans (conventional ultrasound, power Doppler ultrasound and duplex ultrasound) were acquired. Co-registered PA and US images of the carotid artery were obtained. The high optical contrast co-registered with high resolution US imaging allows real-time in vivo imaging of carotid artery with detailed anatomical analysis. The multi-linear array based co-registered high frequency PA and multi-modal US imaging has been found promising in terms of non-invasiveness, high spatial and temporal resolution at sufficient depths, sensitivity, adaptability and imaging speed for the carotid artery imaging.

9689-128, Session PSun

Optical evaluation of myocardial infarction and its treatment effects using PS-OCT, fluorescence mapping and SHG microscopy

Yong-Guk Kang, Korea Univ. College of Health Sciences (Korea, Republic of); Mirim Kim, Korea Univ. College of Medicine (Korea, Republic of); Min-Gyu Hyeon, Korea Univ. (Korea, Republic of); Yong-Doo Park, Korea Univ. College of Medicine (Korea, Republic of); Beop-Min Kim, Korea Univ. College of Health Sciences (Korea, Republic of) and Korea Univ. (Korea, Republic of)

We investigated the treatment effect of several drugs for chronic myocardial infarction (CMI) using multi-modal optical methods. First, we observed action potential propagation of retrograde-perfused CMI model of rat heart with ratiometric optical mapping. Using this method, both normal action potential and fibrillation in the left ventricle wall were successfully observed, and conduction velocities were also calculated. Second, birefringence of ventricular walls of intact and infarcted myocardial tissues was evaluated using polarization-sensitive, swept-source optical coherence tomography (CP OCT) and non-linear microscopy based on second harmonic generation and two-photon-excited fluorescence to assess collagen and elastin fibers in the development of the atherosclerotic plaque. The study shows potential of CP OCT for the assessment of collagen and elastin fibers condition in atherosclerotic arteries. It is shown that high coherent cross-scattering indicates the predominance of high content of thick and organized collagen fibers of fibrous plaques. The typical heterogeneous cross-scattering in the form of “bright spots”, with the prevalence of the disorganized fibrous structures in inflammation, indicated an increase in the vulnerability of the plaque. Specifically, the additional information afforded by CP OCT, related to birefringence and cross-scattering properties of arterial tissues, may improve the robustness and accuracy of assessment about the microstructure and composition of the plaque for different stages of atherosclerosis. However it can also create a difficulty with the image interpretation, because backscattering contrast may have a similar appearance to the birefringence related fringes. They can be separated using Pythagorean sum of the channel, there only backscattering-related contrast sustains.

9689-129, Session PSun

Characterization of atherosclerotic plaques by cross-polarization optical coherence tomography

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Development of high resolution imaging technologies opened new possibilities for studying spatial organization of tissues in different pathologies. Inflammation, typical for atherosclerosis, disorganises collagen and elastin fibers, which, in turn, affects polarization light propagating through the tissue and can be used to characterize the atherosclerotic plaques. We combined cross-polarization optical coherence tomography (CP OCT) and non-linear microscopy based on second harmonic generation and two-photon-excited fluorescence to assess collagen and elastin fibers in the development of the atherosclerotic plaque. The study shows potential of CP OCT for the assessment of collagen and elastin fibers condition in atherosclerotic arteries. It is shown that high coherent cross-scattering indicates the predominance of high content of thick and organized collagen fibers of fibrous plaques. The typical heterogeneous cross-scattering in the form of “bright spots”, with the prevalence of the disorganized fibrous structures in inflammation, indicated an increase in the vulnerability of the plaque. Specifically, the additional information afforded by CP OCT, related to birefringence and cross-scattering properties of arterial tissues, may improve the robustness and accuracy of assessment about the microstructure and composition of the plaque for different stages of atherosclerosis. However it can also create a difficulty with the image interpretation, because backscattering contrast may have a similar appearance to the birefringence related fringes. They can be separated using Pythagorean sum of the channel, there only backscattering-related contrast sustains.
Quantitative imaging of lipid volume fraction in atherosclerotic plaque phantom under saline by a multispectral angioscope at wavelengths around 1200 nm

Katsunori Ishii, Daichi Matsui, Kunio Awazu, Osaka Univ. (Japan)

A method that provides objective and quantitative evaluation about stability of atherosclerotic plaques is required for diagnosis and treatment of atherosclerosis. Several spectroscopic techniques have been researched for intravascular diagnostic imaging of plaques. Near-infrared (NIR) light efficiently penetrates biological tissues, and the NIR region contains the characteristic absorption range of lipid that is one of the important factor to analyze stability of plaques. In this study, we focused on NIR multispectral imaging (NIR-MSI). The NIR-MSI can obtain the spatial and spectroscopic information of plaques simultaneously by combining with an angioscopy. The objective of this study is the development of NIR multispectral angioscope using at two or three wavelengths around 1200 nm. In order to evaluate the developed angioscope, atherosclerotic plaque phantoms with various lipid volume fractions were measured. Data were obtained in saline to simulate an angioscopic environment. Multispectral images of the phantom were constructed using a spectral angle mapper algorithm. As a result, the simulated plaque area that could not be detected with the angioscopic visible image was emphatically observed under 11 mm saline layer. In addition, the lipid volume fractions up to 10 vol.% in atherosclerotic plaque phantoms were quantitatively evaluated. A multispectral angioscope at wavelengths around 1200 nm have a potential to identify atherosclerotic plaques in saline infusion.
Three-photon imaging of ovarian cancer (Invited Paper)

Jennifer K. Barton, The Univ. of Arizona (United States); Elizabeth J. Swan, College of Optical Sciences, The Univ. of Arizona (United States); B. Amirsolaimani, Photini Faith Rice, The Univ. of Arizona (United States); Khanh Q. Kieu, College of Optical Sciences, The Univ. of Arizona (United States)

Optical imaging methods have the potential to detect ovarian cancer at an early, curable stage. Optical imaging has the disadvantage that high-resolution techniques require access to the tissue of interest, but miniature endoscopes that traverse the natural orifice of the reproductive tract, or access the ovaries and fallopian tubes through a small incision in the vagina, can provide a minimally-invasive solution.

We have imaged both rodent and human ovaries and fallopian tubes with a variety of endoscope-compatible modalities. The recent development of fiber-coupled femtosecond lasers will enable endoscopic multiphoton microscopy (MPM). We demonstrated two- and three-photon excited fluorescence (2PEF, 3PEF), and second- and third-harmonic generation microscopy (SHG, THG) in human ovarian and fallopian tube tissue. A study was undertaken to understand the mechanisms of contrast in these images. Six patients (normal and ovarian adenocarcinoma) provided ovarian and fallopian tube biopsies. The tissue was imaged with three-dimensional optical coherence tomography, multiphoton microscopy, and frozen for histological sectioning. Tissue sections were stained with hematoxylin and eosin, Masson's trichrome, and Sudan black. 2PEF yielded ~1 µm resolution images dominated by collagen and lipofuscin fluorescence. SHG signal was mainly from collagen. 3PEF and THG signal came from a variety of sources, including a strong signal from fatty connective tissue and red blood cells. Cancer was characterized by loss of signal intensity and disorder of the tissue structure. There was limited overlap of two- and three-photon signals, suggesting that three-photon imaging can provide additional information for early diagnosis of ovarian cancer.

Improved selection of cortical ovarian strips for autotransplantation of ovarian tissue using full-field optical coherence tomography (FFOCT)

Paulien L. Stegehuis, Inge T. Peters, Tjalling Bosse, Peter J. K. Kuppen, Baptist J. Trimbos, Boudewijn P. F. Lelieveldt, Cornelis J. H. van de Velde, Leiden Univ. Medical Ctr. (Netherlands); Carina G. J. M. Hilders, Erasmus MC (Netherlands) and Reinier de Graaf Hospital (Netherlands); Jouke Dijkstra, Alexander L. Vahrmeijer M.D., Leiden Univ. Medical Ctr. (Netherlands)

Premature ovarian failure is a major concern in women of reproductive age who undergo gonadotoxic cancer treatment. Autotransplantation of frozen-thawed cortical ovarian tissue allows the immediate start of cancer treatment, but risks re-introduction of cancer. Current tumor detection methods compromise the ovarian tissue's viability and can therefore only be used to exclude the presence of metastases in the cortical ovarian strips that are not transplanted. A non-invasive method is needed that can be used to exclude metastases in the actual ovarian autografts without affecting the tissue's viability. In this study we applied FFOCT – a non-fixative technique that uses white light interferometry to make high-resolution images (1um isotropic) of fresh tissue – to study healthy and malignant ovarian tissue. We created an image atlas of 13 healthy ovarian tissues from premenopausal patients and 19 ovarian tissues with metastases from several tumor types. To get the best possible match between hematoxylin-and-eosin stained slides and FFOCT images formalin-fixed paraffin-embedded tissue samples were deparaffinized and FFOCT images were acquired within a few minutes. FFOCT images were compared with histology images. All normal structures such as follicles in all phases, inclusion cysts, blood vessels, corpora lutea, and corpora albicantia were clearly recognizable. Ovarian metastases could be well distinguished from normal ovarian tissue. Currently we investigate the detection limit of FFOCT.

FFOCT is a promising technique in the field of fertility preservation: metastases can be detected and additionally cortical ovarian strips can be selected on the basis of high follicle density.

Functional optical coherence tomography for high-resolution mapping of cilia beat frequency in mouse oviduct in vivo

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The oviduct cilia beat periodically to support mammalian reproductive process, such as fertilization and oocytes transportation. Since mouse is a superior model for mammalian reproduction, studying ciliary behavior in the mouse oviduct has significant implications on further understanding and treatment of female infertility. The relation between ciliary activities and the dynamic reproductive processes currently remains unclear, largely owing to the lack of live imaging approaches to assess the oviduct ciliary behavior. Here, we report in vivo micro-scale mapping of cilia location and cilia beat frequency (CBF) in the intact mouse oviduct using optical coherence tomography (OCT). Our functional imaging technique is based on spectral analysis of the OCT speckle variations produced by the beat of cilia in the oviduct, which does not require exogenous contrast agents. Surgical procedures similar to the ones used for production of transgenic mice are utilized to expose the reproductive organs for imaging in anesthetized females. Validation of this approach for cilia location imaging and CBF measurement was performed through comparative analysis with traditionally-used immunofluorescence staining and bright-field microscopy, respectively. For the feasibility demonstration, in vivo CBF mapping in mouse oviduct was performed at different anatomical locations through different post-conception stages. Our results indicate this imaging approach could be a useful tool for a variety of live investigations of mammalian reproduction and infertility.

Cervical Intraepithelial Neoplasia treatment: a non-invasive translational technology

Natalia M. Inada, Fernanda M. Carbinatto, Univ. de São Paulo
Polarimetry for margin assessment of breast cancer

Adam Gribble, Univ. of Toronto (Canada) and Ontario Cancer Institute (Canada); Manuela Ventura, Univ. Health Network (Canada); Milan Ganguly, STTARR Innovation Ctr. (Canada); Alessandra Tata, Emma Bluemke, Univ. Health Network (Canada); Arash Zarrine-Afsar, Univ. Health Network (Canada) and Univ. of Toronto (Canada) and St. Michael’s Hospital (Canada); Alex Vitkin, Univ. of Toronto (Canada) and Ontario Cancer Institute (Canada)

Intraoperative pathology assessment for breast cancer patients is imperative to determine that the entire tumour has been removed. If tumour margins are ill-defined and there is residual cancer following surgery (incomplete resection), then there is an increased risk of recurrence. Furthermore patients commonly await results under general anesthesia. Thus, there is an unmet clinical need to improve intraoperative histology by developing faster and more sensitive techniques to discern cancerous tissues from healthy ones. Mueller matrix polarimetry, a type of polarized light imaging, is a fast, label-free, optical technique that can distinguish between different tissue types based on their interactions with polarized light. Here we demonstrate that Mueller matrix polarimetry is capable of revealing tumour margins in breast cancer. Human breast cancer cells were first injected into the quadriceps muscle of mice. Following tumour growth, sections of tissue containing the tumour and a margin of healthy surrounding tissue were excised and sliced for analysis with polarimetry and histology. Mueller matrix polarimetry imaging was followed by polar decomposition to measure the depolarization of the tissue samples. We found that depolarization is reduced in cancerous regions compared to the surrounding healthy muscle tissue. The tumour margins measured with polarimetry were confirmed with histology. Thus, the use of polarimetry to measure cancer margins may have potential to improve the speed and efficacy of intraoperative pathology assessment, either through direct measurement or as rapid guidance for more sensitive analysis techniques (such as mass spectrometry imaging).
Towards intra-operative visualization of breast tumour margins using low-cost epi-fluorescence endomicroscopy

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Breast conserving surgery (BCS) is often the treatment of choice for patients with early-stage breast cancer, as it allows for complete tumour excision while still maintaining acceptable cosmesis. However, 20-30% of BCS patients require one or more re-operative interventions to obtain oncologically clear resection margins. It may be possible to reduce the re-occurrence rates by using novel intraoperative imaging tools that provide a high degree of sensitivity and specificity for margin detection during BCS.

Epifluorescence endomicroscopy (also known as high resolution microendoscopy) is a low cost, high resolution imaging technique that provides in-vivo histology of tissue in real-time. In this paper we investigate the feasibility of using epifluorescence endomicroscopy for visualization of tissue morphology during breast surgery. Imaging is performed on 10 freshly excised and topically stained human breast tissue specimens and image mosaics are analyzed to assess the visual distinction between neoplastic and non-neoplastic samples. We show that the characteristic cellular and architectural features of the breast tissue can be visualized in real-time using endomicroscopy imaging. The findings are correlated with standard histology and the possibility of using epifluoresence endomicroscopy as an affordable intra-operative tool for real-time visualization and assessment of breast tumour margins is discussed.

Dispersion analysis of collagen fiber networks in cervical tissue using optical coherence tomography

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Understanding the human cervical collagen fiber network is critical to delineating the physiology of cervical remodeling during pregnancy. Previously, we presented our methodology to study the ultrastructure of collagen fibers over an entire field of transverse slices of human cervix tissue using optical coherence tomography. Here, we present a pixel-wise fiber orientation method to enable dispersion analysis on entire slices of human cervical tissues.

We obtained en face images that were parallel to the surface. In each en face image, we masked the collagen fiber region based on signal noise ratio. Then, we extracted fiber orientations in each pixel using a weighted summation scheme and generated a pixel-wise directionality map within the entire region. The weight was determined by intensity variations between a pixel of interest and its neighboring pixels and their corresponding distances. We divided the directionality map into regions of 400°m x 400°m along radial direction in all four quadrants. In each region, we fit von-Mises distribution to fiber orientations of pixels with mode θ and dispersion b. We compared dispersions among regions and samples. Using IRB approved protocols, we obtained whole transverse slices of cervical tissue from pregnant (n = 2) and non-pregnant (n = 13) women. We observed higher dispersion in pregnant samples compared to non-pregnant samples and higher dispersions in patient’s right/left zones than posterior/ anterior zones within an axial slice. Future studies will analyze how collagen fiber dispersion patterns change from the internal to the external os.

Visualization of tumor vascular reactivity in response to respiratory challenges by optical coherence tomography

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We previously reported the potential of using vascular reactivity during respiratory challenges as a marker to predict the response of breast tumor to chemotherapy in a rat model by using a continuous wave near-infrared spectroscopy. However, it cannot visualize how the vascular reactivity from tumor vessel can predict the tumor response to its treatment. In this study, we utilized a spectral domain optical coherence tomography (SD-OCT) system to visualize vascular reactivity of both tumor and normal vasculature during respiratory challenges in a mouse model. We adapted intensity based Doppler variance algorithm to draw angiogram from the ear of mouse (8-week-old Balb/c nu/nu). Animals were anesthetized using 1.5% isoflurane, and the body temperature was maintained by a heating pad. Inhalational gas was switched from air (10min) to 100% oxygen (10min) and a pulse oximeter was used to monitor arterial oxygen saturation and heart rate. OCT angiograms were acquired 5 min after the onset of each gas. The vasoconstriction effect of hyperoxic gas on vasculature was shown by subtracting an en-face image acquired during 100% oxygen from the image acquired during air inhalation. The quantitative change in the vessel diameter was measured from the en-face OCT images of the individual blood vessels. The percentage of blood vessel diameter reduction varied from 1% to 12% depending on arterial, capillary, or venous blood vessel. The vascular reactivity change during breast tumor progression and post chemotherapy will be monitored by OCT angiography.

Redox subpopulations and the risk of breast cancer progression

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It is established that a malignant tumor is akin to a complex organ comprising of various cell populations including tumor cells that are genetically, metabolically and functionally different. Our redox imaging data have demonstrated intra-tumor redox heterogeneity in all mouse xenografts derived from human melanomas, breast, prostate, and colon cancers. Based on the signals of NADH and oxidized flavoproteins and their ratio, i.e., the redox ratio, which is an indicator of mitochondrial metabolic status, we have discovered up to seven distinct redox subpopulations in xenograft breast tumors potentially recapitulating functional heterogeneity within the tumor. Furthermore, xenograft breast tumors with higher metastatic potential tend to have a redox subpopulation whose redox ratio is significantly different from that of tumors with lower metastatic potential and usually have a bi-modal distribution of the redox ratio. The redox subpopulations from human breast cancer samples can also be very complex. For example, the redox ratio of a breast cancer clinical specimen can have a very wide distribution that may be divided into 3 to 5 distinct redox states (tri- to penta-modal) as determined by fitting the redox ratio histograms with multi-Gaussian functions, although some tumors did have relatively homogeneous distribution of redox ratio. We observed that the larger the redox deviation is, the higher the metastatic risk is suggesting that the redox ratio difference may have potential prognostic value in both animal models and clinical breast cancer patients (N=29). We plan to further confirm and validate the hypothesis with a larger patient sample size.
High-throughput autofluorescence flow cytometry of breast cancer metabolism

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Tumor heterogeneity poses challenges for devising optimal treatment regimens for cancer patients. In particular, subpopulations of cells can escape treatment and cause relapse. There is a need for methods to characterize tumor heterogeneity of treatment response. Cell metabolism is altered in cancer (Warburg effect), and cells use the autofluorescent cofactor NADH in numerous metabolic reactions. Previous studies have shown that microscopy measurements of NADH autofluorescence are sensitive to treatment response in breast cancer, and these techniques typically assess hundreds of cells per group. An alternative approach is flow cytometry, which measures fluorescence on a single-cell level and is attractive for characterizing tumor heterogeneity because it achieves high-throughput analysis and cell sorting in millions of cells per group.

Current applications for flow cytometry rely on staining with fluorophores. This study characterizes flow cytometry measurements of NADH autofluorescence in breast cancer cells. Preliminary results indicate flow cytometry of NADH is sensitive to cyanide perturbation, which inhibits oxidative phosphorylation, in nonmalignant MCF10A cells. Additionally, flow cytometry is sensitive to higher NADH intensity for HER2-positive SKBr3 cells compared with triple-negative MDA-MB-231 cells. These results agree with previous microscopy studies. Finally, a mixture of SKBr3 and MDA-MB-231 cells were sorted into each cell type using NADH intensity.

Sorted cells were cultured, and microscopy validation showed the expected morphology for each cell type. Ultimately, flow cytometry could be applied to characterize tumor heterogeneity based on treatment response and sort cell subpopulations based on metabolic profile. These achievements could enable individualized treatment strategies and improved patient outcomes.

Using a reflectance-based correction on Cherenkov images to strengthen in vivo correlation with surface dose in whole-breast radiotherapy patients

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Purpose: The purpose of this study was to investigate a new method for improving the in vivo correlation between Cherenkov emission intensity and surface radiation dose. Superficial dosimetry through Cherenkov imaging could improve patient safety and aid in monitoring treatment response.

Methods: A silicone breast phantom was affixed to an anthropomorphic phantom of the torso. CT simulation was used to create two radiation treatment plans, one at 18MV and another at 6MV, composed of two opposing radiation beams tangent to the chest wall. A set of 10 thermoluminescent dosimeters were placed on the surface of the phantom to provide experimental measurements of surface dose. The phantom was aligned on the linac treatment couch, and three reflectance images were captured before irradiation using different illumination sources: i) LED flash ring around the camera lens, ii) LED flash ring with linear polarizing filter and camera lens with orthogonal linear polarizing filter, iii) ambient room lighting. Cherenkov images were then corrected following protocols while the radiation was delivered. Analysis of uncorrected versus the three different corrected images was conducted with post-processing in MATLAB.

Results: The orthogonally polarized source and detector(ii) removed surface glare that degraded correlation following reflectance correction without polarizers from the LED ring(i). The corrected images have a stronger correlation to surface dose calculated in the Eclipse treatment plan than the uncorrected Cherenkov images.

Conclusion: Pixel-by-pixel correction for clinical Cherenkov images of complex volumes causes increased correlation between Cherenkov emission intensity and surface radiation dose.

Diffuse optical tomography with structured-light patterns to quantify breast density

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Considering the cost of breast cancer screening, its frequency is an important choice for women for early diagnosis. The recent use of risk models has helped women choose when to begin screening and whether costly exams, such as MRI, are necessary. Breast density is an independent risk factor for breast cancer and can strengthen risk assessment. Current methods to quantify the volumetric percentage of dense, fibroglandular tissue within the breast use 2D mammographic images. Segmentation on 3D magnetic resonance images provides a more accurate quantification, but it prevents its widespread implementation. Optical-based techniques provide a suitable and affordable alternative.

We explored the projection and detection of various spatial light patterns (SLPs) for diffuse optical tomography of breast. For our FEM-based simulations, we generated realistic digital breast phantoms from MR images, including centralized and dispersed dense tissue morphologies. Measurements at five wavelengths enabled reconstruction of water, lipid, oxy- and deoxyhemoglobin chromophore maps. Fibroglandular tissue has been found to have a higher and lower amount of water and lipid, respectively, compared to adipose tissue. For our preliminary studies, information from the water and lipid maps was used to form a regression model to estimate the volumetric percentage of dense tissue. Meanwhile, preliminary phantom studies with a SLP-projector source and a CCD camera showed that we were able to recover inclusions. Currently, we are expanding our system to multiple wavelengths by replacing the CCD camera with digital micro-mirrors that focus SLPs into a photomultiplier tube, allowing us to image lipid and water.

Photoacoustic spectroscopy based investigatory approach to discriminate breast cancer from normal: a pilot study

Mallika Priya, Bola Sadashiva Satish Rao, Subhash Chandra, Satadru Ray, Stanley Mathew M.D., Anirbit Datta, Manipal Univ. (India); Subramanya G. Nayak, Manipal Univ. (India) and Manipal Institute of Technology (India); Krishna Kishore Mahato, Manipal Univ. (India)

In spite of many efforts for early detection of breast cancer, there is still lack of technology for immediate implementation. In the present study, we have attempted to evaluate the potential of photoacoustic spectroscopy in discriminating breast cancer from normal, involving blood serum samples seeking early detection. Three photoacoustic spectra in time domain were recorded from each of 20 normal and 20 malignant samples at 28nm pulsed laser excitations and a total of 120 spectra were generated. The time domain spectra were then Fast Fourier Transformed into frequency domain and 116.5625 - 206.875 kHz region was selected for further analysis using
a combinational approach of wavelet, PCA and logistic regression. Initially, wavelet analysis was performed on the FFT data and seven features (mean, median, area under the curve, variance, standard deviation, skewness and kurtosis) from each were extracted. Principal Component Analysis (PCA) was then performed on the feature matrix (7x120) for discriminating malignant samples from the normal by plotting a decision boundary using logistic regression analysis. The unsupervised mode of classification used in the present study yielded specificity and sensitivity values of 100% in each respectively with a ROC - AUC value of 1. The results obtained have clearly demonstrated the capability of photoacoustic spectroscopy in discriminating cancer from the normal, suggesting its possible clinical implications.

9689-500, Session HT

Imaging cellular heterogeneity in cancer
Melissa C. Skala, Vanderbilt Univ. (United States)

No Abstract Available

9689-134, Session PSun

Design of an everting balloon to deploy a microendoscope to the fallopian tubes
Molly Keenan, Caitlin Howard, Tyler Tate, The Univ. of Arizona (United States); John Black, Giannaventa, Inc. (United States); Urs Utzinger, Jennifer K. Barton, The Univ. of Arizona (United States)

The 5-year survival rate for ovarian cancer is only 45%, largely due to lack of effective screening methods. Current methods include palpation, transvaginal ultrasound, and the CA-125 blood test. Finding disease reliably and at an early stage increases 5-year survival to 92%. We have designed and built a 0.7 mm endoscope for the early detection of ovarian cancer. Inserted transvaginally through the working channel of a hysteroscope, the falloposcope creates a minimally invasive procedure for the screening of high risk women. To improve the ease-of-use and safety of falloposcope deployment, we have created a dual-chamber everting balloon. Currently, the falloposcope would require a skilled user to operate due to the challenging anatomy of the fallopian tubes – a small opening from the uterus (<1 mm), tortuous path, and delicate lumenal features. A balloon delivery system would gently open the fallopian tube and guide the falloposcope down the center of lumen. The novel two chamber design allows the user to deflate one side and place the endoscope in contact with the wall, taking advantage of its unique optical properties. We show balloon design and integration with the falloposcope prototype. We test possible mechanical damage to the tissue due to scraping, puncture, or overstretching. Successful introduction of the everting balloon to simplify falloposcope delivery could expand screening beyond specialized centers to smaller clinical locations.

9689-151, Session PSun

Large area 3-D optical coherence tomography imaging of lumpectomy specimens for radiation treatment planning
Cuihuan Wang, Rutgers, The State Univ. of New Jersey (United States); Leonard Kim, Atif Khan, Rutgers Cancer Institute of New Jersey (United States); Mark C. Pierce, Rutgers, The State Univ. of New Jersey (United States)

Our long term goal is to develop a high-resolution imaging method for comprehensive assessment of tissue removed during lumpectomy procedures. By identifying regions of high-grade disease within the excised specimen, this method may be used to develop patient-specific post-operative radiation treatment regimens.

We have assembled a benchtop spectral-domain optical coherence tomography (SD-OCT) system with 1320 nm center wavelength. The axial and lateral resolution were measured to be 6 microns and 16 microns, respectively. Automated XY beam scanning enables sub-volumes spanning 5 mm x 5 mm x 2 mm (500 A-lines x 500 B-scans) to be collected in under 15 seconds. A motorized sample positioning stage enables multiple sub-volumes to be acquired across an entire specimen. Sub-volumes are reconstructed from individual B-scans in 3D Slicer software and en face (XY) images are extracted from specific depths. These en face images are then tiled together using MosaicJ software to produce a large area en face view (up to 40 mm x 25 mm). After imaging, specimens were subsequently sectioned and stained with H&E, allowing comparison between OCT image features and established disease markers on histopathology.

We have reconstructed large area en face images of tissue microarchitecture in 20 unstained lumpectomy specimens using OCT. We are currently assessing the accuracy of tissue classification based on OCT image features, against histopathology as the gold standard. Future goals include developing image reconstruction algorithms for mapping an entire sample, and registering OCT image volumes with clinical CT and MRI images for post-operative treatment planning.

9689-152, Session PSun

Cervical collagen imaging for determining preterm labor risks using a colposcope with full mueller matrix capability
Susan Stoff, Jessica C. Ramella-Roman, Florida

Wide-field lifetime-based FRET imaging for the assessment of early functional distribution of transferrin-based delivery in breast tumor-bearing small animals
Nattawut Sinsuebphon, Rensselaer Polytechnic Institute (United States); Alena Rudkouskaya, Margarida Barroso, Albany Medical College (United States); Xavier Intes, Rensselaer Polytechnic Institute (United States)

Targeted drug delivery is a key concept for cancer therapy. Assessment of dynamic distribution of the drug provides relative concentration and bioavailability at the target tissue. The common approach of the assessment is intensity-based imaging which provides only anatomical distribution information and can be corrupted by heterogeneous tissue attenuation. Lifetime-based imaging enable to sense functional local state of tissues as well as mitigate intensity-based bias. Especially, lifetime-based Förster resonance energy transfer (FRET) enable to observe the interaction of biomolecules at the nanoscale. In this study, we used wide-field lifetime-based FRET imaging for the study of early functional distribution of transferrin delivery in breast tumor in small animals. Transferrin is a carrier for cancer drug delivery. Its interaction with the receptor is within a few nanometers which is suitable for FRET. Alexa Fluor® 700 and Alexa Fluor® 750 were conjugated to holo-transferrin which then were administered via tail vein to the mice implanted with T47D breast cancer xenograft. Images were continuously acquired for 60 minutes post injection. The results showed that transferrin was primarily distributed to the liver, the urinary bladder and the tumor. The cellular uptake of transferrin, which was indicated by the level of FRET, was high in the liver but very low in the urinary bladder, and in-between for the tumors. The results also suggested that the intensity and FRET were independent.
Conference 9689E: Diagnosis and Treatment of Diseases in the Breast and Reproductive System

International Univ. (United States); Amir Gandjbakhche, Viktor V. Chernomordik, National Institutes of Health (United States)

Preterm birth is a worldwide health issue, as the number one cause of infant mortality and neurological disorders. Although affecting nearly 10% of all births, an accurate, reliable diagnostic method for preterm birth has, yet, to be developed. The primary constituent of the cervix, collagen, provides the structural support and mechanical strength to maintain cervical closure, through specific organization, during fetal gestation. As pregnancy progresses, the disorganization of the cervical collagen occurs to allow eventual cervical pliability so a baby can be birthed through the cervical opening. This disorganization of collagen affects the mechanical properties of the cervix and, if the changes occur prematurely, may be a significant factor leading to preterm birth. The organization of collagen can be analyzed through the use of Mueller Matrix Polarimetric imaging of the characteristic birefringence of collagen. In this research, we have built a full Mueller Matrix Polarimetry attachment to a standard colposcope to enable imaging of human cervixes during standard prenatal exams at various stages of fetal gestation. Analysis of the polarimetric images provides information of quantity and organization of cervical collagen at specific gestational stages of pregnancy. This quantitative information may provide an indication of risk of preterm birth.

9689-153, Session PSun
Photodynamic therapy of Cervical Intraepithelial Neoplasia (CIN) high grade
Fernanda M. Carbinatto, Instituto de Física de São Carlos (Brazil); Natalia M. Inada, Univ. de São Paulo (Brazil); Wellington Lombardi, Eduardo V. da Silva, UNIARA (Brazil); Renata Belotto, Hospital Perola Byington (Brazil); Cristina Kurachi, Vanderlei Bagnato, Univ. de São Paulo (Brazil)

Cervical intraepithelial neoplasia (CIN) is the precursor of invasive cervical cancer and associated with human papillomavirus (HPV) infection. Photodynamic therapy (PDT) is a technique that has been used for the treatment of tumors. PDT is based on the accumulation of a photosensitizer in target cells that will generate cytotoxic reactive oxygen species upon illumination, inducing the death of abnormal tissue and PDT is a technique which induces less damaging to normal tissues than surgery, radiation, or chemotherapy and seems to be a promising alternative procedure for CIN treatment. The CIN high grades (II and III) presents potential indications for PDT due the success of PDT for CIN low grade treatment. The aim of the study was to evaluate efficacy of the new clinic protocol of PDT for treatment of CIN high grade using two session of PDT and a new design of tip treatment with LED 630 nm. The patient with CIN high grade that were treated with new clinic protocol shows lesion regression for CIN low grade 30 days after the treatment. The new clinical protocol using for treatment CIN high grade shows great potential to become a public health technique and chemical therapy within one single platform to fight against Her2-positive breast cancer. Gold nanorods (GNR) has been used as a promising photothermal therapeutic agent due to their high photothermal conversion efficiency. However, GNRs-assisted phototherapy in single-modality format is often thought to be insufficient to ablate tumor cells completely owing to energy loss via scattering and absorption during penetrating into the tissues, which often result in low therapeutic efficiency. Herein, we aimed to design a multi-modality therapeutic platform to overcome the drawbacks of single modality therapy. To this end, a layer of mesporous SiO2 was encapsulated onto the GNRs followed by conjugation of Kadcyla to the silica shell. Kadcyla is an antibody-drug conjugates (ADC). It consists of one molecule of chimeric monoclonal antibody Trastuzumab, that can target Her2 and disrupt Her2 signalling pathway, and several molecules of cytotoxic agent DM1 linked to the antibody, which enters cells and destroys them by binding to tubulin. Our results demonstrated that the so-designed nanodrug retained the properties of Trastuzumab. Drug therapy followed by photothermal therapy using NIR laser of 793nm specifically ablated Her2-positive breast cancer cells with high efficiency. Further in vivo work will be performed to more completely evaluate the performance of this agent. In addition, due to the excellent optical properties of GNR, the designed nanodrug can also be utilized for two photo imaging and photoacoustic imaging in live animals. Thus it holds great potential for multi-modality imaging guided therapy.

9689-157, Session PSun
The lavender procedure: how we do it
Phillip Bretz M.D., Richard Lynch, David Mantik, The Visionary Breast Ctr. (United States)

Breast cancer continues to take its toll on women around the world. An alternative to current methods of diagnosis and treatment needs to be researched to bring definitive care to women globally. Data on our first 1000 patients will be presented including a genetics test that not only predicts a woman’s risk for breast cancer but when that risk will likely manifest within ten years during her lifetime. A personalized surveillance program is then generated. Heterogeneity is the cause for treatment failure. Using non-radiation modalities including genomics, infrared with artificial intelligence and neural network, ultrasound, a pressure device and Halo we have the capability to ferret out ultra-small cancers (3-5mm). We can preempt heterogeneity and metastatic potential and kill the tumor in a 20 minute office procedure using liquid nitrogen probe. The patient resumes normal activity immediately without a single stitch being used. Preliminary evidence suggests an active anti-body specific response against the tumor as it is absorbed since we don’t remove the tumor. The Lavender Procedure can eliminate the need for surgery, chemotherapy and radiation.

9689-158, Session PSun
A fiber-delivered optoacoustic guide for precise breast-conserving surgery
Lu Lan, Purdue Univ. (United States); Kaiming Liu, Tsinghua Univ. (China); Rui Li, Pu Wang, Purdue Univ. (United States); Linda K. Han M.D., Indiana Univ. Melvin and Bren Simon Cancer Ctr. (United States); Ji-Xin Cheng, Purdue Univ. (United States)

Breast-conserving surgery (BCS) is a well-accepted breast cancer treatment. To prevent local cancer recurrence after BCS, sufficient margin of normal tissue surrounding the tumor must be obtained. Currently re-operation rate for BCS ranges from 20% to 70%. Lack of accurate real-time surgical guidance to locate the exact cancerous area and confirm expansion of sufficient margins is a critical factor for such a high re-operation rate. Current gold-standard of pre-operative wire-localization of the tumor site, in which a thin wire is inserted into the tumor mass center, can only provide a rough estimation of tumor location in surgery and does not provide...
the crucial information of tumor margins. Here, we demonstrate a fiber-delivered optoacoustic guide that can help improve the accuracy of BCS and reduce re-operation rates. The optoacoustic guide, having a diameter of 0.42 mm, is composed of one multimodal optical fiber with the proximal end coated by absorbers. By placing the coated end into the tumor mass center and coupling laser pulse from the distal end, an acoustic wave is generated and detected by transducer at the tissue surface, providing accurate spatial localization information of tumor. Our simulation and experiment results show that omnidirectional detection with a high dynamic range can be achieved. Our optoacoustic guide is easy to fabricate, cost-effective, and naturally compatible with a commercial ultrasound system currently used in BCS.

9689-159, Session PSun

**Morphologic 3D scanning of fallopian tubes to assist ovarian cancer diagnosis**

Wendy-Julie Madore, Étienne De Montigny, Andréanne Deschênes, Fouzi Benboujja, Mikael Leduc, Ecole Polytechnique de Montréal (Canada); Anne-Marie Mes-Masson, Diane Provencher M.D., Kurosh Rahimi M.D., Ctr. Hospitalier de l’Univ. de Montréal (Canada); Caroline Boudoux, Nicolas Godbout, Ecole Polytechnique de Montréal (Canada)

In women with a hereditary risk of ovarian cancer, prophylactic surgeries can be performed as a prevention strategy. For women with suspicious lesions, diagnosis follows debulking surgery. As the majority of high-grade serous ovarian cancers are now believed to originate in the fallopian tubes, pathological examination should include a thorough examination of the excised ovaries and oviducts. Within the tubes, intraepithelial lesions can be smaller than a few hundred micrometers, and can be missed on routine pathological evaluation. To circumvent this limitation, we present an imaging system allowing for ex vivo diagnostic exploration of the oviducts. This system is based on optical coherence tomography (OCT), a laser imaging modality giving access to sub-epithelial tissue architecture. This system produces cross-sectional images of 1 to 3 mm in depth, with a lateral resolution of \( \approx 20 \mu m \) and an axial resolution of \( \approx 15 \mu m \). An single fiber probe was developed to fit in a human oviduct. This 2 mm probe produces 3D volume data of the entire inner tube within a few minutes.

To demonstrate the clinical potential of OCT, we studied 5 different tubal lesions. We imaged 52 paraffin-embedded human surgical biopsies with a benchtop system and compared these images with histology. We also imaged healthy oviducts from 3 animal models to find one resembling the human anatomy and to develop a functional ex vivo imaging procedure with in which we image ex vivo fallopian tubes with the probe.

9689-160, Session PSun

**Spectroscopic imaging system for high-throughput viability assessment of ovarian microtumors in a microfluidic system**

Amélie St-Georges-Robillard, Mathieu Masse, Ecole Polytechnique de Montréal (Canada) and Ctr. Hospitalier de l’Univ. de Montréal Research Ctr. (Canada); Jennifer Kendall-Dupont, Ctr. Hospitalier de l’Univ. de Montréal Research Ctr. (Canada) and Institut du Cancer de Montréal (Canada); Mathias Strupler, Ecole Polytechnique de Montréal (Canada); Bishnubrata Patra, Polytechnique Montreal (Canada) and Centre Hospitalier de l’Universite de Montreal Research Center (Canada) and Institut du cancer de Montreal (Canada); Michael Jermyn, McGill Univ. (Canada) and Ecole Polytechnique de Montréal (Canada); Anne-Marie Mes-Masson, Ctr. Hospitalier de l’Univ. de Montréal Research Ctr. (Canada) and Institut du Cancer de Montréal (Canada) and Univ. de Montréal (Canada); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada) and Ctr. Hospitalier de l’Univ. de Montréal Research Ctr. (Canada); Thomas Gervais, Ecole Polytechnique de Montréal (Canada) and Ctr. Hospitalier de l’Univ. de Montréal Research Ctr. (Canada) and Institut du cancer de Montreal (Canada)

There is a growing effort in the biomicrosystems community to develop a personalized treatment response assay for cancer patients using primary cells, patient-derived spheroids, or live tissues on-chip. Recently, our group has developed a technique to cut tumors in 350 \( \mu m \) diameter microtissues and keep them alive on-chip, enabling multiplexed in vitro drug assays on primary tumor tissue. Two-photon microscopy, confocal microscopy and flow cytometry are the current standard to assay tissue chemosensitivity on-chip. While these techniques provide microscopic and molecular information, they are not adapted for high-throughput analysis of microtumors.

We present a spectroscopic imaging system that allows rapid quantitative measurements of multiple fluorescent viability markers simultaneously by using a liquid crystal tunable filter to record fluorescence and transmittance spectra. As a proof of concept, 16 microtissues composed of ovarian cancer cell line TOV112D were trapped in a microfluidic system, stained with a live cell marker (CellTrackerTM Green or Orange) and a dead cell marker (propidium iodide), and imaged. System response, background noise and autofluorescence were removed. Fluorescence intensity was normalized by the white-light spectrum at excitation to isolate the markers’ intrinsic fluorescence from tissue absorption and scattering. Spectral un-mixing was applied to separate each fluorophore’s contribution. We have demonstrated that rapid and simultaneous viability measurements on multiple microtissues can be achieved, which will have a significant impact to predict a tumor’s response to multiple treatment options as well as in drug discovery to assess the potential of a drug candidate directly on human primary tissue.
9689-162, Session 1

**Determining early markers of disease using Raman spectroscopy in a rat combat-trauma model of heterotopic ossification**

Katherine E. Cilwa, Naval Medical Research Ctr. (United States) and The Henry M. Jackson Foundation for the Advancement of Military Medicine (United States); Jonathan A. Forsberg, Naval Medical Research Ctr. (United States) and Uniformed Services Univ. of the Health Sciences (United States) and Walter Reed National Military Medical Ctr. (United States); Nicole J. Crane, Naval Medical Research Ctr. (United States) and The Henry M. Jackson Foundation for the Advancement of Military Medicine (United States)

Heterotopic ossification (HO) is the pathological formation of bone in soft tissue and is a debilitating sequela of burns and traumatic injuries. Over 60% combat related injuries and severe burns develop HO; sometimes resulting in reduced mobility, chronic pain, ulceration, tissue entrapment, and reduced ambulation. Detection and prognosis is limited by current clinical imaging modalities (computed tomography, radiography, and ultrasound). We report the use of Raman spectroscopy as a non-invasive, non-destructive method for detection of pre-HO tissue in a rat combat-trauma model of HO at early time points post injury. HO was induced in Sprague-Dawley rats via blast over pressure injury, mid-femoral fracture, 60 second soft tissue crush injury, and finally amputation through the zone of injury. Sham animals were not subjected to injury. Rats were sacrificed and immediately examined via Raman spectroscopy. Spectra were collected at the amputation site using a fiberoptic probe with 6 mm spot size and 830 nm excitation. Early HO and pre-HO tissue exhibited changes in Raman spectra indicative of apatitic mineral formation and collagen deposition, respectively. Tissue changes were confirmed via histology and compared with microCT results. This study demonstrates the non-invasive ability of Raman spectroscopy to detect changes in tissue associated with early HO development before mineralization events necessary to diagnose via current clinical imaging modalities have occurred.

9689-163, Session 1

**Photoacoustic imaging of inflammatory arthritis in human joints**

Janggun Jo, Guan Xu, April Marquardt, Sheeja Francis, Univ. of Michigan (United States); Jie Yuan, Nanjing Univ. (China); Dhanuj Girish, Huron High School (United States); Gandikota Girish, Xueding Wang, Univ. of Michigan (United States)

With the capability of assessing highly sensitive optical information in human soft tissues at imaging depth up to several centimeters, innovative biomedical photoacoustic imaging (PAI) offers potential benefits in diagnosis and treatment monitoring of inflammatory arthritis. PAI system combined with US has been developed for facilitating two imaging functions simultaneously in real-time fashion. The dual-modality system has been adapted to imaging of active synovitis in metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints of arthritis patients. Photoacoustic (PA) images of the joints were acquired at 580-nm laser wavelength providing a desired balance between the optical contrast of hemoglobin over bone cortex and the imaging depth. When working with multiple wavelengths, this system is also capable of displaying blood oxygen saturation in patients' joints. Our initial results from arthritis patients and normal volunteers have demonstrated the feasibility of PAI in identifying the enhanced flow as a result of joint inflammation based on the endogenous optical contrast in the synovial tissues. At the current stage, the resolution and sensitivity of PAI for detecting joint inflammation are comparable to the established US Doppler imaging.

9689-164, Session 1

**Photoacoustic 3D imaging in bone assessment (Invited Paper)**

Ting Feng, Univ. of Michigan Medical School (United States) and Nanjing Univ. (China); Ken Kozloff, Joseph Perosky, Univ. of Michigan (United States); Sidan Du, Jie Yuan, Nanjing Univ. (China); Cheri Deng, Xueding Wang, Univ. of Michigan (United States)

Osteoporosis is a progressive bone disease that is characterized by both bone quantity and microstructure. Photoacoustic (PA) imaging and sensing techniques evaluate bone based on highly sensitive optical contrast which holds distinctive advantages in evaluating the bone health. In previous work, the capability of PA techniques for bone evaluation has been demonstrated on small-animal models. However, before translating the techniques to clinical management of osteoporosis, the feasibility of PA imaging and sensing in dealing with human bones with relatively larger sizes still needs to be investigated. In this study, we first quantitatively evaluated the optical and ultrasound penetration in large bovine bones. Next, we investigated the feasibility in generating 3D PA images of bones including both cortical and trabecular parts. We have also characterized the porous trabecular bones by using our recently developed PA spectrum analysis (PASA) method. The results show that the bone after demineralization has thinner trabeculae which can be quantitatively evaluated by the spectral parameter slope. PASA results were validated by the gold standard microCT. The findings from this study demonstrate that PA techniques can offer sufficient penetration depth for clinical assessment of bone health. Both bone mineral density (BMD) and bone microstructure, the two key parameters determining the bone strength, can be objectively assessed. Considering that PA measurement is non-ionizing and non-invasive, these new techniques hold promise for clinical translation.

9689-165, Session 1

**A portable cross-shape near-infrared spectroscopic detector for bone marrow lesions diagnosis**

Yu Su, Ting Li, Yunlong Sun, Kai Li, Yuan Gao, Univ. of Electronic Science and Technology of China (China)

Bone marrow lesions (BMLs) is a incidence-increasing disease which seriously hazard to human health and possibly contribute to paralysis. Delayed treatment often occurred to BMLs patients due to its characteristics such as complex and diverse clinical manifestations, non-specific, easy to misdiagnosis and etc. The conventional diagnosis methods of BMLs mainly rely on bone marrow biopsy/aspiration, which are invasive, painful, high health risk, and discontinuous which disabled monitoring and during-surgery guidance. Thus we proposed to develop a noninvasive, real-time, continuous measurement, easy-operated device aimed at detecting bone marrow diseases. This device is based on near-infrared spectroscopy and
the probe is designed with a cross-shape to tightly and comfortably attach human spine. Space-resolved source-detector placement and measurement algorithm are employed. Four selected wavelength were utilized here to extract BMLs-related component contents of oxy-, deoxy- hemoglobin, fat, scattering index corresponding to fibrosis. We carried out a phantom experiment, in-vivo physiological tests, and one clinical measurement to verify the feasibility of our device. The potential of NIRS in BMLs clinics is revealed.

9689-166, Session 1

**Optical diagnostics of osteoblast cells and osteogenic drug screening**

Deepak K. Khajuria, Elayaraja Kolanti, Sarath C. Veerla, D. Roy Mahapatra, Indian Institute of Science (India)

Microfluidic device based diagnostics involving optical fibre path, in situ imaging and spectroscopy are gaining importance due to recent advances in diagnostics instrumentation and methods, besides other factors such as low amount of reagent required for analysis, short investigation times, and potential possibilities to replace animal model based study in near future. It is possible to grow and monitor tissues in vitro in microfluidic lab-on-chip. It may become a transformative way of studying how cells interact with drugs, pathogens and biomaterials in physiologically relevant microenvironments. To a large extent, progress in developing clinically viable solutions has been constrained because of (i) contradiction between in vitro and in vivo results and (ii) animal model based and clinical studies which is very expensive. Our study here aims to evaluate the usefulness of microfluidic device based 3D tissue growth and monitoring approach to better emulate physiologically and clinically relevant microenvironments in comparison to conventional in vitro 2D culture. Moreover, the microfluidic methodology permits precise high-throughput investigations through real-time imaging while using very small amounts of reagents and cells. In the present study, we report on the details of an osteoblast cell based 3D microfluidic platform which we employ for osteogenic drug screening. The drug formulation is functionalized with fluorescence and other biomarkers for imaging and spectroscopy, respectively. Optical fibre coupled paths are used to obtain insight regarding the role stress/flow pressure fluctuation and nanoparticle-drug concentration on the osteoblast growth and osteogenic properties of bone.

9689-167, Session 1

**Fourth NIR optical window for assessment of bone abnormalities and other diseases**

Diana C. Sordillo, Laura A. Sordillo, Peter P. Sordillo M.D., Robert R. Alfano, Institute for Ultrafast Spectroscopy and Lasers (United States)

Due to the wavelength dependence of the scattering coefficient (reduction in photon scattering) at longer near-infrared (NIR) wavelengths and minimal absorption, there exists a new fourth NIR optical window from 2100 nm to 2300 nm between major peaks of water absorption. Water molecules, hemoglobin, and deoxy-hemoglobin, in particular, greatly affect image quality and penetration depth. Recently, NIR optical windows other than the first or therapeutic NIR optical window (from 650 nm to 950 nm), which are located from 1100 nm to 1350 nm (window II) and from 1600 nm to 1870 nm (window III), between water peak maxima at approximately 1400 nm and 1900 nm, were utilized to penetrate more deeply through turbid media and to image abnormalities hidden beneath thick tissue. Wavelengths of light in the fourth NIR optical window may offer similar penetration depth benefits as the first three NIR optical windows. In this report, tissue optical properties of normal and diseased tissues, of bone abnormalities, as well as of intralipid solution, which serves as a tissue phantom, in the fourth NIR optical window are presented. Optical properties of biological tissues can be described in terms of their absorption and scattering properties using Lambert-Beer's equation. These properties are wavelength dependent and can affect the amount of light which can penetrate tissue.

9689-168, Session 2

**In-situ photopolymerized and monitored implants: successful application to an intervertebral disc replacement**

Andreas Schmocker, Christophe Moser, Azadeh Khoushabi, Pierre-Etienne Bourbon, Dominique Pioletti, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Photopolymerization is a common tool to harden materials initially in a liquid state. A surgeon can directly trigger the solidification of a dental implant or a bone or tissue filler. Traditionally, photopolymerization has been used mainly in dentistry. Over the last decade advances in material development including a wide range of biocompatible gel- and cement-systems open up a new avenue for in-situ photopolymerization. We designed a miniaturized light probe where a photoactive material can be 1) mixed, pressurized and injected 2) photopolymerized or photoactivated and 3) monitored during the chemical reaction. The device enables surgeries to be conducted through a hole smaller than 500 mm in diameter. Using a combination of Raman and fluorescence spectroscopy, the current state of the photopolymerization was inferred and monitored in real time within an in-vitro tissue model. It was also possible to determine roughly the position of the probe within the tissue cavity by analyzing the fluorescence signal. Using the technique hydrogels were successfully implanted into a bovine intervertebral disc model. Mechanical tests in a biomechanical test platform (0.5 million loading cycles) could not obstruct the functionality of the implant. Finally, the device was also used for other application such as the implantation of a hydrogel into an aneurysm tissue cavity which will be presented at the conference.

9689-173, Session 2

**Noninvasive assessment of fracture healing using spatially offset Raman spectroscopy (Invited Paper)**

Hao Ding, Guijin Lu, Christopher West, Gloria Gogola, James Kellam, Catherine Ambrose, Xiaohong Bi, The Univ. of Texas Health Science Ctr. at Houston (United States)

Fracture non-unions and bone re-fracture are the most common challenges for post-fracture management. To achieve better prognosis and treatment evaluation, it is important to be able to assess the quality of the healing bone tissue, especially over the time course of healing. The goal of this study is to investigate the potential of Raman spectroscopy in assessing callus quality non-destructively and predicting resistance to re-fracture by exploring the correlation between material and mechanical properties at the calluses. Spatially offset Raman spectroscopy was exploited to study the fracture healing process. Fractures were created in vivo in one femur of rats and fixed with an intramedullary k-wire, while the contralateral femur served as an un-fractured control. After euthanasia at two or four weeks post-fracture, Raman spectra were collected from the deimplanted thighs at both sides transcutaneously in situ with various source/detection offsets. Bone signals were recovered from each spectrum that contains contribution from atop soft tissue and buried bone, and then compared with those collected from bare bones. The relative intensity of mineral from fractured bone was markedly decreased compared to the control. The fractured bones demonstrated lower mineral and carbonate level and higher collagen content in the callus at both time points. At four weeks, the carbonation from fractured bone was not significantly different from the control, indicating improved bone quality with time. The results from Raman
Simultaneous fluorescence-Raman imaging tools for in vivo investigation of biomineralization processes (Invited Paper)

Admir Masic, Max-Planck-Institut für Kolloid- und Grenzflächenforschung (Germany)

No Abstract Available

Supercontinuum ballistic imaging of bone using the fourth NIR optical window (Invited Paper)

Laura A. Sordillo, The City College of New York (United States) and Institute for Ultrafast Spectroscopy and Lasers (United States); Diana C. Sordillo, Lingyan Shi, Peter P. Sordillo M.D., Robert R. Alfano, Institute for Ultrafast Spectroscopy and Lasers (United States)

In this study, we investigated a new near-infrared (NIR) optical window, a fourth NIR optical window located from 2100 nm to 2300 nm, using a supercontinuum (SC) laser light source for imaging abnormalities hidden beneath tissue. Due to the wavelength dependence of the scattering coefficient (reduction in photon scattering) at longer NIR wavelengths and minimal absorption, the fourth NIR optical window may provide deeper penetration through tissue than with wavelengths of light in the conventional therapeutic or first NIR optical window. A SC laser has a spectral range from 700 nm to 2,500 nm and can provide wavelengths of light in the fourth NIR optical window with between 200- 500 microwatt/nm power, can provide a greater number of outgoing photons than conventional lamp light sources, and can highlight its ballistic photons. Ballistic photons, unlike snake or diffusive photons which are scattered or absorbed, pass through turbid media uninterrupted, and carry valuable information to the detector about the objects they encounter along their path. These photons of light are ideal for reaching greater penetration depths through tissue. Using the SC laser and IR-CCD InGaAs camera detector (Goodrich Sensors Inc. camera SU320KT5W-1.7RT with spectral response highlighting the second and third optical windows) and IR-CCD InSb detector, images of bone abnormalities with overlying tissue of various thicknesses were studied in the second, third and fourth NIR optical windows. Optical properties, absorption and scattering coefficients from bone and other tissues were also investigated in this study using the fourth NIR window.
Reliability analysis of instrument design of noninvasive bone marrow disease detector

Yu Su, Ting Li, Yunlong Sun, Yuan Gao, Kai Li, Univ. of Electronic Science and Technology of China (China)

Bone marrow is an important hematopoietic organ, and bone marrow lesions (BMLs) may cause a variety of complications with high death rate and short survival time. Early detection and follow up care are particularly important. But the current diagnosis methods rely on bone marrow biopsy/puncture, with significant limitations such as invasion, complex operation, high risk, and discontinuous. It is highly in need of a non-invasive, safe, easily operated, and continuous monitoring technology. So we proposed to design a device aimed for detecting bone marrow lesions, which was based on near infrared spectrum technology. Then we fully tested its reliabilities, including the sensitivity, specificity, signal-to-noise ratio (SNR), stability, and etc. Here, we reported this sequence of reliability test experiments, the experimental results, and the following data analysis. This instrument was shown to be very sensitive, with distinguishable concentration less than 0.002 and with good linearity, stability and high SNR. Finally, these reliability-test data supported the promising clinical diagnosis and surgery guidance of our novel instrument in detection of BMLs.
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9690-1, Session 1
Assessing the feasibility of time-resolved fNIRS to detect brain activity during motor imagery

Androu Abdalmalak, Western Univ. (Canada) and Lawson Health Research Institute (Canada); Daniel Milej, Mamadou Diop, Western Univ. (Canada) and Lawson Health Research Institute (Canada); Lorina Naci, Adrian M. Owen, Brain and Mind Institute, Western Univ. (Canada); Keith St. Lawrence, Western Univ. (Canada) and Lawson Health Research Institute (Canada)

Functional near-infrared spectroscopy (fNIRS) is a non-invasive optical technique that can detect brain activity and has been previously used during motor and motor executive tasks. There is an increasing interest in using fNIRS as a brain computer interface (BCI) for patients who lack the physical (but not the mental) ability to respond to commands. The goal of this study was to assess the feasibility of time-resolved fNIRS to detect brain activity during a motor imagery task (i.e. imagining playing tennis). Two pulsed picosecond lasers (760 and 830 nm) were used, and the NIRS probes were placed on the scalp over the premotor cortex and supplementary motor area, as these areas are responsible for motion planning. The experimental paradigm consisted of five cycles of 30 s rest, 30 s motor imagery, followed by 30 s rest. To validate the fNIRS results, subjects also underwent functional magnetic resonance imaging (fMRI) while performing the same task. Statistical moment analysis was performed on the time-resolved fNIRS data, as this method has been previously shown to improve depth sensitivity. The activation in the supplementary motor area and the premotor cortex was first confirmed by fMRI, then motor imagery activity was measured by fNIRS. The fNIRS results showed a decreased in the statistical moments for the 830 nm wavelength during motor imagery, which returned to the baseline level during rest. These preliminary results highlight the potential of time-resolved fNIRS as a BCI, which could be adapted for bedside studies of patients with disorders of consciousness.

9690-2, Session 1
Applying support vector machine on hybrid fNIRS/EEG signal to classify driver’s conditions

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Driver’s condition plays a critical role in driving safety. The fact that about 20 percent of automobile accidents occurred due to driver fatigue leads to a demand for developing a method to monitor driver’s status. In this study, we acquired brain signals such as oxy- and deoxy-hemoglobin and neuronal electrical activity by a hybrid fNIRS/EEG system. Experiments were conducted with 11 subjects under two conditions: Normal condition, when subjects had enough sleep, and sleep deprivation condition, when subject did not sleep previous night. During experiment, subject performed a driving task with a car simulation system for 30 minutes. After experiment, oxy-hemoglobin and deoxy-hemoglobin changes were derived from fNIRS data, while beta and alpha band relative power were calculated from EEG data. Decrement of oxy-hemoglobin, beta band power, and increment of alpha band power were found in sleep deprivation condition compare to normal condition. These features were then applied to classify two conditions by Fisher’s linear discriminant analysis (FLDA). The ratio of alpha-beta relative power showed classification accuracy with a range between 62% and 99% depending on a subject. However, utilization of both EEG and fNIRS features increased accuracy in the range between 68% and 100%. The highest increase of accuracy is from 63% using EEG to 99% using both EEG and fNIRS features. In conclusion, the enhancement of classification accuracy is shown by adding a feature from fNIRS to the feature from EEG using FLDA which provides the need of developing a hybrid fNIRS/EEG system.

9690-3, Session 1
Functional connectivity during phonemic and semantic verbal fluency test: a multi-channel near infrared spectroscopy study

Chun-Jung Huang, Chia-Wei Sun, National Chiao Tung Univ. (Taiwan); Po-Han Chou, Taichung Veterans General Hospital (Taiwan); Ching-Cheng Chuang, National Chiao Tung Univ. (Taiwan)

Verbal fluency tests (VFT) are widely used neuropsychological tests of frontal lobe and have been frequently used in various functional brain mapping studies. There are two versions of VFT based on the type of cue: the letter fluency task (LFT) and the category fluency task (CFT). However, the fundamental aspect of the brain connectivity across spatial regions of the fronto-temporal regions during the VFTs has not been elucidated to date. In this study we hypothesized that different cortical functional connectivity over bilateral fronto-temporal regions can be observed by means of multi-channel fNIRS in the LFT and the CFT respectively. Our results from fNIRS (ETG-4000) showed different patterns of brain functional connectivity consistent with these different cognitive requirements. We demonstrate more brain functional connectivity over frontal and temporal regions during LFT than CFT, and this was in line with previous brain activity studies using fNIRS demonstrating increased frontal and temporal region activation during LFT and CFT and more pronounced frontal activation by the LFT.

9690-4, Session 1
Deception detection by EEG and fNIRS

Hong Di, Univ. of International Relations (China)

That the deception requires cognitive processes has been demonstrated. To detect a deception, an effective way is to explore the pattern of cognitive functions on the brain. Electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) have been involved to record signals of functional activity on the brain during a lie detection task. They have been verified the accuracy of detecting deception up to 85% by one of them. However, practical application of fMRI remains constrained by its limited accessibility and low affordability and application of EEG was restricted at its lower accuracy by itself. In order to improve the accuracy of deception detection, we propose to involve two brain functional modalities in the detection, EEG and functional near infrared spectroscopy (fNIRS). EEG could measure the electrophysiological signals on the scalp and fNIRS could record the hemodynamic response signals. The hemodynamic response could complement the limitation of single functional signals by EEG only. What’s more important that the fNIRS can provide signals within a brain area. The modality was not affected by the volume conduct effect, while
Eeg was. The combination of two complementary functional activities could provide more psychological signals during lying and the complete information would improve the accuracy of deception detection to 90%.

9690-5, Session 2

Visualization of microhemorrhages with optical histology in mouse model of cerebral amyloid angiopathy

Patrick Lo, Christian Crouzet, Beckman Laser Institute and Medical Clinic (United States); Vitaly Vasilevko, Univ. of California, Irvine (United States); Bernard Choi, Beckman Laser Institute and Medical Clinic (United States)

Cerebral amyloid angiopathy (CAA) is a neurovascular disease that is strongly associated with an increase in the number and size of spontaneous microhemorrhages. Conventional methods, such as magnetic resonance imaging (MRI), can detect microhemorrhages while positron emission tomography (PET) with Pittsburgh Compound B can detect amyloid deposits. MRI and PET can separately demonstrate the presence of microhemorrhages and CAA in affected brains in vivo; however, there is still a lack of strong evidence for the direct involvement of CAA in the presence of microhemorrhage formation. In this study, we use optical histology, a method which combines histochemical staining, chemical optical clearing, and optical imaging, in a Tg2576 mouse model of Alzheimer's disease to enable simultaneous, co-registered three-dimensional visualization of cerebral microvasculature, microhemorrhages, and amyloid deposits. Our data strongly suggest that microhemorrhages are localized within the brain regions affected by amyloid deposits. All but two observed microhemorrhages (n=18) were closely localized with vessels affected by CAA whereas no microhemorrhages or amyloid deposits were observed in wild type mouse brain sections. Our data also suggest that the predominant type of CAA-related microhemorrhage is associated with leaky or ruptured hemorrhagic microvasculature within the hippocampus and cerebral cortex rather than occluded ischemic microvasculature. The proposed optical histology method will allow future studies about the relationship between CAA and microhemorrhages during disease development and in response to treatment strategies.

9690-6, Session 2

Novel fiber-optic Imaging platform reveals behaviorally-relevant astrocyte network activation in the brain of freely-moving animals

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Mapping complex brain activities to distinct animal behaviors is fundamental in neuroscience. Recent advances in optical imaging, such as two-photon microscopy and genetically-encoded Ca2+ indicators (known-as GCaMP), facilitate the process with large-scale activity sampling in brains of behaving animals. However, these studies generally confine their scope to neurons, disregarding the enormous cellular diversity. Moreover, requiring animals to be restrained for bench-top microscopes inevitably restricts or alters their behaviors.

Here we demonstrated a platform that allowed real-time and non-invasive imaging in the intact CNS of freely-behaving animals. This system coupled a 30,000 core, 650-micron-diameter optic fiber-bundle to a 488nm laser and a CCD camera, approaching high resolution at 3-micron. The animal behaviors were tracked in concert using a custom-software-driven NIR camera. In addition, we presented imaging results from our recently-generated transgenic mouse, GLAST-CreER; GCaMP3, expressing Ca2+ indicators selectively in one type of non-neural cells: astrocyte. Certain evidence suggests astrocytes as an elemental part of the brain. These cells tile throughout CNS, extend ramified processes that ensheathe neuronal connections and release neuroactive substances. Nonetheless, prevailing concept remains that astrocytes are merely "passive" supporting cells. Our fiber-optic freely-moving imaging revealed that, for the first time, behaviorally-relevant astrocyte activation in natural settings, grounding for the significance of astrocyte network in brain.

This work incorporated a fiber-optic microscope that can be head-mounted on freely-behaving animals, enabling functional brain imaging in normal conditions. Together with other cutting-edge techniques like genetics, this could open avenues to understanding of functional correlations between the heterogeneous brain and animal behaviors.

9690-7, Session 2

Adaptive optics microscopy enhances image quality in deep layers of CLARITY processed brains of YFP-H mice

Xiaodong Tao, Florian Ermini, Univ. of California, Santa Cruz (United States); Laurent A. Bentolila, Dustin G. Roberts, Allan MacKenzie-Graham, Univ. of California, Los Angeles (United States); Joel Kubby, Univ. of California, Santa Cruz (United States)

Optical sectioning of biological tissues has become the method of choice for three dimensional histological analysis. This is particularly important in the brain where neurons can extend processes over large distances and often whole brain tracing of neuronal processes is desirable. To allow deeper optical penetration, which in fixed tissue is limited by scattering and refractive index mismatching, tissue clearing procedures such as CLARITY have been developed. CLARITY processed brains have a nearly uniform refractive index and three dimensional reconstructions at cellular resolution have been published. However, when imaging in deep layers at submicron resolution some limitations caused by residual refractive index mismatching become apparent, as the resulting wavefront aberrations distort the microscopic image. The wavefront can be corrected with adaptive optics. Here, we investigate the wavefront aberrations at different depths in CLARITY processed mouse brains and demonstrate the potential of adaptive optics to enable higher resolution and a better signal to noise. Our adaptive optics system achieves high-speed measurement and correction of the wavefront with an open loop control using a wave front sensor and a deformable mirror. Using adaptive optics enhanced microscopy we demonstrate improved image quality wavefront, point spread function and signal to noise in the cortex of YFP-H mice.

9690-8, Session 2

Effects of cranial window on monitoring neurovasculature using laser speckle contrast imaging

Hang Yu, Janaka Senaratna, Johns Hopkins Univ. (United States); Betty M. Tyler, The Johns Hopkins Hospital (United States); Arvind P. Pathak, Nitish V. Thakor, Johns Hopkins Univ. (United States)

Cranial window provides optical access to the rodent brain using high-resolution optical imaging tools. Two types of cranial windows are commonly employed, either open-skull window or thinned-skull window. However, the long-term characteristics of the windows have not been revealed. Laser speckle contrast imaging (LSCI) is a widely used optical imaging technique to monitor neurovasculature and cerebral blood flow in neurovascular studies. In this study, we applied LSCI to investigate the impacts of cranial windows on neurovascular structure and functions. We equally divided ten mice into open-skull window group and thinned-skull window group (3 mm diameter). The neurovasculature underlying
the windows were monitored consecutively using LSCI for fourteen days. The imaging outcomes of the two windows were assessed by parameters such as contrast-to-noise ratio (CNR) and microvessel density (MVD). For the thinned-skull window, CNR and MVD first increased with time probably recovering from the surgical procedures. After six days, both the parameters began to decrease due to the skull regrowth. Contrary to that, the open-skull window exhibited a monotonic decrease in CNR and MVD during the monitor period. We concluded that the optimal imaging time for thinned-skull window is four days after the surgery. A longer recovery period (more than fourteen days) is needed for the open-skull window due to the more invasive nature of the procedure. This study allows researchers to choose the suitable cranial window type based on their specific experimental paradigm.

9690-9, Session 3

First multiphoton tomography of brain in man

Karsten König, Univ. des Saarlandes (Germany); Sven Rainer Kantelhardt M.D., Alf Giese M.D., Johannes Gutenberg Univ. Mainz (Germany)

We report on the first two-photon imaging study of the brain in man during surgery using the flexible certified multiphoton tomograph MPTflex. High resolution optical biopsies were taken label-free during surgery in order to gain information on tumor borders. Furthermore, fast screening of tissue biopsies was performed in the operation theatre. One optical section (512x512 pixels) with a microsecond beam dwell time per pixel took 6 seconds using low-power near infrared 80 MHz femtosecond laser pulses. The most interesting information was obtained by time-resolved single photon counting of the two-photon excited autofluorescence.

9690-10, Session 3

Neural networks improve brain cancer detection with Raman spectroscopy in the presence of light artifacts

Michael Jermyn, Montreal Neurological Institute and Hospital (Canada); Joannie Desroches, Jeanne Mercier, Karl St-Arnaud, Ecole Polytechnique de Montréal (Canada); Marie-Christine Guiot, Kevin Petrecca, McGill Univ. (Canada); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada)

It is often difficult to identify cancer tissue during brain cancer (glioma) surgery. Gliomas invade into areas of normal brain, and this cancer invasion is frequently not detected using standard preoperative magnetic resonance imaging (MRI). This results in enduring invasive cancer following surgery and leads to recurrence. A hand-held Raman spectroscopy is able to rapidly detect cancer invasion in patients with grade 2-4 gliomas. However, ambient light sources can produce spectral artifacts which inhibit the ability to distinguish between cancer and normal tissue using the spectral information available. To address this issue, we have demonstrated that artificial neural networks (ANN) can accurately classify invasive cancer versus normal brain tissue, even when including measurements with significant spectral artifacts from external light sources. The non-parametric and adaptive model used by ANN makes it suitable for detecting complex non-linear spectral characteristics associated with different tissues and the confounding presence of light artifacts. The use of ANN for brain cancer detection with Raman spectroscopy, in the presence of light artifacts, improves the robustness and clinical translation potential for intraoperative use. Integration with the neurosurgical workflow is facilitated by accounting for the effect of light artifacts which may occur, due to operating room lights, neuronavigation systems, windows, or other light sources. The ability to rapidly detect invasive brain cancer under these conditions may reduce residual cancer remaining after surgery, and thereby improve patient survival.

9690-11, Session 3

Optical coherence tomography and fluorescence spectroscopy for brain tumor detection

Neda Haj-Hosseini, Peter Milos, Camilla Hildesjö, Martin Hallbeck, Johan Richter, Karin Wårdell, Univ. Hospital Linköping (Sweden)

Resection of brain tumor is a challenging task as the tumor does not have clear borders and the malignant types specifically have often a diffuse and infiltrative pattern of growth. We have previously implemented and evaluated a fluorescence spectroscopy based handheld probe for detecting the 5-aminolevulinic acid induced protoporphyrin IX (PpIX) in the gliomas in 50 patients. The results show a significantly high sensitivity for differentiating tumor from the healthy tissue. However, knowledge on association of the quantified fluorescence signals specifically in the intermediate inflammatory zone with the infiltrative tumor cells can be complemented with volumetric tissue imaging and a higher precision histopathological analysis. In this work, a spectral domain optical coherence tomography (OCT) system has been used to image the tissue volume that the fluorescence is collected from and is evaluated against histopathological analysis with a higher precision. The results show that although healthy brain has a homogeneous structure in the OCT images, the brain tumor shows a distinguished texture in the images correlated with the PpIX fluorescence intensity and histopathology.

9690-12, Session 3

Increasing the efficacy of antitumor glioma vaccines by photodynamic therapy and local injection of allogeneic glioma cells

Steen J. Madsen III, Univ. of Nevada, Las Vegas (United States); Catherine E. Christie M.D., Beckman Laser Institute and Medical Clinic (United States); Qian Peng M.D., Oslo Univ. Hospital (Norway); Henry Hirschberg M.D., Beckman Laser Institute and Medical Clinic (United States)

Immunotherapy of brain tumors involves the stimulation of an antitumor immune response. This type of therapy can be targeted specifically to tumor cells thus sparing surrounding normal brain. Due to the presence of the blood-brain barrier, the brain is relatively isolated from the systemic circulation and, as such, the initiation of significant immune responses is more limited than other types of cancers. The purpose of this study was to show that the efficacy of tumor primed antigen presenting macrophage vaccines could be increased by: (1) PDT of the priming tumor cells, and (2) injection of allogeneic glioma cells directly into brain tumors. Experiments were conducted in an in vivo brain tumor model using Fisher rats and BT4C (allogeneic) and F98 (syngeneic) glioma cells. Preliminary results showed that vaccination alone had significantly less inhibitory effect on F98 tumor growth compared to the combination of vaccination and allogeneic cell (BT4C) injection.

9690-14, Session 4

An intraoperative spectroscopic imaging system for quantification of Protoporphyrin IX during glioma surgery

Leticia M. Angulo-Rodríguez, Audrey Laurence, Ecole
Polytechnique de Montréal (Canada); Michael Jermy, Ecole Polytechnique de Montréal (Canada) and Montreal Neurological Institute and Hospital (Canada); Guillaume Sheehy, Ecole Polytechnique de Montréal (Canada); Mira Sibai, Princess Margaret Cancer Ctr. (Canada) and Univ. of Toronto (Canada); Kevin Petrecca, Montreal Neurological Institute and Hospital (Canada); David W. Roberts M.D., Dartmouth Hitchcock Medical Ctr. (United States); Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States); Brian C. Wilson, Princess Margaret Cancer Ctr. (Canada) and Univ. of Toronto (Canada); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada)

Cancer tissue often remains after brain tumor resection due to the inability to detect the full extent of cancer during surgery, particularly near tumor boundaries. Commercial systems are available for intra-operative real-time aminolevulenic acid (ALA)-induced protoporphyrin IX (PpIX) fluorescence imaging. These are standard white-light neurosurgical microscopes adapted with optical components for fluorescence excitation and detection. However, these instruments lack sensitivity and specificity, which limits the ability to detect low levels of PpIX and distinguish it from tissue auto-fluorescence. Current systems also cannot provide repeatable and un-biased quantitative fluorophore concentration values because of the unknown and highly variable light attenuation by tissue. We present a highly sensitive spectroscopic fluorescence imaging system that is seamlessly integrated into a neurosurgical microscope. Hardware and software were developed to achieve through-microscope spatially-modulated illumination for 3D profilometry and to use this information to extract tissue optical properties to correct for the effects of tissue light attenuation. This gives pixel-by-pixel quantified fluorescence values and improves detection of low PpIX concentrations. This is achieved using a high-sensitivity Electron Multiplying Charge Coupled Device (EMCCD) with a Liquid Crystal Tunable Filter (LCTF) whereby spectral bands are acquired sequentially; and a snapshot camera system with simultaneous acquisition of all bands is used for profilometry and optical property recovery. Sensitivity and specificity to PpIX is demonstrated using brain tissue phantoms and intraoperative human data acquired in an on-going clinical study using PpIX fluorescence to guide glioma resection.

9690-15, Session 4
Fiber-based tissue identification for electrode placement in deep brain stimulation neurosurgery

Damon T. DePaoli, Nicolas Lapointe, Laurent Goetz, Institut Univ. en Santé Mentale de Québec (Canada); Martin Parent, Univ. Laval (Canada); Michel Prudhomme M.D., Ctr. Hospitalier Univ. Laval (Canada); Léo Cantin M.D., Univ. Laval (Canada); Tigran Galstian, Younès Messaddeq, Ctr. d’Optique, Photonique et Laser (Canada); Daniel C. Côté, Ctr. de Recherche de l’Univ. Laval Robert-Giffard (Canada) and Ctr. d’Optique, Photonique et Laser (Canada)

Deep brain stimulation’s effectiveness relies on the ability of the stimulating electrode to be properly placed within a specific target area of the brain. Optical guidance techniques that can increase the accuracy of the procedure, without causing any additional harm, are therefore of great interest.

We have designed a cheap optical fiber-based device that is small enough to be placed within commercially available DBS stimulating electrodes’ hollow cores and that is capable of sensing biological information from the surrounding tissue, using low power white light. With this probe we have shown the ability to distinguish white and grey matter as well as blood vessels, in vitro, in human brain samples and in vivo, in rats. We have also repeated the in vitro procedure with the probe inserted in a DBS stimulating electrode and found the results were in good agreement.

We are currently validating a second fiber optic device, with micro-optical components, that will result in label free, molecular level sensing capabilities, using CARS spectroscopy. The final objective will be to use this data in real-time, during deep brain stimulation neurosurgery, to increase the safety and accuracy of the procedure.

9690-16, Session 4
Effective transvascular drug delivery to glioma in rats by using a pulsed laser-induced photomechanical wave

Yusuke Akutsu, Keio Univ. (Japan); Shunichi Sato, Arata Tomiyama, National Defense Medical College (Japan); Yasuyuki Tsuno, Keio Univ. (Japan); Satoko Kawachi, Kentaro Mori, National Defense Medical College (Japan); Mitsuhiro Terakawa, Keio Univ. (Japan)

Glioma is one of the most aggressive cancers, for which efficacy of conventional chemotherapy is often limited due to the blood-tumor barrier (BTB). Thus, the development of a method for enhancing the BTB permeability is strongly desired. In this study, we applied a photomechanical wave (PMW), which was generated by the irradiation of a light-absorbing material with a nanosecond laser pulse, to transiently open the BTB in a rat intracranial glioma model using C6 cells. A tumor was grown in the both hemispheres, and a solution of Evans blue (EB), as a test drug, was injected into the tail vein. Thereafter, we applied a PMW generated at a laser fluence of 0.2 J/cm^2 (averaged peak pressure, ~27 MPa), 0.4 J/cm^2 (~54 MPa) or 0.6 J/cm^2 (~78MPa), to one hemisphere through the cranial window, while the other hemisphere served as a control. Four hours later, the rat was perfused, and we compared intensity distributions of EB fluorescence between the both hemispheres. Intensities of EB fluorescence both in the peritumoral and tumor core regions were increased with increasing the laser fluence, but hemorrhage was observed at the highest fluence. Thus, 0.4 J/cm^2 would be optimum for efficient and safe BTB opening. On the basis of fluorescence microscopy with the use of enhanced green fluorescent protein-expressing C6 cells, we confirmed that a drug was delivered into targeted glioma cells in the peritumoral region. These results show the validity of the present transvascular drug delivery method to glioma.

9690-17, Session 4
Intraoperative brain hemodynamic response assessment with real-time hyperspectral optical imaging

Audrey Laurence, Julien Pichette, Leticia M. Angulo-Rodriguez, Catherine St. Pierre M.D., Frédéric Lesage, Ecole Polytechnique de Montréal (Canada); Alain Bouthillier M.D., Univ. de Montréal (Canada); Dang Khoa Nguyen, Ctr. Hospitalier de l’Univ. de Montréal (Canada); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada)

Following normal neuronal activity, there is an increase in cerebral blood flow and cerebral blood volume to provide oxygenated hemoglobin to active neurons. For abnormal activity such as epileptiform discharges, this hemodynamic response may be inadequate to meet the high metabolic demands. To verify this hypothesis, we developed a novel hyperspectral imaging system able to monitor real-time cortical hemodynamic changes during brain surgery. The imaging system is directly integrated into a surgical microscope, using the white-light source for illumination. A snapshot hyperspectral camera is used for detection (4x4 mosaic filter array detecting 16 wavelengths simultaneously). We present calibration experiments where phantoms made of intralipid and food dyes were
A number of noninvasive/minimally invasive in vivo imaging modalities have
been applied in neuroscience studies. Among them, two-photon microscopy and
magnetic resonance imaging are the major tools of choice, but they
either lack high resolution or large imaging size. Overall, an imaging tool
with a micron level resolution that can provide a millimeter level field of
view with a high frame rate is desired for in vivo rodent brain imaging.
Optical coherence tomography (OCT) is a non-invasive method for imaging
three-dimensional biological tissues with high resolution (~10 μm), and
without requiring the use of contrast agents. Despite the requirements of in
vivo rodent brain imaging are hard to satisfy for aforementioned traditional
technologies, OCT can easily reach at these speeds and provide high
resolution volumetric images with a large field of view using the available
electronics. In this work, we provide the overview of recent developments of
OCT based imaging techniques for neuroscience applications on rodents. We summarize today's technology alternatives of OCT based
microangiography for neuroscience and provide a road map for the future
challenges and opportunities. Moreover, comprehensive summary of OCT
angiography studies for stroke and traumatic brain injury cases on rodents
are provided.

From chance to neurophotonics
David A. Boas, Massachusetts General Hospital, Harvard Medical School (United States)
No Abstract Available

OCT imaging of acute vascular changes following mild traumatic brain injury in mice
Isabel Chico-Calero D.V.M., Milen Shishkov, Jonathan Welt, Cedric Blatter, Wellman Ctr. for Photomedicine (United States); Benjamin J. Vakoc, Massachusetts General Hospital (United States)
While most people recover completely from mild traumatic brain injuries
(mTBIs) and concussions, a subset develop lasting neurological disorders. Understanding the complex pathophysiology of these injuries is critical to
developing improved prognostic and therapeutic approaches. Multiple
studies have shown that the structure and perfusion of brain vessels are
altered after mTBI. It is possible that these vascular injuries contribute to
or trigger neurodegeneration. Intravital microscopy and mouse models of
TBI offer a powerful platform to study the vascular component of mTBI.
Because optical coherence tomography based angiography is based on
perfusion contrast and is not significantly degraded by vessel leakage
or blood brain barrier disruption, it is uniquely suited to studies of brain
perfusion in the setting of trauma. However, existing TBI imaging models
require surgical exposure of the brain at the time of injury which conflates
TBI-related vascular changes with those caused by surgery. In this work, we
describe a modified cranial window preparation based on a flexible,
transparent polyurethane membrane. Impact injuries were delivered directly
through this membrane, and imaging was performed immediately after
injury without the need for additional surgical procedures. Using this model,
we demonstrate that mTBI induces a transient cessation of flow in the
capillaries and smaller vessels near the injury point. Reperfusion is observed
in all animals within 3 hours of injury. This work describes new insight into
the transient vascular changes induced by mTBI, and demonstrates more
broadly the utility of the OCT/polyurethane window model platform in
preclinical studies of mTBI.

Application of optical coherence tomography based microangiography for cerebral imaging
Utku Baran, Ruikang K. Wang, Univ. of Washington (United States)
The brain is a complex system, consisted of multiple layers and components.
A number of noninvasive/minimally invasive in vivo imaging modalities have
been applied in neuroscience studies. Among them, two-photon microscopy
and magnetic resonance imaging are the major tools of choice, but they
either lack high resolution or large imaging size. Overall, an imaging tool
with a micron level resolution that can provide a millimeter level field of
view with a high frame rate is desired for in vivo rodent brain imaging.
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resolution volumetric images with a large field of view using the available
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OCT based imaging techniques for neuroscience applications on rodents. We summarize today's technology alternatives of OCT based
microangiography for neuroscience and provide a road map for the future
challenges and opportunities. Moreover, comprehensive summary of OCT
angiography studies for stroke and traumatic brain injury cases on rodents
are provided.

Non-destructive optical clearing technique enhances optical coherence tomography (OCT) for real-time, 3D histomorphometry of brain tissue
Akshay Paul, Beckman Laser Institute and Medical Clinic (United States) and OCT Medical Imaging Inc. (United States); Theodore H. Chang, Li-Dek Chou, OCT Medical Imaging Inc. (United States) and Beckman Laser Institute and Medical Clinic, Univ. of California, Irvine (United States); Tirunelveli S. Ramalingam, OCT Medical Imaging Inc. (United States)
Evaluation of neurodegenerative disease often requires examination of
brain morphology. Volumetric analysis of brain regions and structures can
be used to track developmental changes, progression of disease, and the
Conference 9690A: Clinical and Translational Neuro photonics

9690-21, Session 6
Polarization properties of amyloid-beta plaques in Alzheimer’s disease
Bernhard Baumann, Adelheid Wöhrer, Gerda Ricken, Michael Pircher, Gabor G. Kovacs, Christoph K. Hitzenberger, Medizinische Univ. Wien (Austria)

In histopathological practice, birefringence is used for the identification of amyloidosis in numerous tissues. Amyloid birefringence is caused by the parallel arrangement of fibrous protein aggregates. Since neurodegenerative processes in Alzheimer’s disease (AD) are also linked to the formation of amyloid-beta (Aβ) plaques, optical methods sensitive to birefringence may act as non-invasive tools for Aβ identification. At last year’s Photons West, we demonstrated polarization-sensitive optical coherence tomography (PS-OCT) imaging of ex vivo cerebral tissue of advanced stage AD patients. PS-OCT provides volumetric, structural imaging based on both backscatter contrast and tissue polarization properties. In this presentation, we report on polarization-sensitive neuroimaging along with numerical simulations of three-dimensional Aβ plaques. High speed PS-OCT imaging was performed using a spectral domain approach based on polarization maintaining fiber optics. The sample beam was interfaced to a confocal scanning microscope arrangement. Formalin-fixed tissue samples as well as thin histological sections were imaged. For comparison to the PS-OCT results, ray propagation through plaques was modeled using Jones analysis and various illumination geometries and plaque sizes. Characteristic polarization patterns were found. The results of this study may not only help to understand PS-OCT imaging of neuritic Aβ plaques but may also have implications for polarization-sensitive imaging of other fibrillary structures.

9690-23, Session 7
Multimodal optical platform for functional monitoring of cerebral response to cardiac arrest and resuscitation
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Treatment in the intensive care unit (ICU) for cardiac arrest survivors is a major clinical challenge, as the majority of these patients enter a state of coma and are unable to ever obtain complete neurological recovery. To provide a quantitative means of assessing the brain’s response to different clinical interventions during and after resuscitation, we have developed an optical imaging platform that combines multispectral spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI). This technology is compatible with the “small animal” OCT monitoring setup developed in the Department of Neurology at UC Irvine. We have employed our device to interrogate brain hemodynamics in a rat model of cardiac arrest and resuscitation. From multispectral SFDI, we can obtain temporally- and spatially-resolved maps of tissue hemoglobin concentration and oxygenation. From LSI, we can obtain temporally- and spatially-resolved maps of speckle flow index, a known indicator of blood flow. Acquiring these two types of data simultaneously allows us to separate the dynamics of perfusion from metabolism in the brain in response to cardiac arrest.
and resuscitation, elucidating mechanisms of reperfusion and neurological recovery. In addition, the optical data is compared with quantitative electroencephalogram (EEG) and standard ICU hemodynamic monitoring obtained simultaneously throughout the experiment, providing insight into neurovascular coupling. Preliminary results suggest a decoupling of perfusion and metabolism (evidenced by different temporal hemodynamics of the speckle flow index and hemoglobin oxygenation variables) during the time period in which quantitative EEG demonstrates gradual recovery of neurological function.

9690-24, Session 7

Study the efficacy of neuroprotective drugs on brain physiological properties during focal head injury using optical spectroscopy data analysis

David Abookasis, Ariel Shochat, Ariel Univ. (Israel)

We present a comparative evaluation of five different neuroprotective drugs in the early phase following focal traumatic brain injury (TBI) in mouse intact head. The effectiveness of these drugs in terms of changes in brain tissue morphology and hemodynamic properties was experimentally evaluated through analysis of the optical absorption coefficient and spectral reduced scattering parameters in the range of 650-1000 nm. Anesthetized male mice (n=50 and n=10 control) were subjected to weight drop model mimics real life focal head trauma. Monitoring the effect of injury and neuroprotective drugs was obtained by using a diffuse reflectance spectroscopy system utilizing independent source-detector separation and location. Result indicates that administration of minocycline improve hemodynamic and reduced the level of tissue injury at an early phase post-injury while hypertonic saline treatment decrease brain water content. These findings highlight the heterogeneity between neuroprotective drugs and the ongoing controversy among researchers regarding which drug therapy is preferred for treatment of TBI. On the other hand, our results show the capability of optical spectroscopy technique to noninvasively study brain function following injury and drug therapy.

9690-25, Session 7

In vivo imaging of cerebral hemodynamics and regional oxygen saturation in rats with a digital red-green-blue camera

Izumi Nishidate, Yoshika Harasaki, Tokyo Univ. of Agriculture and Technology (Japan); Satoko Kawauchi, Shunichi Sato, National Defense Medical College (Japan); Manabu Sato, Yasuaki Kokubo, Yamagata Univ. (Japan)

We propose a method to monitor the spatial distribution of total hemoglobin concentration (CHbT) and the regional oxygen saturation (rSO2) in cerebral cortex using a digital red-green-blue camera. In the method, the RGB-values are converted into the tristimulus values in CIEXYZ color space which is a device-independent color system and compatible with the common RGB working spaces (NTSC, sRGB, etc). Monte Carlo simulation (MCS) for light transport in tissue is used to specify a relation among the tristimulus XYZ-values and the concentrations of oxygenated hemoglobin (CHbO) and deoxygenated hemoglobin (CHbR). In the present study, we performed in vivo recordings of RGB images for exposed brain of rats while varying the fraction of inspired oxygen (FiO2). The values of CHbO and CHbR were increased and decreased, respectively, during hypoxemia, which caused the increase in rSO2. After the onset of anoxia, the values of CHbO and CHbR decreased and increased, respectively. Consequently, the value of rSO2 was dramatically decreased. The value of CHbT begins to increase before respiratory arrest (RA) and reaches a maximum amplitude approximately 1 min after RA, which is indicative of an increase in blood flow for compensating hypoxia. Immediately after RA, the values of both CHbO and CHbT decreased rapidly. The time courses of CHbO, CHbR, CHbT, and rSO2 are consistent with the well-known hemodynamic responses to the change in FiO2. The results in this study indicate potential of the method to evaluate the physiological conditions and loss of tissue viability in brain tissue.

9690-26, Session 7

Spatiotemporal characteristics of spreading depolarization, hypoxemia and vasoconstriction caused by a laser-induced shock wave in the rat brain

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Mild blast-induced traumatic brain injury has been a major concern in neurotraumatology; however, its pathophysiology and mechanism are poorly understood. We previously performed real-time optical monitoring of the rat brain exposed to a laser-induced shock wave (LISW). Spreading depolarization (SD) occurred immediately after LISW application, which was accompanied by transient hemoglobin oxygenation and hyperemia, being followed by long-lasting hypoxemia and oligemia (Sato et al., PLoS one, 2014). In this study, we used multispectral imaging method to investigate the shock wave-induced events in more details, i.e., spatiotemporal changes in scattering amplitude, tissue oxygen saturation (StO2) and diameters of arteriole for the same model. An LISW was applied to the left frontal cortex, and the brain tissue with a diameter of ~2.5 mm in the ipsilateral parietal region was subjected to multispectral imaging. During propagation of SD over the cortex, StO2 was increased by > 30%, which was accompanied by dilatation of arterioles and parenchymal hyperemia as observed in the previous study. In this phase, however, we found that a few arterioles showed distinct constriction; the vessel diameters were reduced by up to -50%. The tissue close to a vessel showing stronger constriction was followed by more evident decrease in StO2 (< 50% of the baseline) for longer duration (~60 min). These results indicate that hypoxemia induced by a shock wave is site-dependent, which would be affected by the initial responses of arterioles to a shock wave exposure.
Non-invasive assessment of cerebral microcirculation with diffuse optics and coherent hemodynamics spectroscopy (Invited Paper)

Sergio Fantini, Angelo Sassaroli, Tufts Univ. (United States); Jana M. Kainerstorfer, Tufts Univ. (United States) and Carnegie Mellon Univ. (United States); Kristen T. Tgavalekos, Xuan Zang, Tufts Univ. (United States)

We used coherent hemodynamics spectroscopy (CHS) to measure the local cerebral blood flow and cerebral autoregulation in the healthy and vulnerable human brain. CHS is a new technique that performs a quantitative assessment of cerebral microcirculation on the basis of the cerebral hemodynamic response to controlled perturbations in the arterial blood pressure. Among a number of options to modulate the arterial blood pressure, we adopted a cyclic inflation (to a pressure of 200 mmHg) and deflation of two pneumatic cuffs placed around the subject’s thighs at frequencies in the range 0.05-0.10 Hz. We also considered a fast deflation of the two pneumatic cuffs after a 2-min period of sustained inflation to induce a dynamic transient in the mean arterial pressure over a time of about 20 s. The resulting cerebral hemodynamic response was measured with near-infrared spectroscopy (NIRS), and quantitatively analyzed with a novel hemodynamic model, resulting in CHS measurements. We have applied CHS on a group of eleven healthy subjects, where CHS detected the enhanced cerebral autoregulation associated with hypocapnic conditions realized by hyperventilation. Specifically, the cutoff frequency of the high-pass filter established by autoregulation for blood flow dynamics induced by changes in cerebral perfusion pressure, increased from 0.017+/−0.002 Hz during normal breathing to 0.034+/−0.005 Hz during hyperventilation. In a clinical study on five hemodialysis patients, we found a longer capillary transit time (1.1+/−0.3 s), indicating a reduced speed of cerebral blood flow, with respect to a group of six healthy controls (0.5+/−0.2 s).

A novel time-domain diffuse correlation spectroscopy (DCS) system for improved transcranial measurement of cerebral blood flow (CBF).

Jason Sutin, Massachusetts General Hospital (United States) and Boston Univ. (United States); Danil Tyulmankov, Athinoula A. Martinos Ctr. for Biomedical Imaging (United States) and Massachusetts Institute of Technology (United States); Bernhard Zimmermann, Massachusetts General Hospital (United States); Juliette Selb, Massachusetts General Hospital (United States) and Harvard Medical School (United States); David A. Boas, Athinoula A. Martinos Ctr. for Biomedical Imaging (United States) and Harvard Medical School (United States); Maria Angela Franceschini, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

We implemented a novel time-domain diffuse correlation spectroscopy (TD-NIRS) system for improved transcranial measurement of cerebral blood flow (CBFi) in the 700-1000 nm range and enables to detect hemodynamic changes (i.e., oxygenated hemoglobin, deoxygenated hemoglobin, blood volume) as a response to various brain processes. In this study, we developed a new, portable, prefrontal fNIRS system which has 12 light sources, 10 detectors and 108 channels with a sampling rate of 2 Hz. The wavelengths of light source are 780nm and 850nm. ATxmega128A1, 8bit of Micro controller unit (MCU) with 200~4095 resolution along with MatLab data acquisition algorithm was utilized.

A portable, multi-channel fNIRS system for prefrontal cortex: Preliminary study on neurofeedback and imagery tasks

Seung-ho Paik, Beop-Min Kim, Korea Univ. (Korea, Republic of)

fNIRS is a neuroimaging technique which uses near-infrared light source in the 700-1000 nm range and enables to detect hemodynamic changes (i.e., oxygenated hemoglobin, deoxygenated hemoglobin, blood volume) as a response to various brain processes. In this study, we developed a new, portable, prefrontal fNIRS system which has 12 light sources, 15 detectors and 108 channels with a sampling rate of 2 Hz. The wavelengths of light source are 780nm and 850nm. ATxmega128A1, 8bit of Micro controller unit (MCU) with 200~4095 resolution along with MatLab data acquisition algorithm was utilized.
In the system, it has one source and four detectors. The source, located in the middle of forehead, can emit two near infrared light, 740nm and 860nm. Two detectors are arranged in one side in 2 centimeters and 3 centimeters from the source. Their measurements are used to calculate the saturation in the cerebral cortex. The system has included the rechargeable lithium battery and Bluetooth smart wireless micro-computer unit.

9690-33, Session 9
A spatially resolved diffuse correlation spectroscopy for cerebral blood flow measurement in the layered structure of head
Yu Shang, Univ. of Kentucky (United States) and North Univ. of China (China); Guoqiang Yu, Univ. of Kentucky (United States)

In developing diffuse correlation spectroscopy (DCS) for cerebral blood flow (CBF) measurements, we found that conventional semi-infinite homogenous solutions for DCS data analysis could lead to substantial errors of CBF in heterogeneous tissues with irregular geometries like the head. To overcome this partial volume influence, we created a novel spatially-resolved DCS (SR-DCS) algorithm integrating a linear model of autocorrelation functions measured at multiple source-detector (S-D) pairs with the Monte Carlo simulation of photon migrations in the layered tissues of the head. With sufficient S-D pairs at different distances, SR-DCS allowed for simultaneous measurements of blood flow indices in scalp, skull, cerebrospinal fluid, and brain cortex. We compared this algorithm with the semi-infinite homogenous solution in a computer model of adult head. Our simulation results demonstrated significantly higher accuracy of SR-DCS (errors < 3%) compared to the semi-infinite homogenous solution (errors > 35%) in extracting CBF. We also conducted a validation study using SR-DCS on a 2-layer head-simulating phantom. Briefly, a realistic human skull was kept inverted and filled with an Intralipid phantom solution mimicking the brain tissue. The Intralipid particles in distilled water provided control of scattering and Brownian motion (flow). The flow change in the “brain” was induced by increasing temperature of the liquid phantom inside the skull. The flow measurement errors using SR-DCS algorithm (< 5%) were found to be much less than those using the semi-infinite homogenous solution (errors > 20%). Future study will test SR-DCS for in vivo CBF measurements.

9690-34, Session 9
Evaluation of time-resolved multi-distance methods to retrieve absorption and reduced scattering coefficients of adult heads in vivo: Optical parameters dependences on geometrical structures of the models used to calculate reflectance
Tadatoshi Tanifuji, Kitami Institute of Technology (Japan)

Time-resolved multi-distance measurements are studied, which have enough sensitivity to determine both absorption and reduced scattering coefficients (myu_a and myu_s') for superficial tissues, gray matter and white matter of adult heads. The finite difference time domain analysis was used to calculate time-resolved reflectance from realistic adult head models composed of four layers (i.e. scalp, skull, gray matter and white matter) with subarachnoid spaces and brain grooves filled with a non-scattering cerebrospinal fluid (CSF). In vivo time-resolved reflectances of human heads were measured by a system composed of a time-correlated single photon counter and diode laser operating at 880 nm. By minimizing the objective functions that compare theoretical and experimental time-resolved reflectances, myu_a and myu_s' of brain were determined. In this method, the geometrical structures of the models significantly influence the accuracy of the retrieved myu_a and myu_s' of the four layers. One of the
critical structural parameters is head surface curvature which is different at the positions of the head even in the identical subject. Measurements were performed by putting the injection and collection fibers on the left semi-sphere of the forehead, with the injection fiber placed toward the temporal region, and by moving the collection fiber between 10 and 60 mm from the central sulcus. It became clear that optical parameters of the forehead at all collection fibers were reasonably determined by selecting the appropriate visibility length of the geometrical head models, which is related to head surface curvature at each position.

9690-35, Session 9

**Chronic monitoring of cortical hemodynamics in behaving, freely-moving rats using a miniaturized head-mounted optical microscope**

Iliya Sigal, Raanan Gad, Univ. of Toronto (Canada); Margaret Koletar, Sunnybrook Research Institute (Canada); Dene Ringuette, Univ. of Toronto (Canada); Bojana Stefanovic, Sunnybrook Health Sciences Ctr. (Canada); Ofer Levi, Univ. of Toronto (Canada)

Growing interest within the neurophysiology community in assessing healthy and pathological brain activity in animals that are awake and freely-behaving has triggered the need for optical systems that are suitable for such longitudinal studies. In this work we report label-free multi-modal imaging of cortical hemodynamics in the somatosensory cortex of awake, freely-behaving rats, using a novel head-mounted miniature optical microscope. The microscope employs vertical cavity surface emitting lasers (VCSELs) at three distinct wavelengths (680 nm, 795 nm, and 850 nm) to provide measurements of four hemodynamic markers: blood flow speeds, HbO, HbR, and total Hb concentration, across a >2mm field of view. Blood flow speeds are extracted using Laser Speckle Contrast Imaging (LSCI), while oxygenation measurements are performed using Intrinsic Optical Signal Imaging (IOSI). Longitudinal measurements on the same animal are made possible over the course of >6 weeks using a chronic window that is surgically implanted into the skull. We use the device to examine changes in blood flow and blood oxygenation in superficial cortical blood vessels and tissue in response to behavioural tasks of reaching, grabbing, and whisker stimulation, while correlating behavior with changes in blood flow and blood oxygenation in the brain. In future experiments we seek to apply this device to the study of coupling between motor function and spatially-resolved hemodynamic response in a chronic model of focal ischemia in a rat.

9690-36, Session 9

**Multi-modal in vivo imaging of brain blood oxygenation, blood flow and neural calcium dynamics during acute seizures**

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Dysfunction of the vascular endothelium has been implicated in the development of epilepsy. To better understand the relation between vascular function and seizure and provide a foundation for interpreting results from functional imaging in chronic disease models, we investigate the relationship between intracellular calcium dynamics and local cerebral blood flow and blood oxygen saturation during acute seizure-like events and pharmacological seizure rescue. To probe the relation between the aforementioned physiological markers in an acute model of epilepsy in rats, we integrated three different optical modalities together with electrophysiological recordings: Laser Speckle Contrast Imaging (LSCI) was used to study changes in flow speeds, Intrinsic Optical Signal Imaging (IOSI) was used to monitor changes in oxygenated, de-oxygenated, and total hemoglobin concentration, and Calcium-sensitive dye Imaging was used to monitor intracellular calcium dynamics. We designed a dedicated cortical flow chamber to remove superficial blood and dye resulting from the injection procedure, which reduced spurious artifacts. The near infrared light used for IOSI and LSCI was delivered via a light pipe integrated with the flow chamber to minimize the effect of fluid surface movement on illumination stability. Calcium-sensitive dye was injected via a glass electrode used for recording the local field potential. Our system allowed us to observe and correlate increases in intracellular calcium, blood flow and blood volume during seizure-like events and provide a quantitative analysis of neurovascular coupling changes associated with seizure rescue via injection of an anti-convulsive agent.

9690-37, Session 10

**Mapping whole-brain activity with cellular resolution by light-sheet microscopy and high-throughput image analysis**

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Mapping neuronal activity patterns across the whole brain with cellular resolution is a challenging task for state-of-the-art imaging methods. Indeed, despite a number of technological efforts, quantitative cellular-resolution activation maps of the whole brain have not yet been obtained. Many techniques are limited by coarse resolution or by a narrow field of view. High-throughput imaging methods, such as light sheet microscopy, can be used to image large specimens with high resolution and in reasonable times. However, the bottleneck is then moved from image acquisition to image analysis, since many TeraBytes of data have to be processed to extract meaningful information.

Here, we present a full experimental pipeline to quantify neuronal activity in the entire mouse brain with cellular resolution, based on a combination of genetics, optics and computer science. We used a transgenic mouse strain (Arc-dVenus mouse) in which neurons which have been active in the last hours before brain fixation are fluorescently labelled. Samples were cleared with CLARITY and imaged with a custom-made confocal light sheet microscope. To perform an automatic localization of fluorescent cells on the large images produced, we used a novel computational approach called semantic deconvolution.

The combined approach presented here allows quantifying the amount of Arc-expressing neurons throughout the whole mouse brain. When applied to cohorts of mice subject to different stimuli and/or environmental conditions, this method helps finding correlations in activity between different neuronal populations, opening the possibility to infer a sort of brain-wide ‘functional connectivity’ with cellular resolution.

9690-38, Session 10

**Fast whole-brain optical tomography capable of automated slice-collection**

Jing Yuan, Tao Jiang, Lei Deng, Beng Long, Jie Peng, Qingming Luo, Hui Gong, Huazhong Univ. of Science and
Technology (China)

Acquiring brain-wide composite information of neuroanatomical and molecular phenotyping is crucial to understand brain functions. However, current whole-brain imaging methods based on technical sectioning haven’t achieved brain-wide acquisition of both neuroanatomical and molecular phenotyping due to the lack of appropriate whole-brain immunostaining of embedded samples. Here, we present a novel strategy of acquiring brain-wide structural and molecular maps in the same brain, combining whole-brain imaging and subsequent immunostaining of automated-collected slices. We developed a whole-brain imaging system capable of automatically imaging and then collecting imaged tissue slices in order. The system contains three parts: structured illumination microscopy for high-throughput optical sectioning, vibratome for high-precision sectioning and slice-collection device for automated collecting of tissue slices. Through our system, we could acquire a whole-brain dataset of agarose-embedded mouse brain at lateral resolution of 0.33 µm with z-interval sampling of 100 µm in 9 h, and automatically collect the imaged slices in sequence. Subsequently, we performed immunohistochemistry of the collected slices in the routine way. We acquired mouse whole-brain imaging datasets of multiple specific types of neurons, proteins and gene expression profiles. We believe our method could accelerate systematic analysis of brain anatomical structure with specific proteins or genes expression information and understanding how the brain processes information and generates behavior.

9690-39, Session 10

High throughput optical imaging of the CLARITY-processed tissue

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Understanding brain function and dysfunction requires integrative knowledge of the brain’s architecture?how neuronal connectivity and molecular machinery orchestrate mental function. Recent advent of tissue processing technologies, such as CLARITY and other optical clearing methods, open the possibility of investigating anatomical and molecular architecture of intact brain without laborious mechanical sectioning and reconstruction. Conventional confocal and two photon microscopies, however, significantly limits their utility due to the slow scanning nature of the imaging process. Here, we introduce a temporally focused line scanning two photon microscope which can provide a resolution comparable to point scanning two photon microscopy while the imaging speed is only limited by the readout rate of the sCMOS sensor. Further increase in depth resolution is achieved by using a rolling shutter mode of the sCMOS sensor as a dynamic confocal slit. This imaging platform could be particularly useful for human brain mapping because any size and shape of the sample (e.g. whole coronal block of the human brain, rat brain, monkey brain, and other organs) can be readily mounted on the upright configuration of the imaging system. The imaging depth will be limited by the working distance of the objective, but when combined with sectioning by vibrating blade microtome, this imaging modality may even enable imaging of whole mounted primate/rat brains in an automated manner.

9690-40, Session 10

Low-light CoDiM super-resolution imaging to observe ultrastuctural effects of cannabinoid receptor activation on neuronal growth and synaptic connections

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Cannabinoids are naturally produced by the body to regulate neuronal growth, ensure proper brain wiring and synaptic function. They are also proving increasingly useful for medical purposes. Cannabinoid receptor activation in neurons can also result both in rapid and lasting morphological modifications in the brain, which may be pathogenic when used in uncontrolled contexts such as recreational drug consumption in adolescents. Rapid cytoskeletal changes downstream of neuronal cannabinoid receptor activation remain elusive and increased imaging resolution is critically needed to address this question. Super-resolution imaging has already been achieved on neurons with remarkable results but at the cost of high illumination levels that are known to be detrimental to live cells. Here we perform super-resolution imaging of fixed and live neurons in physiological low-light conditions using the new CoDiM system. This approach may now unveil previously unobserved morphological changes in the synaptic cleft and neuronal growth cones following cannabinoid-receptor activation. Our results will improve our understanding of the mechanisms driving these effects and pave the ways towards physiologically-relevant low-light super-resolution imaging.

9690-41, Session 10

Two-photon multiplane imaging of neural circuits

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Imaging the neuronal activity throughout the brain with high temporal and spatial resolution is an important step in understanding how the brain works. Two-photon laser scanning microscopy with fluorescent calcium indicators has enabled this type of experiments in vivo. Most of these microscopes acquire images serially, with a single laser beam, limiting the overall imaging speed. To overcome this limit, multiple beamlets can be used to image in parallel multiple regions. Here, we demonstrate a novel scheme of a two-photon laser-scanning microscope that can simultaneously record neuronal activity at multiple planes of the sample with a single photomultiplier tube. A spatial light modulator is used to generate the designated multiple beamlets, and a constrained non-negative matrix factorization algorithm is used to demix the signals from multiple scanned planes. We simultaneously record neuronal activity of multiple layers of a mouse cortex at 10 fps in vivo. This novel imaging scheme provides a powerful tool for mapping the brain activity.
9690-42, Session 11

Fluorescent nanodiamond and lanthanide labelled in situ hybridization for the identification of RNA transcripts in fixed and CLARITY-cleared central nervous system tissues

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Despite significant advancement in the methodology used to conjugate, incorporate and visualize fluorescent molecules at the cellular and tissue levels, biomedical imaging predominantly relies on the limitations of established fluorescent molecules such as fluorescein, cyanine and AlexaFluor dyes or genetic incorporation of fluorescent proteins by viral or other means. These fluorescent dyes and conjugates are highly susceptible to photobleaching and compete with cellular autofluorescence, making biomedical imaging unreliable, difficult and time consuming in many cases. In addition, some proteins have low copy numbers and/or poor antibody recognition, further making detection and imaging difficult. We are developing better methods for imaging central nervous system neuroinflammatory markers using targeted mRNA transcripts labelled with fluorescent nanodiamonds or lanthanide chelates. These tags have increased signal and photostability and can also discriminate against tissue/cell autofluorescence. Brains and spinal cords from BALB/c mice with a chronic constriction model of neuropathic pain (neuroinflammation group) or that have undergone sham surgeries (control group) were collected. A subset of brains and spinal cords were perfused and fixed with paraformaldehyde (n=3 sham and n=3 pain groups) prior to sectioning and in situ hybridization using nanodiamond or lanthanide chelate conjugated complementary RNA probes. Another subset of brains and spinal cords from the same cohort of animals were perfused and processed for CLARITY hydrogel based clearing prior to in situ hybridization with the same probes. We will present our findings on the photostability, sensitivity and discrimination from background tissue autofluorescence of our novel RNA probes, compared to traditional fluorophore tags.

9690-44, Session 11

Novel phosphorescent materials for in-vivo imaging of brain structure and function

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A number of approaches have been developed for in-vivo imaging of neural function at the time scale of action potentials and at the spatial resolution of individual neurons. Remarkable results have been obtained with optogenetics, although the need for genetic modification is an important limitation of these approaches. Similarly, voltage and ion-sensitive dyes allow for optical imaging of action potentials but toxicity remains a problem. Additionally, optical techniques are often only able to be used up to a limited depth.

Our preliminary work has shown that nanoparticles of common phosphorescent materials, believed to be generally non-toxic, specifically Lutetium oxide and Strontium aluminate, can be utilized for cellular imaging, for tomographic imaging, and that the particles can be designed to adhere to neurons. Additionally, Lutetium oxide has been shown to be highly X-ray luminescent, potentially allowing for imaging deep within the brain, if the particles can be targeted properly.

In ex-vivo experiments, we have shown that the phosphorescence of Strontium aluminate particles is significantly affected by electric fields similar in strength to those found in the vicinity of the cellular membrane of a neuron. This phenomenon is consistent with early published reports in the electroluminescence literature, namely the Gudden-Pohl effect. We will show results of the ex-vivo imaging and dynamic electrical stimulation experiments. We will also show some preliminary in vivo cell culture results, and will describe plans for future research, focusing on in vivo potential in both cell cultures and in animal models.

9690-45, Session 11

Network inference from functional experimental data

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Functional connectivity maps of neuronal networks are critical tools to understand how neurons form circuits, how information is encoded and processed by neurons, how memory is shaped, and how these basic processes are altered under pathological conditions. Current light microscopy allows to observe calcium or electrical activity of thousands of neurons simultaneously, yet assessing comprehensive connectivity maps directly from such data remains a non-trivial analytical task. There exist simple statistical methods, such as cross-correlation and Granger causality, but they only detect linear interactions between neurons. Other more involved inference methods inspired by information theory, such as mutual information and transfer entropy, identify more accurately connections between neurons but also require more computational resources.

We carried out a comparative study of common connectivity inference methods. The relative accuracy and computational cost of each method was determined via simulated fluorescence traces generated with realistic computational models of interacting neurons in networks of different topologies (clustered or non-clustered) and sizes (10-1000 neurons). To bridge the computational and experimental works, we observed the intracellular calcium activity of live hippocampal neuronal cultures infected with the fluorescent calcium marker GCaMP6f. The spontaneous activity of the networks, consisting of 50-100 neurons per field of view, was recorded from 20 to 50 Hz on a microscope controlled by a homemade software. We implemented all connectivity inference methods in the software, which rapidly loads calcium fluorescence movies, segments the images, extracts the fluorescence traces, and assesses the functional connections (with strengths and directions) between each pair of neurons. We used this software to assess, in real time, the functional connectivity from real calcium imaging data in basal conditions, under plasticity protocols, and epileptic conditions.

9690-46, Session 11

Multi-channel fiber photometry for population neuronal activity recording

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The development of calcium fluorescent probes, especially the genetically encoded calcium indicators (GECIs) has promoted the optical imaging and recording methods of measuring calcium levels as the proxies of cell-type specific neuronal activities. Compared to electrophysiology and other
impairments for better understanding of OSAH pathophysiology. Hemodynamics may adversely impact brain function, future study will found during OSAH. Since frequent variations/disturbances in cerebral oxygen saturation (SaO2) measured by finger pulse oximeter were also on both CBF and cerebral oxygenation. Large variations in arterial blood concentration: [HbO2]/[Hb]/[THC] during overnight nocturnal polysomnography (NPSG) diagnostic test for obstructive sleep apnea-hypopnea (OSAH). A fiber-optic probe was fixed on subject’s frontal head and connected to the DCS flow-oximeter through a custom-designed fiber-optic connector, which allowed us to easily connect/detach the optical probe from the subject when needed (e.g., the subject went to bathroom). To minimize the disturbance to the subject, the DCS flow-oximeter was remotely operated by a desktop located in the control room. The results showed that apneic events caused significant variations in cerebral oxygen saturation (SaO2) measured by finger pulse oximeter were also found during OSAH. Since frequent variations/disturbances in cerebral hemodynamics may adversely impact brain function, future study will investigate the correlations between these cerebral variations and functional impairments for better understanding of OSAH pathophysiology.

Obstructive sleep apnea-hypopnea results in significant variations in cerebral hemodynamics detected by diffuse optical spectroscopies

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The objective of this study was to adapt a novel near-infrared diffuse correlation spectroscopy (DCS) flow-oximeter for simultaneous and continuous monitoring of relative changes in cerebral blood flow (CBF) and cerebral oxygenation (i.e., oxygenated/deoxygenated/totai hemoglobin concentration: [HbO2]/[Hb]/[THC]) during overnight nocturnal polysomnography (NPSG) diagnostic test for obstructive sleep apnea-hypopnea (OSAH). A fiber-optic probe was fixed on subject’s frontal head and connected to the DCS flow-oximeter through a custom-designed fiber-optic connector, which allowed us to easily connect/detach the optical probe from the subject when needed (e.g., the subject went to bathroom). To minimize the disturbance to the subject, the DCS flow-oximeter was remotely operated by a desktop located in the control room. The results showed that apneic events caused significant variations in cerebral variables were significantly correlated with the severity of OSAH as determined by the apnea-hypopnea index (AHI), demonstrating the OSAH influence on both CBF and cerebral oxygenation. Large variations in arterial blood oxygen saturation (SaO2) measured by finger pulse oximeter were also found during OSAH. Since frequent variations/disturbances in cerebral hemodynamics may adversely impact brain function, future study will investigate the correlations between these cerebral variations and functional impairments for better understanding of OSAH pathophysiology.
the blood oxygenation level-dependent activity or cerebral blood flow or oxygenated hemoglobin (HbO) will occur in brain areas in order to support the behaviors such as synergy, sequencing and deftness. Most functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) studies to investigate the mechanisms underlying motor skill learning are using fingers or foot movements of subjects lying in the scan machines. However, functional near-infrared spectroscopy (fNIRS) can overcome this disadvantage that it enables participants to perform tasks either sitting in a chair or walking or running. Furthermore, using the near-infrared light in the spectrum of 700-900 nm, fNIRS can show the brain dynamic changes in regional cerebral blood flow which are elicited by neural activity by measuring the concentration of brain oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR). Besides, fNIRS has some advantages in reducing the electrical noise, low cost, easy and portability to use compared to other non-invasive measurement techniques.

In this study, we aim to explore the hemoglobin changes in activation around the primary motor cortex (M1), premotor cortex (PMC), supplementary motor area (SMA) and prefrontal cortex (PFC) during motor sequence learning using fNIRS. The results from the present study validated hemoglobin changes in the channels covering those brain areas.

9690-67, Session PMon
The hemodynamic changes in the human prefrontal cortex during the Flanker and Simon tasks: a fNIRS study
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To date, the neural mechanism underlying the executive function is unclear and still under extensive investigations. The aim of present study is to reveal the different brain activity pattern of adult subjects during the Flanker and Simon tasks underlying different conditions so as to identify the basic neural mechanism of executive function. In the study, we utilized fNIRS to explore the hemodynamic changes including the changes of HbO2, HbR and HbT in the prefrontal cortex, especially the dorsolateral prefrontal cortex (DLPFC) of human brain under the stimuli of two typical tasks, namely Flanker and Simon task. The Flanker task was employed here to reveal the neural features of selective attention while the Simon effect could capture the function of spatial incompatibility. Our pilot results showed that the hemodynamic changes in prefrontal cortex between the congruent condition and incongruent conditions during both the Flanker and Simon tasks are significantly different.

9690-68, Session PMon
Combination of hyperspectral imaging and skull optical clearing for dynamic oxygen saturation in cortical microvessels
Wei Feng, Chao Zhang, Rui Shi, Yanjie Zhao, Dan Zhu, Huazhong Univ. of Science and Technology (China)

The cerebral oxygen saturation is one of the most important parameters, which can affect the function of brain, such as neuronal activity. Hyperspectral imaging methods could extract the map of tissue oxygen saturation. However, the turbid skull limits the penetration of light, thus, the imaging quality was too bad to obtain the high-resolution map of cerebral oxygen saturation. Here, the skull optical clearing technique was used to make skull transparent temporarily without removing the skull or opening cranial windows through craniotomy. Firstly, the effect of skull optical clearing method on blood dynamics was assessed. The results suggested that the skull optical clearing solution has no significant influence on blood dynamics. Moreover, we quantitatively analyze the diameter, total blood flow (aCBF) and oxygen saturation in cortical microvessels during task performance. After injection, vasoconstriction was observed, besides, both HbT and SO2 decreased significantly. Therefore, combination of hyperspectral imaging and skull optical clearing techniques provides a method for dynamically monitoring the cerebral blood dynamics.

9690-69, Session PMon
Image intensity restoration for whole brain dataset of micro-optical sectioning tomography
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Optical microscopic imaging for whole brain tissue is a new filed in recent years. Systematic full-volume imaging is time-consuming, often takes about several days for one cubic centimeter of brain tissue. It is almost impossible for optical and mechanical scanning modules to keep consistent state during the data collection, and frequently distorts the image intensity information. The distortion, or degradation, will brings unacceptable errors in the following image analysis. We study the intensity restoration methods based on a lot of whole brain imaging datasets, which collected via the serial techniques of micro-optical sectioning tomography (MOST). We have corrected the intensity errors caused by uniform illumination, motion artifact and inadequate tissue staining at different levels, such as single field-of-view, complete section and image sequence. The setup of processing procedure is also automatic for different image features. Several whole mouse brain dataset were tested, and the intensity of the processed images were uniformly distributed throughout different brain areas. And these processed datasets are now fundamental atlas for automatic image analysis, including soma detecting, vascular tracing and neuron tracing. We also developed a parallel application based on Message Passing Interface (MPI) in a cluster computer to speed up the process for big image volume, and the optimized time-consumption is less than one day for 10-teravoxel raw data.

9690-70, Session PMon
Investigating vascular remodeling in the cerebral cortex of mice due to chronic cranial window
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Introduction: Microscopic imaging in awake mice has increasingly drawn interest in neuroscience studies. This is because, measurement acquired from awake animals reflect natural brain activity free of influences of anesthesia. Chronic cranial windows allow longitudinal studies in the same subject, increasing statistical power for investigation of normal neuronal plasticity, progression of brain disease, or effects of treatment. However, the procedure of chronic window implantation may cause perturbation of brain physiology with long lasting effects. Objective: Our goal would be to evaluate potential vascular remodeling and perfusion following standard implantation procedures. Animal Preparation: We implanted chronic cranial windows with or without removal of dura under Ketamine/Xylazine anesthesia. Vascular structure and perfusion were assessed in six imaging sessions up to one month after the implantation. Method & Experiment: Spectral-domain optical coherence tomography (SD-OCT) was employed to monitor the changes in vascular morphology and absolute cerebral blood flow (aCBF). For each mouse, first, SD-OCT angiogram was acquired covering an area of 1.8 x 1.8 mm within the chronic window to assess vascular morphology. More, volumetric Doppler-OCT imaging was conducted to estimate the aCBF. Our results provide evidence of the remodeling of cerebral microvasculature due to the cranial window installation. Careful evaluation of microvascular structure and function are important for
Functional brain imaging of moving mouse using fiber-based multi-channels near infrared spectroscopy (NIRS)

Young Kyu Kim, Seung-ho Paik, Beop-Min Kim, Korea Univ. (Korea, Republic of)

Near-infrared spectroscopy (NIRS) is a portable and non-invasive method for monitoring cerebral hemodynamic activities and it has been used extensively in small animal studies. Since the optical signals are very vulnerable to motion artifacts, NIRS studies have been limited to the immobile animals under general anesthesia fixed by stereotaxic apparatus. To overcome this limitation, we devised a new tool to measure cerebral hemodynamic activities in moving mice.

Our NIRS system comprises twelve laser diode light sources and twelve photodiode detectors with customized flexible fiber-optic delivery system at two wavelengths (785nm and 850nm). A customized two wavelengths mixer module has been used to account for the small size of the mouse brain. Therefore, a single fiber delivers both wavelengths at the same spot on the brain. To secure stable fiber tissue interface, we scan the scalp-removed mouse head using a 3D scanner. The resultant 3D model of skull is 3D printed which later modified to accommodate the fiber probes. The complete probe with optical fibers is attached on top of the skull and the interface is no longer affected by the movement of the mouse. We found that our NIRS system for small animals is a promising tool for various animal studies.

A single camera, two channel hemodynamic imaging of the mouse brain without removing skull

Sedef Erdogan, Seung-ho Paik, Young Kyu Kim, Beop-Min Kim, Korea Univ. (Korea, Republic of)

Various studies suggest that the hemodynamic responses in brain is strongly connected to neuronal activities. Cerebral hemodynamic activities are inferred by observing optical property changes around the isosbestic point (~800 nm) in the near infrared region. We devised a single camera based two channel imaging system that can detect the light at 780nm and 850nm reflected from the same area on the brain. In this way, similarly to the functional near infrared spectroscopy (fNIRS), the oxy-, deoxy- and total hemoglobin information can be extracted from the cortical surfaces. The camera that we used in this study is versatile and has a large number of pixels (up to 2048x2048 pixels) with high speed (up to 2565fps, when 87N pixels). The reflected signal from the cortical window is divided into two different paths, which are filtered by two different bandpass filters (780 and 850) and directed to the two different regions of the same camera. All the optics are adjusted to deliver the same sized images to the camera. We perform all the data analyses with MATLAB program. Due to a large penetration depth of the infrared light and the thin skull of the mouse, we were able to show real time imaging of hemodynamic changes within mouse brain in vivo without removing skull. Optical signals from the specific cortical regions corresponding to specific stimulus were successfully observed.

Cerebral hemodynamic correlates of blood-brain barrier integrity in hyperacute focal ischemia

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Blood-brain barrier (BBB) integrity is a key factor in the ischemic cascade and plays an important role in hyperacute ischemia recovery. Cerebral hemodynamic changes are closely linked with a potential for either exacerbating or evading BBB damage. Hence, it is crucial to understand the relationship between cerebral hemodynamic changes and integrity of the BBB. In this study, we adopted functional photoacoustic microscopy (fPAM) imaging to investigate the functional hemodynamic changes after focal ischemia in a middle cerebral artery occlusion (MCAo) model in the rat brain. In addition, a novel non-toxic nanoparticle was applied as a probe for evaluating BBB integrity. A 50-MHz dark field confocal acoustic-resolution PAM system was employed to image functional changes of the brain with 32 µm axial resolution and 61 µm lateral resolution. Two visible wavelengths of laser pulses, 560 and 570 nm (?560 and ?570), were used for PA wave excitation to acquire information on cerebral blood volume (CBV) and hemoglobin oxygen saturation (SO2) for evaluating the cerebrovascular changes following MCAo stroke. The results indicated that cerebrovascular changes following reperfusion at different timings were conversely proportional to the trend of BBB integrity in the hyperacute phase of ischemia. Moreover, delayed reperfusion caused more damage than permanent ischemia. Thus, the combined evaluation of vascular and BBB integrity by the fPAM system opens a new window into the understanding of hyperacute ischemia and therapy.
Ablation efficiency and thermal damage of infrared lasers on ex vivo lamp brain tissues

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The objective of this investigation is to guide to select the most sufficient infrared laser for the neurosurgery. For this reason, 1940-nm thulium ??fiber laser, 1470- nm diode laser, 1070-nm ytterbium ??fiber laser and 980-nm diode laser were operated with the ex vivo lamb brain tissues. Combination of some parameters such as brain tissue (subcortical and cortical tissues), laser output power, energy density, mode of operation (continuous and pulsed-modulated modes) and exposure time were applied. Pre-dosimetry study was conducted to determine coagulation and carbonization onset times for the lamb brain tissues. In this way, safe operation zone could be described for the dosimetry study. In the dosimetry study, both tissues were exposed to some energy densities (2J-4J) and power levels which are 200mW-400mW-600mW-800 mW and 0.5W-1W-1.5W-2W for 1940-nm and 1470-nm laser applications, respectively. The last two laser emitted light to both brain tissues with some power levels (1W-2W-3W-4W) and energy densities (20J-40J). After each laser application, coagulation and ablation diameters were calculated under a light microscope. It was aimed to ??and suitable laser parameter so as to perform the greatest ablation efficiency which is determined as ablation diameter over coagulated diameter. Consequently, 1940-nm and 1470-nm lasers created ablated and coagulated areas while the other two lasers made only coagulated areas. Ablation efficiencies were calculated for 1940-nm and 1470-nm lasers. It was found that the former and the latter can be used as a subcortical and cortical tissue ablator, respectively.

Ultra-high resolution polarization-sensitive optical coherence microscopy for brain imaging at 6 um, 3.4 um and 1.3 um resolution

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Neuroanatomical pathways form the basis for functional activity of brain circuits. In the past, we developed a polarization-sensitive optical coherence tomography with serial scanning to achieve large-scale brain imaging. The system was able to visualize 3D fiber tracts of ~20 um in diameter. To investigate the neuroanatomical pathways at finer scales, we have now built a polarization-maintaining fiber-based ultra-high resolution polarization-sensitive optical coherence microscope (PS-OCM) at 1300 nm. The PS-OCM has an axial resolution of 3.5 um in tissue. The detection setup consists of two spectrometers, acquiring spectral interference on orthogonal polarization channels. With a single measurement, the setup generates four contrasts: reflectivity, cross-polarization, retardance and optic axis orientation. To investigate the capability of PS-OCM at different resolutions, we used three microscope objectives that yield lateral resolutions of 6.0 um, 3.4 um and 1.3 um. Blocks of formalin fixed mouse brain and human brain were scanned. The cross-polarization and retardance images clearly depict the neuronal fiber structures, which are comparable with that generated by the maximum projection of volumetric reflectivity data. The optic axis orientation quantifies the in-plane fiber orientation. With the lateral resolution of 1.3 um, the retardance contrast is weak in white matter due to the shallow depth of focus. Overall, the ultra-high resolution PS-OCM provides a new tool to reveal neuroanatomical maps in the brain at cellular resolution.

Assessing the effects of electrical stimulation on peripheral nerve vasculature using speckle variance optical coherence angiography

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The peripheral nervous system (PNS) performs bidirectional communication between the central nervous system and distal organs. PNS stimulation has been used widely in medical devices for therapeutic indications such as bladder control and seizure cessation. Investigational uses of PNS stimulation include providing sensory feedback for improved control of prosthetic limbs. While nerve safety has been well documented for stimulation parameters used in marketed devices, novel PNS stimulation devices may require alternative stimulation parameters to achieve maximum therapeutic benefit. Improved testing paradigms to assess the safety of stimulation will expedite the development process for novel PNS stimulation devices. The objective of this research is to develop a test platform using optical coherence angiography (OCA) and optical Doppler tomography, to assess peripheral nerve injury in real-time. In this paradigm, the harmful effects of ischemia are predicted through monitoring of vascular constriction and blood flow during stimulation of various intensities. A 1300nm OCA system was used to image blood vasculature changes around electrodes in the rat sciatic nerve in-vivo. The sciatic nerve was exposed and stabilized using a 3D-printed nerve-holder, designed to align a segment of the nerve for imaging, while minimizing motion-artifact. Stimulation...
A platinum electrode was applied through a cuff electrode on the distal end to record the compound action potential in an ex vivo Xenopus Laevis sciatic nerve. The model nerve is electrically large axons (e.g., giant squid axon), and in this study, we explore this technique for imaging and observing changes in neuron structure that has been hypothesized to be expanded to functional imaging at a single neuron level. Considering these results, it has been observed that, due to its high spatial and temporal resolution, OCT is widely used in tissues including the brain.

**Quantifying axis orientation in 3D using polarization-sensitive optical coherence tomography**

Chao Liu, Adam J. Black, Hui Wang, Taner Akkin, Univ. of Minnesota, Twin Cities (United States)

The optic axis of birefringent tissues indicates the direction of structural anisotropy. Polarization-sensitive Optical Coherence Tomography (PS-OCT) can provide reflectivity contrast as well as retardance and optic axis orientation contrasts that originate from tissue birefringence. We introduce imaging 3D tissue anisotropy by using a single-camera and polarization-maintaining fiber (PMF) based PS-OCT, which utilizes normal and angled illuminations.

Because environmental factors such as the movement of PMF and temperature fluctuations induce arbitrary phase changes, the optic axis orientation measurement has a time-varying offset. In order to measure the absolute axis orientation, we add a calibration path which dynamically provides the arbitrary offset to be subtracted from the relative axis orientation values.

The axis orientation on the normal plane is the 2D projection of the fiber direction in 3D space. We propose to characterize the axis orientation in different planes (xy, x'y and x'y planes) by using normal and angled illuminations. This allows calculation of the polar angle that completes the orientation information in 3D. Polarization-based optical systems relying on one illumination angle measure the “apparent birefringence” that light encounters rather than the “true birefringence”. Birefringence as a measure of anisotropy is quantified with the orientation information in 3D. The method and validation with a biological tissue are presented. The study can facilitate imaging and mapping the structural connections in anisotropic tissues including the brain.

**Optical coherence tomography for detection of compound action potential in Xenopus Laevis sciatic nerve**

Francesca Troiani, Konstantin Nikolic, Timothy G. Constandinou, Imperial College London (United Kingdom)

Optical Coherence Tomography (OCT) is a minimally invasive technique that, due to its high spatial and temporal resolution, has been widely used to study different biological specimens, including cardiac, gastric, and brain tissues. Astonishing results have been obtained in the latter, as it has been possible to image at a single neuron level. Considering these results, it has been hypothesized that this technique might be expanded to functional imaging and be used to observe the changes in neuron structure that happen during neuronal activity. While the state of the art research on the use of OCT for real-time action potential detection focuses on single large axons, in this study, we explore this technique on ex vivo Xenopus Laevis sciatic nerve. The model nerve is electrically stimulated using a cuff electrode wrapped around its proximal end. Another cuff electrode on the distal end records the compound action potential generated in the nerve. The optical setup is a free space time domain OCT whose light source is a halogen lamp and the detector is a Si photodiode with low rise and fall time. An OCT image of the nerve is taken before and during stimulation to observe the changes in the detected interferometric pattern. The study includes theoretical investigation into light illumination and noise tolerance requirements. Regarding the proposed technique and our experimental setup, we believe this approach can pave the way to new peripheral nerve recordings, in which signals from single fibers could be detected.

**Statistical parametric mapping of stimuli-evoked changes in quantitative blood flow using extended-focus optical coherence microscopy**


Magnetic Resonance Imaging has revolutionised our understanding of brain function through its ability to image human cerebral structures non-invasively over the entire brain. By exploiting the different magnetic properties of oxygenated and deoxygenated blood, functional MRI can indirectly map areas undergoing neural activation. Alongside the development of fMRI, powerful statistical tools have been developed in an effort to shed light on the neural pathways involved in processing of sensory and cognitive information. In spite of the major improvements made in fMRI technology, the obtained spatial resolution of hundreds of microns prevents MRI in resolving and monitoring processes occurring at the cellular level. In this regard, Optical Coherence Microscopy is an ideal instrumentation as it can image at high spatio-temporal resolution. Moreover, by measuring the mean and the width of the Doppler spectra of light scattered by moving particles, OCM allows extracting the axial and lateral velocity components of red blood cells. The ability to assess quantitatively total blood velocity.
as opposed to classical axial velocity Doppler OCM, is of paramount importance in brain imaging as a large proportion of cortical vascular is oriented perpendicularly to the optical axis. We combine here quantitative blood flow imaging with extended-focus Optical Coherence Microscopy and Statistical Parametric Mapping tools to generate maps of stimuli-evoked cortical hemodynamics at the capillary level.

9690-54, Session 13

Optical microangiography enabling visualization of change in meninges after traumatic brain injury in mice in vivo

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Traumatic brain injury (TBI) is a form of brain injury caused by sudden impact on brain by an external mechanical force. Following the damage caused at the moment of injury, TBI influences secondary pathophysiology in brain that take place within the minutes or hours including alterations in the brain tissue, cerebral blood flow (CBF), and pressure within skull, which become important contributors to morbidity after TBI. Despite many studies for the TBI pathophysiology, the effect of trauma on intracranial tissues has been less well studied. Here, we report use of high-resolution optical microangiography (OMAG) to monitor the changes in cranial meninges beneath the skull of mice after TBI. TBI is induced on a brain of anesthetized mouse by thinning the skull using a soft drill where a series of drilling exert mechanical stress on the brain through the skull, resulting in mild brain injury. Intracranial OMAG imaging of the injured mouse brain during post-TBI phase shows interesting pathophysiological findings in the meningeal layers such as widening of subdural space as well as vasodilation of subarachnoid vessels. These processes are acute and reversible within hours. The results indicate potential of OMAG to explore mechanism involved following TBI on small animals in vivo.

9690-55, Session 13

Study the neurovascular effects of the integrated therapeutic intervention for ischemic stroke by using the ECoG-fPAM system

Yu-Hang Liu, Aishwarya Bandla, Nitish V. Thakor, Lun-De Liao, National Univ. of Singapore (Singapore)

Study of cerebral neurovascular functions affected by ischemic stroke explicates post-stroke brain plasticity. In this study, we report an innovative combination of the functional photoacoustic microscopy (fPAM) imaging with electrocorticography (ECoG) recordings (i.e., ECoG-fPAM system) to not only investigate the hemodynamics but also neural activities after photothermal stroke in the rat brain. In addition, an integration of transcranial direct current stimulation (tDCS) with peripheral sensory stimulation (PSS) is applied as a therapeutic intervention for protecting the affected ischemic region. A 50-MHz dark field confocal acoustic-resolution PAM system is used to image functional changes of the brain with 32 µm axial resolution and 61 µm lateral resolution, while two visible wavelengths of laser pulses, 560 and 570 nm (7600 and 7570), are employed for PA wave excitation. For ECoG recordings, 2 stainless steel epidural electrodes are secured on the skull to acquire somatosensory-evoked potentials (SEEPs) and resting-state ECoG signals, which are pre-amplified and recorded using a commercial bio-signal processor with 1 kHz sampling rate. Using the ECoG-fPAM system, the information of neurovascular functions, including SEEPs, alpha-to-delta ratio (ADR), cerebral blood volume (CBV) and hemoglobin oxygen saturation (SO2) can be acquired for evaluating the influence of tDCS, as well as effects of the proposed intervention. The results demonstrate that the integrated treatment can promote neural protection significantly via inhibition of cortical spreading depression and reversed cortical functions, suggesting effective recovery. Overall, the ECoG-fPAM system holds great potential in disease models involving impairments in neurovascular functions.

9690-56, Session 13

In vitro and in vivo analysis and characterization of engineered spinal neural implants

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Spinal cord injury is a devastating medical condition. Recent developments in pre-clinical and clinical research have started to yield neural implants inducing functional recovery after spinal cord transection injury. However, the functional performance of the transplants was assessed using histology and behavioral experiments which are unable to study cell dynamics and the therapeutic response. Here, we use neurophotic tools and optogenetic probes to investigate cellular level morphology and activity characteristics of neural implants over time at the cellular level. These methods were used in-vitro and in-vivo, in a mouse spinal cord injury implant model. Following previous attempts to induce recovery after spinal cord injury, we engineered a pre-vascularized implant to obtain better functional performance. To image network activity of a construct implanted in a mouse spinal cord, we transfected the implant to express GCAMP6 calcium activity indicators and implanted these constructs under a spinal cord chamber enabling 2-photon chronic in vivo neural activity imaging. Activity and morphology analysis image processing software was developed to automatically quantify the behavior of the neural and vascular networks. Our experimental results and analyses demonstrate that vascularized and non-vascularized constructs exhibit very different morphologic and activity patterns at the cellular level. This work enables further optimization of neural implants and also provides valuable tools for continuous cellular level monitoring and evaluation of transplants designed for various neurodegenerative disease models.

9690-57, Session 14

Short infrared (IR) laser pulses can cause nanoporation-induced activation of the IP3 pathway

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Short infrared (IR) laser pulses on the order of hundreds of microseconds to single milliseconds with typical wavelengths of 1800-2100 nm, have shown the capability of reversibly stimulating action potentials in neuronal cells. While the IR stimulation technique has proven successful for several applications, the exact mechanism(s) underlying the action potential generation has remained elusive. To better understand how IR pulses cause action potential stimulation, we first determined the threshold for the formation of nanopores in the plasma membrane. Using a surrogate calcium ion, thallium, which is roughly the same shape and charge, but lacks the biological functionality of calcium, we recorded the flow of thallium ions into an exposed cell in the presence of a battery of channel blockers. The entry of thallium into the cell indicated that the ions entered via nanopores. We believe that the flow of calcium into the cell via these same outer membrane nanopores leads to the activation of specific intracellular signaling pathways, in particular the inositol triphosphate (IP3) signaling pathway, which in
Infrared laser light radiation can be used to depolarize neurons and to stimulate neural activity. The absorption of infrared radiation and heating of biological tissue is the underlying mechanism of this phenomenon through local temperature increases in the plasma membrane of cells and either direct effects on membrane properties or temperature sensitive ion channels. Action potentials are typically measured electrically in neurons with microelectrodes, but they can also be observed using fluorescence microscopy techniques using synthetic or genetically encoded calcium indicators. In this work, we studied the impact of infrared laser light on neuronal calcium signals to address the mechanism of these thermal effects. Cultured primary mouse hippocampal neurons expressing the genetically encoded calcium indicator GCaMP6s were used in combination with the temperature sensitive fluorophore Rhodamine B to measure calcium signals and temperature changes at the cellular level. Here we present our all optical approach for studying the influence of infrared laser light neuronal activity.

Selective control of small versus large diameter axons using infrared laser light

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Sensory information is conveyed to the central nervous system via small diameter unmyelinated fibers. In general, smaller diameter axons have slower conduction velocities. Selective control of such fibers could create new clinical treatments for chronic pain, nausea in response to chemotherapy agents, or hypertension. Electrical stimulation can control axonal activity, but induced axonal current is proportional to cross-sectional area, so that large diameter fibers are affected first. Physiologically, however, synaptic inputs generally affect small diameter fibers before large diameter fibers (the size principle). A more physiological modality that first affected small diameter fibers could have fewer side effects (e.g., not recruiting excitatory cells). However, studies of infrared light for excitatory cell inhibition have been constrained by the use of invasive and cumbersome electrodes for cell excitation and/or action potential recording. Here, we present an all optical experimental design for neuronal excitation, inhibition, and action potential detection. Primary murine neurons were transfected with plasmids containing the light sensitive ion channel, hChR2. hChR2 has a peak excitation around 480 nm, allowing excitation of transfected neurons by fiber delivery of pulsed blue light from a diode. Calcium imaging was performed at high temporal resolution on a confocal microscope. Neurons were excited by fiber delivery of pulsed blue light from a diode. Calcium imaging was performed at high temporal resolution on a confocal microscope. Neurons were excited by fiber delivery of pulsed blue light from a diode. Calcium imaging was performed at high temporal resolution on a confocal microscope.

Modeling the effect of elevated temperatures on action potential propagation in unmyelinated axons

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Infrared lasers (1.87 µm) are capable of inducing a thermally mediated nerve block in Aplysia and rat nerves. While this block is spatially precise, reversible and safe in sensory and motor neurons, the mechanism is not clearly understood. A model combining NEURON with Python is presented that can simulate the behavior of unmyelinated nerve axons in the presence of spatially and temporally varying temperature distributions. Model predictions show that as a function of temperature, the rates of opening and closing of the voltage gated ion channels are disrupted and normal functioning of the gates is hindered. The threshold temperature required to produce complete arrest of a propagating action potential decreases as the length of temperature elevation along the nerve (block width) increases which can be controlled by the diameter of the fiber optic delivering the light. Both the block width and distribution of the temperature profile influence blocking thresholds. For example, a top-hat temperature distribution will require a smaller block width for inhibition compared to a Gaussian distribution. The threshold block temperature escalates with increase in the axon diameter. This explains the observation of preferential block of smaller diameter axon fibers in Aplysia. The ability to combine the photothermal interaction of laser light and the temperature dependence of the ion channels in-silico will help speed explorations of parameter space and guide future experiments testing the feasibility of selectively blocking pain conduction fibers (Photonic Analgesia of Nerves (PAIN)) in humans.

All optical experimental design for neuron excitation, inhibition, and action potential detection

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Recently, infrared light has been shown to both stimulate and inhibit excitatory cells. However, studies of infrared light for excitatory cell inhibition have been constrained by the use of invasive and cumbersome electrodes for cell excitation and/or action potential recording. Here, we present an all optical experimental design for neuronal excitation, inhibition, and action potential detection. Primary murine neurons were transfected with plasmids containing the light sensitive ion channel, hChR2. hChR2 has a peak excitation around 480 nm, allowing excitation of transfected neurons with blue light. Action potentials were detected by fluorescence imaging of a calcium dye, Calcium Orange, selected to minimize spectral overlap with hChR2 during simultaneous neuron excitation and calcium imaging. Neurons were excited by fiber delivery of pulsed blue light from a diode. Calcium imaging was performed at high temporal resolution on a confocal microscope. Calcium flux analysis confirmed blue-light induced action potentials of the transfected neurons. Finally, neuron inhibition was achieved by fiber delivery of pulsed infrared light at 1868 nm. Neurons were exposed to simultaneous pulses of blue light (for stimulation) and infrared light (for inhibition), and the parameters of infrared laser power, pulse duration, and
pulse frequency to inhibit neuron excitation defined. When used in concert, these optical techniques enable electrode-free neuron excitation, inhibition, and action potential recording, enabling research of neuronal behaviors with high spatial fidelity.

9690-62, Session 14

**Analysis of optical neural stimulation effects on neural networks affected by neurodegenerative diseases**

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The number of people in risk of developing a neurodegenerative disease increases as the life expectancy grows due to medical advances. Multiple techniques have been developed to improve patient’s condition, from pharmacological to invasive electrodes approaches, but no definite cure has yet been discovered.

In this work Optical Neural Stimulation (ONS) has been studied. ONS stimulates noninvasively the outer regions of the brain, mainly the neocortex. The relationship between the stimulation parameters and the therapeutic response is not totally clear. In order to find optimal ONS parameters to treat a particular neurodegenerative disease, mathematical modeling is necessary. Neural networks models have been employed to study the neural spiking activity change induced by ONS. Healthy and pathological neocortical networks have been considered to study the required stimulation to restore the normal activity. The network consisted of a group of interconnected neurons, which were assigned 2D spatial coordinates. The optical stimulation spatial profile was assumed to be Gaussian. The stimulation effects were modeled as synaptic current increases in the affected neurons, proportional to the stimulation fluence. Pathological networks were defined as the healthy ones with some neurons being inactivated, which presented no synaptic conductance. Neurons’ electrical activity was also studied in the frequency domain, focusing specially on the changes of the spectral bands corresponding to brain waves. The complete model could be used to determine the optimal ONS parameters in order to achieve the specific neural spiking patterns or the required local neural activity increase to treat particular neurodegenerative pathologies.
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9690-76, Session 15

**Engineering brighter probes for advanced cellular imaging** *(Keynote Presentation)*
Luke Lavis, Howard Hughes Medical Institute (United States)

No Abstract Available

9690-77, Session 15

**Design considerations for miniaturized optical neural probes**
Linda Rudmann, Juan S. Ordonez, Thomas Stiegliitz, Univ. of Freiburg (Germany)

Neural probes are designed to selectively record from or stimulate nerve cells. Optogenetics is a method that allows cell type specific activation or inhibition of genetically modified nerve cells depending on the wavelength of light. It is desirable to build miniaturized and long-term stable optical neural probes, in which the light sources (i.e. LED chips) can be directly and chronically implanted into the animals to allow free movement and behavior. Because of the size and the beam shape of the available light sources, it is difficult to target single cells, whose sizes vary in the range of 4 to 100 µm, as well as spatially localized networks. We therefore propose design considerations for packages, which encapsulate the light source hermetically and have integrated hemispherical lens structures that enable to focus the light onto the desired region. Considered lens materials are fused silica (n=1.46 @ 470nm), sapphire (n=1.77 @ 470nm) and silicon carbide (n=2.69 @ 488nm). Parameters taken into account are diameter and focal lengths of the lenses, as well as the best suited distance of the light source to the lens and the lens to the tissue. Available lens diameters range from 25 µm to 1300 µm and offer therefore a high degree of freedom in terms of focal lengths. A single as well as a two lens setup is investigated over the visible range (400nm–700nm). The theoretical calculations are compared with the results of optical ray tracing simulations and lenses fabricated on our own by thermal reflow of photoresists.

9690-78, Session 15

**Modulation of Channelrhodopsin-2 mediated currents by femtosecond pulse shaping** *(Invited Paper)*
Kush Paul, Univ. of Illinois at Urbana-Champaign (United States); Eugene D. Ark, Univ. of Illinois at Urbana Champaign (United States); Haohua Tu, Youbo Zhao, Yuan-Zhi Liu, Javier I. Suarez, Parijat Sengupta, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Coherent control of broadband femtosecond pulses by an optical pulse shaper can spectrally/temporally shape or “tailor” light. We demonstrate that pulse compression of broadband femtosecond light pulses generated by pumping a nonlinear photonic crystal fiber (PCF) produces significantly larger whole-cell ionic currents in ChR2-expressing neurons. Further, the addition of a linear chirp to the spectral phase function can modulate this current, and differences between up- and down-chirped pulses evoke different responses that cannot be explained by two-photon absorption alone. A laser source (High-Q) generated ~250 fs pulses at a center wavelength of 1040 nm, which were coupled into a PCF and stably broadened before pulse shaping. Whole-cell patch-clamp recordings were obtained from pyramidal neurons from slices obtained from the prefrontal and somatosensory neocortex of mice (P12-45). Light evoked ChR2 currents in the voltage clamp configuration were also measured in the presence of TTX (0.5 µm). We systematically varied the coefficients of the second, third, and fourth order terms in the spectral phase function to produce complex temporal pulse shapes, and measured the response of the neuron to these complex pulses. Varying the third and fourth order terms alone produced little or no change in the neuronal response, but in combination with linear chirp, produced significant changes in the current response properties. These results suggest that principles of coherent control and femtosecond pulse shaping may enable new capabilities for modulation and control of cells that have been genetically modified to express ChR2.

9690-79, Session 15

**Activation of cells using femtosecond laser beam**
Subrata Batabyal, Sarmishtha Satpathy, Young-tae Kim, Samarendra K. Mohanty, The Univ. of Texas at Arlington (United States)

Study of communication in cellular systems requires precise activation of targeted cell(s) in the network. In contrast to chemical, electrical, thermal, mechanical stimulation, optical stimulation is non-invasive and is better suited for stimulation of targeted cells. As compared to visible lasers, the near infrared (NIR) microsecond/nanosecond pulsed laser beams are being used as preferred stimulation tool as they provide higher penetration depth in tissues. Femtosecond (FS) laser beams in NIR are also being used for direct and indirect (i.e. via two-photon optogenetics) stimulation of cells. Here, we present a comparative evaluation of efficacy of NIR FS laser beam for direct (no optogenetic sensitization) and 2ph optogenetic stimulation of cells. Further, for the first time, we demonstrate the use of blue (~450 nm, obtained by second harmonic generation) FS laser beam for stimulation of cells with and without Channelrhodopsin-2 (ChR2) expression. Comparative analysis of photocurrent generated by blue FS laser beam and continuous wave blue light for optogenetics stimulation of ChR2 transfected HEK cells will be presented. The use of ultrafast laser micro-beam for focal, non-contact, and repeated stimulation of single cells in a cellular circuitry allowed us to study the communication between different cell types.

9690-80, Session 15

**Targeted illumination and tracking using optical fiber probe for optogenetics application**
Anant B. Shinde, Sandeep M. Perinchery, Vadakke Matham Murukeshan, Nanyang Technological Univ. (Singapore)

In life science, imaging systems for tracking are widely used in various research applications. Nonspecific illumination during tracking can have adverse effects such as heating of sample, reduced image contrast and photo bleaching. In fact, currently available targeted tracking and imaging systems for freely moving particles cannot selectively illuminate only the particle being tracked. To fill this void, we have developed a fiber optic probe with controlled illumination on the targeted particle. The probe
consists of an imaging fiber bundle which performs the dual function of illumination and that of an image conduit. Spatial light modulator (SLM) is used to couple light into fiber probe for selective illumination. GRIN lens is attached at the tip of the fiber bundle for optimized illumination and collection. Adaptive tracking algorithm is used to track particle which changes its shape dynamically. Output of the tracking algorithm is designed to control the spatial light modulator in order to target the illumination point or location in accordance with the particle movement and size variation. The excitation source used is a multi-line laser light source which enables illumination of particle simultaneously with different wavelengths. Further with this probe, particles can be illuminated with light pulses of controllable duty cycle and frequency. Particle tracking with controlled multispectral illumination method will be of great significance in many areas such as optogenetic studies, cell signalling studies and cell migrations.

9690-81, Session 15
Spatially controlled optogenetic light stimulation and recording platform via imaging fiber bundles
Javier I. Suárez, Parijat Sengupta, Jonathan Guo-Han Mun, Rajashekar Iyer, Justin Rhodes, Martha U. Gillette, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Current methods for light delivery in in vivo optogenetic applications are typically accomplished via a single multimode fiber that diffuses light over a large area of the brain, and relies on the spatial distribution of transected light-sensitive neurons for targeted control.

In our investigations, an imaging fiber bundle (Schott) containing 4,500 individual fibers, each with a diameter of 7.5 µm, and an overall outer bundle diameter of 530 µm, served as a conduit for light delivery and optical recording/imaging. The use of this fiber bundle, in contrast to a single multimode fiber, allows for individually-addressable fibers, spatial selectivity at the stimulus site, more precise control of light delivery, and full field-of-view imaging and/or optical recordings of individual neurons in local neural circuits. An objective coupled the two continuous wave diode laser sources (561nm/488nm) (Coherent) for stimulation and imaging into the fiber bundle while a set of galvanometer-scanning mirrors was used to couple the light stimulus to distinct fibers within the proximal end of the imaging fiber bundle. In our study, C1V1(E122T/E162T)-TS-p2A-mCherry (Karl Deisseroth, Stanford) and GCaMP6s transgenic mice (Jackson Labs) were utilized for this all-optical approach.

The results of our investigation demonstrate that imaging fiber bundles provide a new level of spatial selectivity and control of light delivery to specific neurons, as well as function as a conduit for optical imaging and recording at the in vivo site of stimulation, in contrast to the use of single multimode fibers that diffusely illuminate neural tissue and lack in vivo imaging capabilities.

9690-82, Session 16
Optogenetic stimulation of myelination
In Hong Yang, Johns Hopkins Univ. (United States) and National Univ. of Singapore (Singapore); Hae Ung Lee, Nitish V. Thakor, National Univ. of Singapore (Singapore)

Myelination is governed by axon-glia interaction which is modulated by neural activity. Currently, the effects of subcellular activation of neurons which induce neural activity upon myelination are not well understood. To identify if subcellular neuronal stimulation can enhance myelination, we developed a novel system for focal stimulation of neural activity with optogenetic in a compartmentalized microfluidic platform. In our systems, stimulation for neurons in restricted subcellular parts, such as cell bodies and axons promoted oligodendrocyte differentiation and the myelination of axons the just as much as whole cell activation of neurons did. The number of premature O4 positive oligodendrocytes was reduced and the numbers of mature and myelin basic protein-positive oligodendrocytes was increased both by subcellular optogenetic stimulation.

9690-83, Session 16
Nano-enhanced optical delivery into targeted cells
Weldon Wright, Sanjay Pradhan, Nanoscope Technologies, LLC (United States)

Nano-enhanced optical field of gold nanoparticles allowed the use of a continuous wave (cw) laser beam for efficient delivery of exogenous impermeable materials into targeted cells. Using this Nano-enhanced Optical Delivery (NOD) method, we show that large molecules could be delivered with low power cw laser with exposure time ~ 1sec. At such low power (and exposure), the non-targeted cells (not bound to gold nanoparticles) were not adversely affected by the laser beam. Further, by varying the size of the gold nanoparticles, cells could be exclusively sensitized to selective wavelengths of laser beam. In contrast other nanoparticles, gold nanoparticles were found to have lower cytotoxicity, making it better suited for clinical NOD. Further, as compared with pulsed lasers, cw (diode) lasers are compact, easy-to-use and therefore, NOD using cw laser beam has significant translational potential for delivery of impermeable bio-molecules to tissues in different organs. We will present optimization of NOD parameters for delivering different molecules to different cells. Success of this NOD method may lead to a new clinical approach for treating AMD and RP patients with geographic atrophy in retina.

9690-84, Session 16
Optogenetic stimulation of multiwell MEA plates for neural and cardiac applications
Isaac P. Clements, Daniel C. Millard, Anthony M. Nicolini, James D. Ross, Axion BioSystems (United States)

Microelectrode array (MEA) plates enable distributed voltage recordings from cultured neuronal and cardiac cell networks. Multwell MEA systems (48, 96, or more MEA wells per microplate) make these recordings possible on a high-throughput scale, at reduced time and cost per experiment. While microelectrodes can be used to stimulate cells electrically, optogenetic stimulation enables targeting of specific cell types, suppression of activity, minimal stimulus artifact, and uniform stimulus delivery across a culture. These capabilities enable enhanced disease modeling, cell and drug screening, and control over developing stem cells.

Here, we describe a multiwell optical stimulation system for independent control of light delivery to each well of a multiwell microplate or MEA. The system delivers up to four wavelengths of light per well with precise control over timing and intensity. Using human induced pluripotent stem cell (hiPSC) derived cardiomyocytes and neurons, we demonstrate multiwell light-based excitation through expression of Channelrhodopsin-2 (ChR2) and suppression of activity through expression of Archaerhodopsin (Arch1). The system’s multiwell architecture enables rapid evaluation of light evoked activity metrics to assay network characteristics in response to pharmacological manipulations or other experiment conditions. Optogenetic stimulation also enables tuning of networks states across cultures (e.g. mean firing rate or cardiac beat rate) to improve assay sensitivity. These findings demonstrate the potential of optically-integrated multiwell MEA systems to enable high-throughput drug screening and phenotypic modeling of disease.
9690-85, Session 16

**Label free detection of optogenetically stimulated cellular activity by low coherence interferometry**

Sarmishtha Satpathy, Subrata Batabyal, Digan P. Dave, Samarendra K Mohanty, The Univ. of Texas at Arlington (United States)

Detecting cellular activity in sub-millisecond timescale and micrometer resolution without using invasive means has been a long standing goal in the study of cellular networks. Here, we have employed phase sensitive low coherence interferometry for detecting optogenetically stimulated activity of cells. Nanoscale changes in optical path length (due to change in refractive index and changes in cell thickness) occur when cells are activated, which we aim to detect by phase sensitive low coherence interferometry. A low coherence interferometry and patch-clamp electrophysiology systems were integrated with an inverted fluorescence microscope. Blue laser beam was coupled to the electrophysiology-interferometric detection system for optogenetic stimulation. The phase-sensitive measurements were carried out on Channelrhodopsin-2 sensitized cells (identified by YFP fluorescence) as well as control cells in reflection mode for different intensities and exposures of optogenetic stimulation beam. This method offers good temporal and spatial resolution without using exogenous labeling. Results of studies on all optical stimulation and detection of cellular activity will be presented. Interpretation of the optical activity signals will be discussed in context with changes in cell physiology during stimulation. We will also discuss the potential sources of various artifacts in optical/electrical detection of cellular activity during optical stimulation.

9690-86, Session 16

**Optogenetic control of the cardiac conduction system**

Claudia Crocini, European Lab. for Non-linear Spectroscopy (Italy); Cecilia Ferrantini, Raffaele Coppini, Univ. degli Studi di Firenze (Italy); Leslie M. Loew, Univ. of Connecticut Health Ctr. (United States); Elisabetta Cerbai, Corrado Poggesi, Univ. degli Studi di Firenze (Italy); Francesco S. Pavone, Leonardo Sacconi, European Lab. for Non-linear Spectroscopy (Italy)

Fatal cardiac arrhythmias are a major medical and social issue in Western countries. Current implantable pacemaker/defibrillators have limited effectiveness and are plagued by frequent malfunctions and complications. Here, we aim at setting up a new method to map and control the electrical activity of whole isolated mouse hearts. We employ a transgenic mouse model expressing Channelrhodopsin-2 (ChR2) in the heart coupled with voltage optical mapping to monitor and control action potential propagation. The whole heart is loaded with the fluorinated red-shifted voltage sensitive dye (di-4-ANBDQPS) and imaged with the central portion (128 x 128 pixel) of a CMOS camera operating at frame rate of 1.6 kHz. The wide-field imaging system is implemented with a random access ChR2 activation developed using two orthogonally-mounted acousto-optical deflectors (AODs). AODs rapidly scan different sites of the sample with a commutation time of 4 Ts, allowing us to design ad hoc ChR2-stimulation pattern. First, we demonstrate the capability of our system in manipulating the conduction system of the whole mouse heart by changing the electrical propagation features. Then, we explore the efficacy of the random access ChR2 stimulation in inducing arrhythmias as well as to restore the cardiac sinus rhythm during an arrhythmic event. This work shows the potentiality of this new method for studying the mechanisms of arrhythmias and reentry in healthy and diseased hearts, as well as the basis of intra-ventricular dyssynchrony.

9690-87, Session 16

**Optogenetic pacing in Drosophila melanogaster**

Aneesh Alex, Jing Men, Chao Zhou, Lehigh Univ. (United States)

A non-invasive, contact-less cardiac pacing technology can be a powerful tool in basic cardiac research and in clinics. Currently, electrical pacing is the gold standard for cardiac pacing. Although highly effective in controlling the cardiac function, the invasive nature, non-specificity to cardiac tissues and possible tissue damage limits its capabilities. Optical pacing of heart is a promising alternative, which is non-invasive and more specific, has high spatial and temporal precision, and avoids shortcomings in electrical stimulation. Optical coherence tomography has been proved to be an effective technique in non-invasive imaging in vivo with ultra-high resolution and imaging speed. In the last several years, non-invasive specific optical pacing in animal hearts has been reported in quail, zebrafish, and rabbit models. However, Drosophila Melanogaster, which is an important model with orthologs of 75% of human disease genes, has rarely been studied concerning their optical pacing in heart. Here, we combined optogenetic control of Drosophila heartbeats with optical coherence microscopy (OCM) technique for the first time. The light-gated cation channel, channelrhodopsin-2 (ChR2) was specifically expressed by transgene as a pacemaker in drosophila heart. By stimulating the pacemaker with 472 nm pulsed laser light at different frequencies, we achieved non-invasive and more specific optical control of the Drosophila heart rhythm, which demonstrates the wide potential of optical pacing for studying cardiac dynamics and development. Imaging capability of our customized OCM system was also involved to observe the pacing effect visually. No tissue damage was found after long exposure to laser pulses, which proved the safety of optogenetic control of Drosophila heart.

9690-88, Session 16

**Temporal and spatial mapping of neuronal signals in brain slice using image-guided recording system**

Jeonghyeon Lee, Ulsan National Institute of Science and Technology (Korea, Republic of); Jaemyung Jang, Seoul National Univ. (Korea, Republic of); Nam Hyun Cho, Songyee Baek, Ulsan National Institute of Science and Technology (Korea, Republic of); Noo Li Jeon, Seoul National Univ. (Korea, Republic of); Woonggyu Jung, Ulsan National Institute of Science and Technology (Korea, Republic of) and Institute for Basic Science (Korea, Republic of)

Micro-electrode Array (MEA) is an emerging device for studying neuronal circuits, because it offers to record extracellular field potentials as well as single action potential in the multiple sites, simultaneously and non-invasively. In particular, it is very effective to investigate temporal and spatial connectivity of neuronal network. For this reason, it has been intensively used as advanced electrophysiological tool to various neuronal studies based on cultured neurons and brain slices. However, it has rarely used in upright microscopy configuration, because it has restrictions to observe position of electrodes located beneath brain tissue. Thus, spatial mapping of neuronal activities on sliced brain tissue is inherently limited. Here, we present new method, optical image-guided recording system which enable to overcome the current limit and realize the temporal and spatial mapping. The platform of our device consists of optical coherence tomography (OCT) and 60-channel extracellular recording system. OCT is label-free, non-destructive and cross-sectional imaging tool in real time. OCT initially captures 3D brain tissue as well as MEA pattern including the features of individual electrodes. The neural signals are then measured.
with a 60-channel extracellular recording system. Thus, our device is able to achieve temporal-sequential neuronal activities and spatial mapping on the volumetric OCT image. In order to demonstrate the performance of our system, hippocampal slice is prepared and its spontaneous and stimulated activities are investigated. Through our research, our system would be a very promising tool to study connectivity of neuronal network in the future.

9690-90, Session 17

Experimental assessment of thermal effects of high power density light stimulation for optogenetics control of deep brain structures

Yann Suhan Senova, C.H.U. Henri-Mondor (France) and INSERM (France) and Univ. Paris-EST (France); Ilona Scisniak, Univ. Paris-Sud 11 (France) and Univ. Paris-Saclay (France); Chih Chieh Chiang, National Tsing Hua Univ. (Taiwan); Isabelle Doignon, Université Paris Sud INSERM, Laboratoire Interactions Cellulaires et PhysiologieHépatique (France); Claire Martin, Institut National de Physique Nucléaire et de Physique des Particules (France) and Univ. Paris-Saclay (France) and Ctr. National de la Recherche Scientifique (France); Stephane Palfi, C.H.U. Henri-Mondor (France) and INSERM (France) and Univ. Paris-Est Crétal Val de Marne (France); Antoine Chaillot, Univ. Paris-Sud 11 (France) and Univ. Paris-Saclay (France) and Lab. des Signaux et Systèmes (France); Frederic Pain, Univ. Paris-Sud 11 (France) and Univ. Paris-Saclay (France) and Ctr. National de la Recherche Scientifique (France)

2D surface maps of light distribution and temperature increase were recorded in wild type anesthetized rats brains during 90s light stimulation at 478nm (blue) and 638nm (red) with continuous or pulsed optical stimulations with corresponding power ranging from 100 up to 1200 mW/mm² at the output of an optical fiber. Post mortem maps were recorded in the same animals to assess the cooling effect of blood flow. Post mortem histological analysis were carried out to assess whether high power light stimulations had phototoxic effects or could trigger non physiological functional activation. Temperature increase remains below physiological changes (0.5 -1°) for stimulations up to 400mW/mm² at 40Hz. Histology did not show significant irreversible modifications or damage to the tissues. The spatial profile of light distribution and heat were correlated and demonstrate as expected a rapid attenuation with diatnce to the fiber.

9690-91, Session 17

Computational modeling of optogenetic neuronal excitation under complex illumination conditions using a Matlab-Neuron interface

Guy Yona, Yonatan Weissler, Nizan Meitav, Eliran Guzi, Dafna D. Rifold, Itamar Kahn, Shy Shoham, Technion-Israel Institute of Technology (Israel)

Optogenetics has in recent years become a central tool in neuroscience research. Creating a realistic model of optogenetic neuronal excitation is of crucial importance for controlling the activation levels of various neuronal populations in different depths, predicting experimental results and designing the optical systems. However, current approaches to modeling light propagation through rodents’ brain tissue suffer from major shortcomings and comprehensive modeling of local illumination levels together with other important factors governing excitation (i.e., cellular morphology, channel dynamics and expression), are still lacking. To address this challenge we introduce a new simulation tool for optogenetic neuronal excitation under complex & realistic illumination conditions that implements a detailed physical model for light scattering (in MATLAB) together with neuron morphology and channelrhodopsin-2 model (in NEURON). These two dispature simulation environments were interconnected using a newly developed generic interface termed ‘NeuroLab’. Applying this method, we show that in a layer-V cortical neuron, the relative contribution of the apical dendrites to neuronal excitation is considerably greater than that of the soma or basal dendrites, when illuminated from the surface.

9690-92, Session 17

Gold nanoparticle plasmonics enhanced ultrafast laser-induced optoporation and stimulation of targeted cells (Invited Paper)

Michel Meunier, Éric Bergeron, Ecole Polytechnique de Montréal (Canada); Flavie Lavoie-Cardinal, Univ. Laval (Canada); Christos Boutopoulos, Ecole Polytechnique de Montréal (Canada); Charleen Salesse, Univ. Laval (Canada); Françoise M. Winnik, Univ. de Montréal (Canada); Paul De Koninck, Univ. Laval (Canada)

Gold nanoparticles (AuNPs) have found numerous applications in nanomedicine in view of their robustness, ease of functionalization and low toxicity. Upon irradiation of AuNPs by a pulsed ultrafast laser, various highly localized phenomena can be obtained including a temperature rise, pressure wave, charge injection and production of nanobubbles close to the cellular membrane [1]. These phenomena can be used to manipulate, optoperforate, transfect and stimulate targeted cells [2-5]. Irradiating at 800 nm in the optically biological transparent window, we demonstrated local optoporation and transfection of cells as well as local stimulation of neurons. Two recent examples will be given: (i) Laser-induced selective optoporation of cells: The technique can be used on various types of cells and a proof of principle will be given on human cancer cells in a co-culture using functionalized AuNPs [6]. (ii) Laser-induced stimulation of neurons and monitoring of the localized Ca2+ signaling: This all optical method uses a standard confocal microscope to trigger a transient increase in free Ca2+ in neurons covered by functionalized AuNPs as well as to measure these local variations optically with the Ca2+ sensor GCaMP6s [7]. The proposed techniques provide a new complement to light-dependent methods in neuroscience. REFERENCES (by our group): (1) Boulais, J. Photochem. Photobiol. C Photochem. Rev. 17, 26 (2013); (2) Baumgart, Biomaterials 33, 2345 (2012); (3) Boulais, NanoLett. 12, 4763 (2012); (4) Boutopoulos, J. Biophotonics (2015); (5) Boutopoulos, Nanoscale 7, 11758 (2015); (6) Bergeron, Biomaterials, submitted (2015); (7) Lavoie-Cardinal, Nature Commun. submitted (2015).

9690-93, Session 17

Hybrid polymer waveguide characterization for microoptical tools with integrated laser diode chips for optogenetic applications at 430 nm and 650 nm

Michael Schwaerzle, Julian Nehlich, Ulrich T. Schwarz, Oliver Paul, Patrick Ruther, Univ. of Freiburg (Germany)

Appropriate micro-optical neural tools are required to exploit the key
advantages of optogenetics, i.e. optical stimulation and inhibition at high spatial and temporal resolutions, providing cell specificity and the opportunity to simultaneously record electrical neural signals. Besides the need for minimally invasive probes to reduce tissue damage, highly flexible or wireless interfaces are demanded for these optrodes specifically for experiments with freely behaving animals. Both requests can be achieved by integrating miniaturized waveguides for light transmission combined with bare laser diode (LD) chips integrated on neural probes.

This paper describes the system concept using integrated, side emitting LD chips directly coupled to miniaturized waveguides on silicon (Si) substrates. It details the fabrication, assembly, and optical as well as electrical characterization of 20?15 µm? waveguides (WG) photolithographically patterned using the hybrid polymer Ormocore®. Bare LD chips are flip chip bonded to electroplated gold (Au) pads with ±5 µm accuracy relative to the WG facets. Transmitted radiant fluxes for blue (430 nm, GaAlInN) and red (650 nm, AlGaNP) LDs are measured to 150 µW (ID=35 mA, duty cycle = 5 %) and 16 µW (ID=165 mA, duty cycle = 0.5 %), respectively. This corresponds to efficiencies of the coupled and transmitted light of 44% for red LDs. Long term measurements with these systems for 24 h showed a decrease of the radiant flux of about 4 % caused by LD aging at stable WG transmission properties. WGs immersed into Ringer’s solution showed no significant change of their transmission properties after four weeks of exposure to the ionic solution.

9690-94, Session 17

In vivo all-optical electrophysiology in mice using two-photon fluorescence microscopy and optogenetic techniques

James R. Mester, Univ. of Toronto (Canada) and Sunnybrook Research Institute (Canada); Paolo Bazzigaluppi, Univ. of Toronto (Canada) and Toronto Western Hospital (Canada); John G. Sled, Univ. of Toronto (Canada) and Toronto Ctr. for Phenonanogomics (Canada); Bojana Stefanovic, Sunnybrook Health Sciences Ctr. (Canada) and Univ. of Toronto (Canada)

Simultaneous stimulation and recording of neurons in vivo using combined optogenetics and calcium imaging has recently been made possible with the development of red-shifted opsins to reduce the spectral overlap in the two-photon (2P) cross-sections of GCaMP and channelrhodopsin-2 (ChR2). In this work, we use GCaMP6f and C1V1, a red-shifted ChR2 variant, in the all-optical interrogation of neurons in deep cortical layers of the mouse brain in vivo. This is performed using simultaneous 2P stimulation of C1V1 and 2P optical interrogation of neurons co-expressing these constructs. Expression was driven in excitatory pyramidal neurons using two adeno-associated viruses (AAVs, UPenn viral vector core) with either the hSyn or CamKIIa promoter, co-transfected in C57BL/6 mice 2-3 weeks prior to imaging for viral expression to occur. On imaging days, mice were equipped with an open cranial window over the injection site, and optical interrogation of single cells was done through simultaneous illumination at wavelengths of 1040nm for C1V1 simulation and 920nm for GCaMP6f imaging. Illumination power did not exceed 20mW to avoid tissue damage. Dual wavelength, spatially-resolved illumination is performed with two Ti:sapphire lasers with acousto-optic modulators (Mai Tai DeepSee, Spectra Physics) attached to a commercial 2P microscope (FV1000, Olympus). Cell-attached recordings were performed on illuminated cells to provide individual action potential (AP) data to correlate to GCaMP6f fluorescence and C1V1 stimulation in co-expressing cells. This work looks to provide a physiological and methodological basis for the use of all-optical electrophysiology as a non-invasive, compartmentalized tool to evaluate neuronal function in vivo.

9690-95, Session 17

Laser nano-surgery for neuronal manipulation

Hori Pada Sarker, Lalit Chudal, Vasu Mahapatra, Young-tae Kim, Samarendra K. Mohanty, The Univ. of Texas at Arlington (United States)

Optical manipulation has enabled study of bio-chemical and bio-mechanical properties of the cells. Laser nanosurgery by ultrafast laser beam with appropriate laser parameters provides spatially-targeted manipulation of neurons in a minimal invasiveness manner with high efficiency. We utilized femto-second laser nano-surgery for both axotomy and sub-axotomy of rat cortical neurons. Degeneration and regeneration after axotomy was studied and with and without external growth-factor(s) and biochemical(s). Further, axonal injury was studied as a function of pulse energy, exposure and site of injury. The ability to study the response of neurons to localized injury opens up opportunities for screening potential molecules for repair and regeneration after nerve injury. Sub-axotomy enabled transient opening of axonal membrane for optical delivery of impermeable molecules to the axoplasm. Fast resealing of the axonal membrane after sub-axotomy without significant long-term damage to axon (monitored by its growth) was observed. We will present these experimental results along with theoretical simulation of injury due to laser nano-surgery and delivery via the transient pore. Targeted delivery of proteins such as antibodies, genes encoding reporter proteins, ion-channels and voltage indicators will allow visualization, activation and detection of the neuronal structure and function.

9690-96, Session 18

Light distribution properties in spinal cord for optogenetic stimulation

Alicja G?secka, Mohamed Bahdine, Nicolas Lapointe, Veronique Rioux, Jimena Perez-Sanchez, Robert P. Bonin, Yves De Koninck, Univ. Laval (Canada); Daniel Côté, Ctr. de Recherche de l’Univ. Laval Robert-Giffard (Canada)

Optogenetics is currently one of the most popular technique in neuroscience. It enables cell-selective and temporally-precise control of neuronal activity. Good spatial control of the stimulated area and minimized tissue damage requires a specific knowledge about light scattering properties. Light propagation in cell cultures and brain tissue is relatively well documented and allows for a precise and reliable delivery of light to the neurons. In spinal cord, light must pass through highly organized while matter before reaching cell bodies present in grey matter, this heterogenous structure makes it difficult to predict the propagation pattern. In this work we investigate the light distribution properties through mouse and monkey spinal cord. The light propagation depends on a fibers orientation, leading to less deep penetration profile in the direction perpendicular to the fibers and lower attenuation in the direction parallel to the fibers. Additionally, the use of different illumination wavelengths results in variations of the attenuation coefficient. Next, we use Monte-Carlo simulation to study light transport. The model gives a full 3-D simulation of light distribution in the use of different scattering properties related to the fibers orientation. These studies are important to estimate the minimum optical irradiance required at the fiber tip to effectively excite the optogenetic proteins in a desired region of spinal cord.
Head-mounted LED for optogenetic experiments of freely-behaving animal

Ki Yong Kwon, Andrew G. Gnade, Alexander D. Rush, Craig D. Patten, Plexon Inc. (United States)

Recent developments in optogenetics have demonstrated the ability to target specific types of neurons with sub-millisecond temporal precision via direct optical stimulation of genetically modified neurons in the brain. In most applications, the beam of a laser is coupled to an optical fiber, which guides and delivers the optical power to the region of interest. Light emitting diodes (LEDs) are an alternative light source for optogenetics and they provide many advantages over a laser based system including cost, size, illumination stability, and fast light switching. However, coupling efficiency of LED’s output light into an optical fiber is lower than a laser due to its non-collimated output light.

In this study, we report and characterize a head-mounted LED for freely-behaving animal experiments. In typical chronic optogenetics experiment, output of the light source is transmitted to the brain through a fiber optic cable and an implanted optical fiber creating power loss in coupling junctions as well as attenuation in the cable. Depending on desired optical power output, the head-mounted LED can be controlled by either tethered (high power) or battery-powered wireless (moderate power) controller. In the tethered system, the LED is controlled through 40 gauge micro coaxial cable which is thinner, more flexible, and more durable than a fiber optic cable. The battery-powered wireless system equips infrared transceiver to achieve real-time control.

Optical, electrical, and thermal characteristics of the head-mounted LED were evaluated. To investigate the capability and reliability of the device, chronic stimulation experiments will be performed.

Digital holographic microscopy for imaging biophysical changes in cells during migration

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It is well known that biochemical changes in cancer cell occur in response to environmental cues and during migration. However, information about changes in the physical properties (e.g., volume, elasticity) of cancer cells during migration and/or in response to physical modulations (confinement and perturbations). We report the use of a near-infrared (NIR) laser microbeam system integrated with a NIR digital holographic microscopy (DHM) to study physical response of cancer cells. The cancer cells were cultured in microfluidic devices and subjected to different physical confinement (controlled by channel geometry), osmolarity changes of extracellular medium and/or laser-induced perturbations. The changes in optical thickness (or phase map) of the cells were monitored with high spatial and temporal resolution during and after the physico-chemical perturbations. A weakly-focused continuous-wave laser microbeam was used to impart radiation pressure on cell membrane and the changes in thickness were monitored using DHM to estimate elasticity. Further, an ultrafast tightly-focused laser microbeam was used to allow extracellular fluid flow into the cell or from the cytoplasm under different osmolarity conditions. Dynamic changes in physical properties of various cells and observed differences in responding to different physical/chemical environment/perturbations will be presented.

Two-photon holographic optogenetics of neural circuits

Weijian Yang, Luis Carrillo-Reid, Darcy S. Peterka, Rafael Yuste, Columbia Univ. (United States)

Optical manipulation of in vivo neural circuits with cellular resolution could be important for understanding cortical function. Despite recent progress, simultaneous optogenetic activation with cellular precision has either been limited to 2D planes, or a very small numbers of neurons over a limited volume. Here we demonstrate a novel paradigm for simultaneous 3D activation using a low repetition rate pulse-amplified fiber laser system and a spatial light modulator (SLM) to project 3D holographic excitation patterns on the cortex of mice in vivo for targeted volumetric 3D photoactivation. This method is compatible with two-photon imaging, and enables the simultaneous activation of multiple cells in 3D, using red-shifted opsins, such as C1V1 or ReaChR, while simultaneously imaging GFP-based sensors such as GCaMP6. This all-optical imaging and 3D manipulation approach achieves simultaneous reading and writing of cortical activity, and should be a powerful tool for the study of neuronal circuits.
A horizon scan on the use of virtual biopsy techniques to study and monitor environmental enteric dysfunction (Invited Paper)

Alex J. Thompson, Michael R. Hughes, Salzitsa Anastasova, Guang-Zhong Yang, Imperial College London (United Kingdom)

Environmental enteric dysfunction (EED) is a widespread condition in low income countries that entails multiple repeated infections leading to various developmental problems in infants (including malnutrition, poor uptake of oral vaccines, long term growth stunting, and cognitive impairment). There is now a burgeoning need for minimally invasive technologies to improve understanding of the causes/effects of EED and to accurately monitor the impact of interventions. In collaboration with the Gates Foundation we are undertaking a horizon scan to assess probe/capsule-based optical imaging/sensing for both purposes. Here we report results from literature reviews and discussions with experts in EED, paediatrics, and biophotonics.

Imaging the intestinal barrier defect in Environmental Enteropathy using confocal laser endomicroscopy (Invited Paper)

M. Paul Kelly, Queen Mary, Univ. of London (United Kingdom)

Environmental Enteropathy (EE) is a subclinical disorder which affects whole populations in Africa, South Asia, and parts of the tropical Americas. EE predisposes to microbial translocation, to malabsorption of micronutrients, and probably to failure of oral vaccines. In a study of 61 Zambian adults with EE, the leakiness of the gut was imaged with confocal laser endomicroscopy, revealing very frequent defects in the epithelial surface which correspond to breaks seen in histological sections and correlate with bacterial molecules in plasma. These data have also been used for transcriptomic analysis, to permit identification of new opportunities for therapy.

High resolution microendoscopy for early detection of esophageal cancer in low-resource settings (Invited Paper)

Rebecca Richards-Kortum, Rice Univ. (United States)

Esophageal squamous cell neoplasia (ESCN) is the sixth leading cause of cancer death worldwide. Most deaths due to ESCN occur in developing countries, with highest risk areas in northern China. Lugol’s chromoendoscopy (LCE) is the gold-standard for ESCN screening; while the sensitivity of LCE for ESCN is >95%, LCE suffers poor specificity (< 65%) due to false positive findings from inflammatory lesions. High resolution microendoscopy (HRME) uses a low-cost, fiber-optic fluorescence microscope to image morphology of the surface epithelium without need for biopsy. We developed a tablet-interfaced HRME with automated, real-time image analysis. In an in vivo study of 177 patients referred for endoscopy in China, use of the algorithm identified neoplasia with a sensitivity and specificity of 95% and 91% compared to the gold standard of histology.
Flexible micro-OCT endobronchial probe for imaging of mucociliary transport

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Mucociliary clearance (MCC) plays a significant role in maintaining the health of human respiratory system by eliminating foreign particles trapped within mucus. Failure of this mechanism in diseases such as cystic fibrosis and chronic obstructive pulmonary disease (COPD) leads to airway blockage and lung infection, causing morbidity and mortality. The volume of airway mucus and the periciliary liquid encapsulating the cilia, in addition to ciliary beat frequency and velocity of mucociliary transport, are vital parameters of airway health. However, the diagnosis of disease pathogenesis and advances of novel therapeutics are hindered by the lack of tools for visualization of ciliary function in vivo.

Our laboratory has previously developed a 1-μm resolution optical coherence tomography method, termed Micro-OCT, which is capable of visualizing mucociliary transport and quantitatively capturing epithelial functional metrics. We have also miniaturized Micro-OCT optics in a first-generation rigid 4mm Micro-OCT endoscope utilizing a common-path design and an apodizing prism configuration to produce an annular profile sample beam, and reported the first in vivo visualization of mucociliary transport in swine. We now demonstrate a flexible 2.5 mm Micro-OCT probe that can be inserted through the instrument channel of standard flexible bronchoscopes, allowing bronchoscopic navigation to smaller airways and greatly improving clinical utility. Longitudinal scanning over a field of view of more than 400 μm at a frame rate of 40 Hz was accomplished with a driveshaft transduced by a piezo-electric stack motor.

We present characterization and imaging results from the flexible micro-OCT probe and progress towards clinical translation. The ability of the bronchoscope-compatible micro-OCT probe to image mucus clearance and epithelial function will enable studies of cystic fibrosis pathogenesis in small airways, provide diagnosis of mucociliary clearance disorders, and allow individual responses to treatments to be monitored.
A piezoelectric microactuator previously proposed by the authors for laser scanning in dual axes confocal endomicroscopy meets two primary challenges for dual axes confocal imaging: large out-of-plane actuation (-500?m) and a relatively high bandwidth (>100Hz). In order to further reach stage positioning error better than desired imaging resolution of 5 ?m and to improve the robustness of actuator performance, a closed-loop controller and thus on-chip sensing, are being incorporated and integrated with system modeling.

This work presents these thin-film PZT based microstages where piezoelectric unimorphs are used not only to actuate its central platform but also to estimate its vertical motion. Initial results from on-chip piezoelectric sensing are presented. Although sensing output shows some feed-through from the actuation signal, testing shows detection of AC motion from various vibration modes of the stage. Meanwhile, 3D profiles of the entire actuator structure at different DC voltage levels were obtained and used to form a nonlinear optimization problem to estimate all forces and moments that each component of the device experiences for the prediction of its deflection. A comparison between modeled and experimental deflection of the actuation beams is included. These results will be used to describe the dynamic behavior of the actuation beams, where the sensors are embedded, and to estimate sensing outputs in order to implement a close-loop controller. Prototype stages are currently being assembled into a handheld dual axes confocal imaging system.

9691-6, Session 3
Surgical multi-color probe based confocal endomicroscopy: when pathologists finally enter the OR
Francois Lacombe, Christof Schäfauer, Sophie Clade, Mauna Kea Technologies (France)

When it was first introduced in 2005 by Mauna Kea Technologies, probe based confocal laser endo-microscopy (pCLE) rapidly proved how the access to key biological information, at cellular level, in vivo and in real time, would now be possible for biologists and physicians.

Shortly after, new avenues were opened: in pre-clinical applications, cancer research, neuroscience, drug-delivery, among others and in the clinics, gastroenterology, pulmonology, urology, which rapidly became the routine practice. Research, neuroscience, drug-delivery, among others and in the clinics, gastroenterology, pulmonology, urology, which rapidly became the routine practice. Short time image of the contacted surface structures. However, its findings have not been established yet.

This paper will present the recent results obtained in the fields of digestive, gynecologic and ENT surgery, in particular, and the way multi-color optical biopsy can now be used to connect surgeons and pathologists for intra-operative and real time tissue assessment.

Various possible tissue labeling strategies, including in the NIR domain, will be detailed with their respective benefits and drawbacks. The capabilities and the versatility of this new technology will be presented with some examples of how it can be combined with roboticized surgical tools, complement or replace needle based tissue sampling or fully be integrated into an hospital network. New perspectives will also be opened and proposed, where high miniaturization and modularity will play a key role: interventional radiology, fusion within integrated operating theaters, and last but not least, automated assistance to image interpretation and diagnosis.

9691-7, Session 3
A prospective cohort: probe based confocal laser endomicroscopy for peripheral pulmonary lesions
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Introduction: The diagnostic value of bronchoscopy for peripheral pulmonary lesions (PPLs) has improved since the application of radial endobronchial ultrasound (R-EBUS). Though R-EBUS indicates the position of the PPL, there is often a discrepancy between the obtained R-EBUS image and the diagnostic outcome. Meanwhile, probe based confocal laser endomicroscopy (pCLE) is a novel technique which provides in vivo real-time imaging of the contacted surface structures. However, its findings have not been established yet.

Methods: Consecutive patients who have undergone bronchoscopy for PPLs were prospectively enrolled. R-EBUS with a guide sheath (GS) was inserted to the target PPL under X-ray fluoroscopic guidance. When an adequate R-EBUS image (within or adjacent to) was obtained, pCLE was sequentially inserted through the GS. Then pCLE image was scanned and biopsy was performed where an abnormal finding was estimated. The pCLE findings of PPLs and the background were recorded and analyzed exploratorily.

Results: We analyzed 19 cases that we could get appropriate tissues. In all cases, bronchial walls showed longitudinal elastic fibers whereas alveolar walls formed grid-like elastic fiber networks. Conversely, discontinuous, crushed or aggregated alveolar structures accompanied by thickened and distorted fibers were detected in PPLs. Some cases showed dark hollow with fragmented or granular fluorescence. On the other hand, 11 cases (57.9%) indicated normal elastic fibers and needed the position change (3 cases; approached other bronchus, 6 cases; adjusted the position, 2 cases; penetrated the covered bronchial wall).

Conclusion: The pCLE has a potential to improve the efficacy of diagnostic bronchoscopy for PPLs.
Two-photon autofluorescence/FLIM/SHGendoscopy to study the oral cavity and wound healing in humans

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Monitoring the oral cavity noninvasively with superior 3D resolution is realized by clinical multiphoton tomography and high NA two-photon endoscopy without the need of additional contrast agents. The technology behind this investigation is based on nonlinear optical contrast of the multiphoton tomograph MPTflex®. Furthermore, the miniaturized GRIN endoscope was used to realize more accessibility for more demanding wound conditions in skin. The MPTflex® distinguishes autofluorescence (AF) signals from second harmonic generation (SHG) signals simultaneously. Fluorescence lifetime imaging (FLIM) based on time correlated single photon counting (TCSPC) technology offers additional information on the functional level of the intratissue fluorophores, their binding status, and the contribution of SHG signals in chronic wounds.

High-resolution and ultra-thin endomicroscopy using a GRIN rod lens

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A graded-index (GRIN) lens is suitable for developing an ultra-thin endoscope due to its small diameter and simplicity for optics design. A GRIN lens, however, generates intrinsic optical aberration causing low resolution and poor imaging quality. Recently, wavefronts of light can be measured with very high precision and the optical aberration can be corrected in numerical ways even for the case of highly scattering media. In this study, based on the high precision wavefront sensing and numerical image processing techniques, we demonstrate a high-resolution and ultra-thin endo-microscope using a GRIN rod lens as a core imaging optics. We constructed a reflection-type interferometric microscope through a GRIN rod lens using a p-polarized Nd:YAG laser (532 nm) as a light source. By recording and processing blank transmission images as a function of various illumination states, the characteristics of the aberration generated by the GRIN lens were obtained. After this pre-calibration, we could numerically compensate the aberration induced onto a reflection image of an object. Consequently, a diffraction limited lateral resolution as well as improved axial resolution could be achieved. Our approach will fascinate the use of GRIN lenses for compact and high-resolution imaging devices including ultra-thin endo-microscopes.

Two-photon lensless endoscopy by controlling the wave front in a multi-core optical fiber

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Typical endoscopes need optical or micromechanical components attached to their tip in order to form an image. These components limit the eventual size of the endoscope probe to around a few millimeters. Recently, developments in the wavefront control have enabled extreme miniaturization of the endoscopic probe, down to size of an optical fiber. This concept would permit imaging at depths inaccessible to conventional endoscopes.

In our approach, we strive for a lensless endoscope capable of nonlinear image contrast using two-photon fluorescence (TPEF). In this case, the constraints on the delivery of an ultrashort short laser pulse also become critical. Here, we demonstrate wavefront shaping techniques in conjunction with a specially designed double clad multi-core fiber bundle that allows for ultrashort pulse delivery and effective collection of the fluorescence signal. We explore concepts that allow for both the measurement and control of the composite wavefront, allowing the generation and the rapid scanning of a focal spot. In addition, the same wavefront shaping elements are also used to compensate the different group delays imposed by the individual fibers of the bundle. Hence, spatio-temporal control of light at the distal tip is possible. We further demonstrate optically sectioned TPEF imaging in an endoscopic configuration with epi-detected signal. We also quantify the improved performance due to the temporal overlap and discuss generalization of these techniques. These concepts would eventually facilitate demanding applications where severe constraints are imposed by limited space and high scattering.

Extra flat, flexible and disposable endoscope for lateral imaging

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We present an innovative disposable endoscope based on extra flat flexible polymer slabs used as multimode waveguides. These latter are compatible with low-cost roll-to-roll production technologies and can be easily customized by patterning, coating and printing techniques according to the specifications of the target application. In order to couple the light (i.e. the image) in and out of the waveguide, diffractive subwavelength gratings are used. These nano-scale optical structures enable a highly efficient and controlled light trapping by total internal reflection, thus minimizing the distortion effects generated by the rough edges. Nano-patterning is obtained using established techniques (i.e. hot embossing and/or UV casting) that are compatible with industrial roll-to-roll production lines.

Unique features of these innovative endoscopes are i) the achievable very thin form that can be reduced to thicknesses below 200 micron, ii) the ability of recording lateral images with respect to the endoscope direction and to image samples (e.g. tissues, tiny objects) in direct contact with the polymer slab, with a minimum imaging distance in the range of few microns, and iii) the access to high volume fabrication techniques that can enable the production of low-cost disposable endoscopes.

A possible device implementation will be shown in details, which consists of a flat line-scanning endoscope enabling the acquisition of 1D images in monochromatic illumination and the reconstruction of 2D images by scanning. Images will be shown and the related technological constraints such as manufacturing tolerances, scattered light, and signal to noise ratio will be discussed. Finally, advantages and disadvantages with respect to other endoscopic techniques will be discussed, thus demonstrating the potential of this innovative approach for endoscopic applications.
Nonlinear endoscope using Kagomé lattice hollow-core fibers

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The development of nonlinear fiber-endoscopes capable of imaging deeper in tissues and accessing internal organs represents a very attractive perspective for application of nonlinear optical microscopes to in-vivo research and diagnostics. The transmission of ultra-short laser pulses within a fiber is a critical issue in the development of such endoscopes. For instance, self-phase modulation (SPM), four-wave mixing (FWM) and Raman scattering occurring in conventional fibers severely affect transmitted pulses profiles in the time and frequency domains. Hollow-core (HC) fibers bring a solution to the problem, since propagation of the pulses in the air core limits nonlinear interactions. We employ here a novel double clad Kagomé-lattice HC fiber for the delivery of ultrafast pulses across a large spectral window (~400nm) with no pulse distortion. The epi-collection of the signal generated at the sample is efficiently performed with a specially designed outer multimode cladding. The fiber is incorporated in a prototype endoscope using a four-quartered piezo-electric tube to scan the laser beam on the sample. The low numerical aperture of the hollow-core (0.02) is efficiently increased by means of a dielectric microsphere attached to the fiber face. This results in tight focusing (~1 micron) of the beam at the HC fiber output. Resonant scanning of the fiber tip allows imaging over a field of 300 microns using low driving voltages. High-resolution images with different contrast mechanisms, such as SHG and TPEF, acquired with the prototype endoscope illustrate the potential of these fibers for nonlinear imaging in regions otherwise inaccessible to conventional optical microscopes.

The integration of single fiber reflectance (SFR) spectroscopy during endoscopic ultrasound-guided fine needle aspirations (EUS-FNA) in pancreatic masses: a feasibility study

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EUS-FNA can be used for pathological confirmation of a suspicious pancreatic mass. However, performance depends on an on-site cytologist and time between puncture and final pathology results can be long. SFR spectroscopy is capable of extracting biologically relevant parameters (e.g. oxygenation and blood volume) in real-time from a very small tissue volume at difficult locations. In this study we determined feasibility of the integration of SFR spectroscopy during EUS-FNA procedures in pancreatic masses.

Patients with benign and malignant pancreatic masses who were scheduled for an EUS-FNA were included. The working guide wire inside the 19 gauge endoscopic biopsy needle was removed and the sterile single fiber (300 μm core and 700 μm outer diameter, wide-angle beam, NA 0.22) inserted through the needle. Spectroscopy measurements in the visible-near infrared wavelength region (400-900 nm) and autofluorescence measurements (excitation at 405 nm) were taken three times, and subsequently cytology was obtained. Wavelength dependable properties were compared to cytology results.

So far, we took measurements in 9 patients at 13 different locations with corresponding cytology results (including mucinous tumor, ductal adenocarcinoma, neuroendocrine tumor, and pancreatitis). Preliminary data already shows differences, e.g. higher blood volume in the neuroendocrine tumor compared with others, and higher autofluorescence of the mucinous tumor compared with others.

Integration of SFR spectroscopy is feasible in EUS-FNA procedures, the workflow hardly requires changes and it takes little time. Currently we are including more patients, analyzing all measurements, and will expand the study to surgical procedures to measure healthy pancreatic tissue.

Optically sectioned spatial-spectral coded holographic fluorescence microscopy

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Wide-field fluorescent imaging severely suffers low resolution and poor contrast from out-of-focus background to image biological samples. In order to enhance optical sectioning capability, Confocal approach has been developed to filter out-of-focus background using point-to-point detection through a spatial pinhole. Recently, active structured illumination in wide-field fashion has been developed to reduce the transversal scanning cost, but still requires scanning in axial direction. Here, we present a wide-field multi-focal fluorescence microscopy incorporating spatial-spectral volume holographic gratings (MVHG's) with 3D active structured illumination to obtain optically sectioned images without scanning is presented. In contrast to conventional holographic techniques, which in general can not obtain fluorescence images, our approach does not require the formation of a hologram during imaging and is compatible with fluorescence based methods of imaging. Our approach requires pair-wise multi-depth resolved images, one with 3D active illumination, and the other with standard uniform illumination. Our approach is configured such that 3D illuminated planes occur inside the specimen, and also serve as the structured modulation for multiple axial planes imaged by MVHGs and display laterally onto the camera. The system can also be combined with micro-objective and relay systems for endoscopic operation. We demonstrate the proposed system's ability to simultaneously obtain wide-field, optically sectioned, and multi-depth resolved images of fluorescently labeled tissue structures.

Clinical experience of using the tethered capsule-based spectrally encoded confocal microendoscopy for diagnosis of eosinophilic esophagitis

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Eosinophilic Esophagitis (EoE) is caused by food allergies, and defined by histological presence of eosinophil cells in the esophagus. The current gold standard for EoE diagnosis is endoscopy with pinch biopsy to detect more than 15 eosinophils/High power field (HPF). Biopsy examinations are expensive, time consuming and are difficult to tolerate for patients. Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance confocal microscopy technology capable of imaging individual eosinophils as highly scattering cells (diameter between 8 µm to 15 µm) in the epithelium. Our lab has developed a tethered SECM capsule that can be swallowed by unsedated patients. The capsule acquires large area confocal images, equivalent to more than 30,000 HPFs, as it traverses through the esophagus. In this paper, we present the outcome of a clinical study using the tethered SECM capsule for diagnosing EoE. To date, 32 subjects have been enrolled in this study. 88% of the subjects swallowed the capsules without difficulty and of those who swallowed the capsule, 95% preferred the tethered capsule imaging procedure to sedated endoscopic biopsy. Each imaging session took about 12 ± 2.4 minutes during which 8 images each spanning of 24 ± 5 cm² of the esophagus were acquired. SECM images acquired from EoE patients showed abundant eosinophils as highly scattering cells in squamous epithelium. Results from this study suggest that the SECM capsule has the potential to become a less-invasive, cost-effective tool for diagnosing EoE and monitoring the response of this disease to therapy.

Reflectance confocal microscopy of red blood cells: simulation and experiment

Adel Zeidan, Daniella Yeheskely-Hayon, Limor Minai, Dvir Yelin, Technion-Israel Institute of Technology (Israel)

The properties of red blood cells are a remarkable indicator of the body’s physiological condition; their density could indicate anemia or polycythemia, their absorption spectrum correlates with blood oxygenation, and their morphology is highly sensitive to various pathologic states including iron deficiency, ovalocytosis, and sickle cell disease. Therefore, measuring the morphology of red blood cells is important for clinical diagnosis, providing valuable indications on a patient’s health. In this work, we simulated the appearance of normal red blood cells under a reflectance confocal microscope and discovered unique relations between the cells’ morphological parameters and the resulting characteristic interference patterns. The simulation results showed good agreement with in vitro reflectance confocal images of red blood cells, acquired using spectrally encoded flow cytometry (SEFC) that imaged the cells during linear flow and without artificial staining. By matching the simulated patterns to the SEFC images of the cells, the cells’ three-dimensional shapes were evaluated and their volumes were calculated. Potential applications include measurement of the mean corpuscular volume, cell morphological abnormalities, cell stiffness under mechanical stimuli, and the detection of various hematological diseases.

Simple, monolithic optical element for forward-viewing spectrally encoded endoscopy

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Spectrally encoded endoscopy (SEE) is a miniature endoscopic technology that can acquire images of internal organs through a hair-thin probe. While most previously described SEE probes have been side viewing, forward-view (FV)-SEE is advantageous in certain clinical applications as it provides more natural navigation of the probe and has the potential to provide a wider field of view. Prior implementations of FV-SEE used multiple optical elements that increase fabrication complexity and may diminish the robustness of the device. In this paper, we present a new design that uses a monolithic optical element to realize FV-SEE imaging. The optical element is specially designed spacer, fabricated from a 500–7µm-glass rod that has a mirror surface on one side and a grating stamped on its distal end. The mirror surface is used to change the incident angle on the grating to diffract the shortest wavelength of the spectrum so that it is parallel to the optical axis. Rotating the SEE optics creates a circular FV-SEE image. Custom-designed software processes FV-SEE images into circular images, which are displayed in real-time. In order to demonstrate this new design, we have constructed the FV-SEE optical element using a 1379 lines/mm diffraction grating. When illuminated with a source with a spectral bandwidth of 420–820 nm, the FV-SEE optical element provides 678 resolvable points per line. The imaging performance of the FV-SEE device was tested by imaging a USAF resolution target. SEE images showed that this new approach generates high quality images in the forward field with a field of view of 58°. Results from this preliminary study demonstrate that we can realize FV-SEE imaging with simple, monolithic, miniature optical element. The characteristics of this FV-SEE configuration will facilitate the development of robust miniature endoscopes for a variety of medical imaging applications.
Image-guided optical measurement of blood oxygen saturation within capillary vessels

Kfir Akons, Adel Zeidan, Daniella Yeheskely-Hayon, Limor Minai, Dvir Yelin, Technion-Israel Institute of Technology (Israel)

Values of blood oxygenation levels are useful for assessing heart and lung conditions, and are frequently monitored during routine patient care. Independent measurement of the oxygen saturation in capillary blood, which is significantly different from that of arterial blood, is important for diagnosing tissue hypoxia and for increasing the accuracy of existing techniques that measure arterial oxygen saturation. Here, we developed a simple, non-invasive technique for measuring the reflected spectra from individual capillary vessels within a human lip, allowing local measurement of the blood oxygen saturation. The optical setup includes a spatially incoherent broadband light that was focused onto a specific vessel below the lip surface. Backscattered light was imaged by a camera for identifying a target vessel and pointing the illumination beam to its cross section. Scattered light from the vessel was then collected by a single-mode fiber and analyzed by a fast spectrometer. Spectra acquired from small capillary vessels within a volunteer lip showed the characteristic oxyhemoglobin absorption bands in real time and with a high signal-to-noise ratio. Measuring capillary oxygen saturation using this technique would potentially be more accurate compared to existing pulse oximetry techniques due to its insensitivity to the patient’s skin color, pulse rate, motion, and medical condition. It could be used as a standalone endoscopic technique for measuring tissue hypoxia or in conjunction with conventional pulse oximetry for a more accurate measurement of oxygen transport in the body.

Reducing the cost of tethered capsule endomicroscopy for Barrett’s esophagus screening

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Optical coherence tomography (OCT) is an imaging technology that forms depth-resolved images by using interferometry to measure the optical delay of backscattered light from a sample. It has been shown in prior studies to be capable of accurately diagnosing Barrett’s Esophagus (BE), a metaplastic change that conveys an increased risk of developing esophageal adenocarcinoma (EAC). Tethered capsule endomicroscopy (TCE) using OCT is a technology developed in our lab where a tethered opto-mechanical capsule that is 11mm x 24.5mm in size obtains 10 μm resolution cross-sectional OCT images of the entire esophageal wall as it traverses the esophagus via peristalsis. Recent work1 improved on this technology by replacing the TCE rotary junction (RJ) and driveshaft by a micro-motor. While this has several advantages, the cost of the motor and consequently that of the capsule is relatively high for a disposable device, hindering wide adoption for BE screening.

In this work we develop a new generation TCE device that is significantly less expensive in both fixed and disposable costs while producing better, quantitative data at the same time. The expensive RJ is replaced by an inexpensive cell-phone micro-motor that costs less than $10. We mitigate challenges arising out of the relatively poor speed characteristics of the inexpensive motors and obtain imaging performance comparable to that from a RJ by performing image segmentation on OCT data, estimating speed characteristics of the micro-motor and using a feedback-loop to correct for imperfections arising from using the inexpensive micro-motor. Consequently, the fixed and disposable costs of the TCE device are reduced dramatically facilitating widespread adoption of TCE for BE screening in primary care settings.

1 Liang et. al. Ultrahigh speed en face OCT capsule for endoscopic imaging, Biomed Opt Express. 6(4): 1146–1163, 2015

Tethered capsule OCT endomicroscopy for upper gastrointestinal tract imaging by using ball lens based probe

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While endoscopy is the most commonly used modality for diagnosing upper GI tract disease, this procedure usually requires patient sedation that increases cost and mandates its operation in specialized settings. In addition, endoscopy only visualizes tissue superficially at the macroscopic scale, which is problematic for many diseases that manifest below the surface at a microscopic scale. Our lab has previously developed technology termed tethered capsule OCT endomicroscopy (TCE) to overcome these diagnostic limitations of endoscopy. The TCE device is a swallowable capsule that contains optomechanical components that circumferentially scan the OCT beam inside the body as the pill traverses the organ via peristalsis. While we have successfully imaged ~100 patients with the TCE device, the optics of our current device have many elements and are complex, comprising a glass ferrule, optical fiber, glass spacer, GRIN lens and prism. As we scale up manufacturing of this device for clinical translation, we must decrease the cost and improve the manufacturability of the capsule’s optical configuration.

In this abstract, we report on the design and development of simplified
TCE optics that replace the GRIN lens-based configuration with an angle-polished ball lens design. The new optics include a single mode optical fiber, a glass spacer and an angle polished ball lens, that are all fusion spliced together. The ball lens capsule has resolutions that are comparable with those of our previous GRIN lens configuration (30µm (lateral) \( \approx 7 \) µm (axial)). Results in human subjects show that OCT-based TCE using the ball lens not only provides rapid, high quality microstructural images of upper GI tract, but also makes it possible to implement this technology inexpensively and on a larger scale.

9691-23, Session 6

Feasibility of optical coherence tomography to detect radiation-induced esophageal damage in small animal models

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Lung cancer survival is poor and radiotherapy patients often suffer serious treatment side effects. The esophagus is particularly sensitive leading to reduced food intake or even fistula formation. Only few direct techniques exist to measure radiation-induced esophageal damage, for which knowledge is needed to improve the balance between risk of tumor recurrence and complications. Optical coherence tomography (OCT) is a minimally-invasive imaging technique that obtains cross-sectional, high-resolution (1-10µm) images and is capable of scanning the esophageal wall up to 2-3mm depth. In this study we investigated the feasibility of OCT to detect esophageal radiation damage in mice.

In total 30 mice were included in 4 study groups (1 main and 3 control groups). Mice underwent cone-beam CT imaging for initial setup assessment and dose planning followed by single-fraction dose delivery of 4, 10, 16, and 20Gy on 5mm spots, spaced 10mm apart. Mice were repeatedly imaged using OCT: pre-irradiation and up to 3 months post-irradiation. The control groups received either OCT only, irradiation only, or were sham-operated. We used histopathology as gold standard for radiation-induced damage.

The study showed edema in both the main and OCT-only groups. Furthermore, radiation-induced damage was primarily found in the highest dose region (distal esophagus). Based on the histopathology reports we were able to identify the radiation-induced damage in the OCT images as a change in tissue scattering related to the type of induced damage. This finding indicates the feasibility and thereby the potentially promising role of OCT in radiation-induced esophageal damage assessment.

9691-24, Session 6

Detection of colon polyps using Doppler optical coherence tomography imaging of microvascular hemodynamics

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Colorectal cancer remains the third deadliest cancer in the United States, despite the high sensitivity and specificity of colonoscopy and sigmoidoscopy. While these standard imaging procedures can accurately detect medium and large polyps, some studies have shown miss rates up to 25% for polyps less than 5 mm in diameter. An imaging modality capable of detecting small dysplastic polyps could potentially improve patient outcomes.

We designed an imaging protocol that uses Doppler optical coherence tomography (OCT) to detect abnormal vasculature characteristic of dysplastic polyps by measuring the hemodynamic response of the microvasculature to heat. It is well known that arterioles dilate under mild-temperature hyperthermia (\( \approx -3 \)°C increase) due to relaxation of the surrounding smooth muscle. However, arterioles developed to support growth of dysplastic polyps lack smooth muscle and have been shown to respond irregularly to heat. To discriminate healthy and dysplastic tissue, we imaged the distal 30 mm of mouse colon before and after heating with a warm-water lavage with OCT. The cancer group consisted of six A/J mice given the carcinogen azoxymethane, and four mice given saline served as the normal group. Doppler variance maps were generated for each image and divided into small subregions. The change in blood flow was calculated as the change in Doppler variance within each subregion before and after heating. These maps of change of blood flow are compared to histology to determine accuracy of our Doppler OCT technique.
9691-26, Session PMon

Optical coherence tomography imaging of colonic crypts in a mouse model of colorectal cancer

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Aberrant crypt foci (ACF) are abnormal epithelial lesions that precede development of colonic polyps. As the earliest morphological change in the development of colorectal cancer, ACF is a highly studied phenomenon. The most common method of imaging ACF is chromoendoscopy using methylene blue as a contrast agent. Methylene blue is not yet FDA approved and has been shown to have some negative side effects.

Narrowband imaging is a contrast-agent-free modality for imaging the colonic crypts. Optical coherence tomography is an attractive alternative to chromoendoscopy and narrowband imaging because it can resolve the crypt structure at sufficiently high sampling while simultaneously providing depth-resolved data. We imaged the distal 30 mm of colon in a mouse model of colorectal cancer using a commercial swept-source OCT system and a miniature endoscope designed and built in-house. We have previously demonstrated early detection of colon adenoma using OCT by detecting minute thickening of the mucosa. By combining mucosal thickness measurement with imaging of the crypt structure, we can correlate ACF and adenoma development in space and time. These results suggest that OCT may be a superior imaging modality for studying the connection between ACF and colorectal cancer, as well as safe ACF detection in humans.

9691-27, Session PMon

Tethered capsule endomicroscopy with capsule position localization for diagnosis of diseases of the upper gastrointestinal tract

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Tethered capsule endomicroscopy (TCE) is a newly developed technique that involves swallowing an opto-mechanically engineered capsule that performs in vivo microscopy. There are many advantages of TCE, including rapid microscopic imaging of the upper gastrointestinal tract, comprehensive volumetric imaging, control of the capsule via manipulation of the tether, and the capability to administer the device in unsedated patients. While we have successfully imaged ~100 patients with this technique, we are currently unable to track the position of the capsule in the patient, which limits our ability to study the organ in three dimensions and evaluate disease progression over time.

In this paper, we describe the incorporation of tracking technology in the TCE device in order to monitor the position of the capsule in the patient in real time. The position-tracking element is a 6DOF miniature electromagnetic sensor (Model 90, NDI Ascension Technology Corporation) that wirelessly communicates with a transmitter (SRT, Ascension) that is near the patient and an electronic unit (driveBAY) in the system console. We have fabricated a prototype position-tracking capsule and tested it in a phantom that mimics the human use case. Results show that the sensor’s tracking precision is better than 1 mm over a range of -30cm to +30cm from the transmitter.

These results indicate that electromagnetic position tracking technology will be suitable for TCE capsule position localization. Once this device is implemented in our TCE devices for human studies, we will be able to assess the longitudinal microscopic natural history of prevalent diseases such as Barrett’s Esophagus and eosinophilic esophagitis, providing new data that will better inform clinical management strategies for these conditions.
Asthma affects hundreds of millions of people worldwide, and the prevalence of the disease appears to be increasing. One of the most important aspects of asthma is the excessive bronchoconstriction that results in many of the symptoms experienced by asthma sufferers, but the relationship between bronchoconstriction and airway morphology is not clearly established. We present the imaging results of a study involving a segmental allergen challenge given to both allergic asthmatic (n = 12) and allergic non-asthmatic (n = 19) human volunteers. Using OCT, we have imaged and assessed baseline morphology in a right upper lobe (RUL) airway, serving as the control, and a right middle lobe (RML) airway, in which the allergen was to be administered. After a period of 24 hours had elapsed following the administration of the allergen, both airways were again imaged and the response morphology assessed. A number of airway parameters were measured and compared, including epithelial thickness, mucosal thickness and buckling, lumen area, and mucus content. We found that at baseline epithelial thickness, mucosal thickness, and mucosal buckling were greater in AAs than ANAs. We also observed statistically significant increases in these values 24 hours after the allergen had been administered for both the ANA and AA sets. In comparison, the control airway which received a diluent showed no statistically significant change.

Peripheral lung nodules found by CT-scans are difficult to localize and biopsy bronchoscopically particularly for those ≤ 2 cm in diameter. In this work, we present the results of endoscopic co-registered optical coherence tomography and autofluorescence imaging (OCT-AFI) of normal and abnormal peripheral airways from 40 patients using 0.9 mm diameter fiber optic rotary pullback catheter. Optical coherence tomography (OCT) can visualize detailed airway morphology endoscopically in the lung periphery. Autofluorescence imaging (AFI) can visualize fluorescing tissue components such as collagen and elastin, enabling the detection of airway lesions with high sensitivity. Results indicate that AFI of abnormal airways is different from that of normal airways, suggesting that AFI can provide a sensitive visual presentation for rapidly identifying possible sites of pulmonary nodules. AFI can also rapidly visualize in vivo vascular networks using fast scanning parameters resulting in vascular-sensitive imaging with less breathing/cardiac motion artifacts compared to Doppler OCT imaging. It is known that tumor vasculature is structurally and functionally different from normal vessels. Thus, AFI can be potentially used for differentiating normal and abnormal lung vasculature for studying vascular remodeling.

Lung cancer patients have a very poor overall 5 year survival rate of ≤17%. This is in contrast to patients found with early stage (0) carcinoma in situ (CIS) or stage IA where the 5 year survival rate is >70%. Currently the most reliable method for localizing early cancer in the central airways is a combined autofluorescence and white light bronchoscopy (AFB+WLB). However the accuracy of a WLB+AFB procedure for detecting CIS and high grade dysplasia (a precursor to CIS) is variable depending on bronchoscopist's experience. On average, demanding a high diagnostic sensitivity results in a low diagnostic specificity. To try and improve on this situation, we have been developing and testing other adjunct optical devices for in vivo early lung cancer detection. Real-time endoscopic Raman spectroscopy (RS) is one possibility due to its ability to detect small biochemical changes non-destructively. These features enable RS to be used to classify tissue pathology with a high sensitivity and specificity. Despite the many challenges (inherently weak signals, poor light collection, and the requirement for fast acquisitions times), several small studies have shown that RS has great promise for real time in vivo tissue classification. In this latest, work spectra were obtained in 1 second from 280 tissue sites (72 high grade dysplasia/malignant lesions, and 208 benign lesions/normal sites) in 80 patients. Using multivariate analyses and waveband selection methods on the Raman spectra, we have demonstrated that high grade dysplasia and malignant lung lesions can be detected with high sensitivity (90%) and good specificity (65%).
A study of airway smooth muscle in asthmatic and non-asthmatic airways using PS-OCT

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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. With standard Optical Coherence Tomography (OCT), imaging ASM is often not possible due to poor structural contrast between the muscle and surrounding tissues. A potential solution to this problem is to utilize additional optical contrast factors intrinsic to the tissue, such as birefringence. Due to its highly ordered structure, ASM is strongly birefringent. Previously, we demonstrated that Polarization Sensitive OCT (PS-OCT) has the potential to be used to visualize ASM as well as easily segment it from the surrounding (weakly) birefringent tissue by exploiting a property which allows it to discriminate the orientation of birefringent fibers. We have already validated our technology with a substantial set of histological comparisons made against data obtained ex vivo. In this work we present a comprehensive comparison of ASM distributions in asthmatic and non-asthmatic human volunteers. By isolating the ASM we parameterize its distribution in terms of both thickness and band width, calculated volumetrically over centimeters of airway. Using this data we perform analyses of the asthmatic and non-asthmatic airways using a broad number and variety of subjects.

Visualization of rat airway damage from toxic chemical agents using an all fiber based OCT imaging probe

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Toxic chemical inhalation exposures are a major risk from industrial accidents and acts of terrorism. Methods for ongoing assessment of resultant airway injury are needed for diagnostic, prognostic, and treatment response determinations. Endoscopy-based Optical Coherence Tomography (OCT) has been utilized to image airway lumen structural changes in various animal models. In this proceeding, we report on the use of a fully fiber based OCT imaging probe in the study of rat models exposed to sulfur mustard (SM) and methyl isocyanate (MIC). The all-optical fiber based imaging probe is contained within a metal tube that has an outer diameter of 400 um and is proximally rotated and translated to generate a helical visualization of the airway lumen structure. Imaging is performed at a speed of 50 frames per second with an axial resolution of 9 um in air. Imaging was performed in animals exposed to sulfur mustard as well as methyl isocyanate, and controls, with substantial injury induced changes evident. Utilization of the OCT images directed sectioning of the airways at appropriate locations in order to capture regions with greatest pathology. Comparison between OCT images and histology sections will be presented.

Using optical frequency domain imaging in the evaluation of airway dynamics in Methacholine challenged sheep

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Asthma is a chronic disease resulting in periodic attacks of coughing and wheezing due to temporarily constricted and clogged airways. The pathophysiology of asthma and the process of airway narrowing are not completely understood. Appropriate in vivo imaging modality with sufficient spatial and temporal resolution to dynamically assess the behavior of airways is missing. Optical coherence tomography (OCT) enables real-time evaluation of the airways during dynamic and static breathing maneuvers. Our aim was to visualize the structure and function of airways in healthy and Methacholine (MCH) challenged lung.

Sheep (n=3) were anesthetized, mechanically ventilated and imaged with OCT in 4 dependent and 4 independent airways both pre- and post-MCH administration. The OCT system employed a 2.4 Fr (0.8 mm diameters) catheter and acquired circumferential cross-sectional images in excess of 100 frames per second during dynamic tidal breathing, 20 second static breath-holds at end-inspiration and expiration pressure, and in a response to a single deep inspiration.

Markedly different airway behavior was found in dependent versus non-dependent airway segments before and after MCH injection. OCT is a non-ionizing light-based imaging modality, which may provide valuable insight into the complex dynamic behavior of airway structure and function in the normal and asthmatic lung.

Monitoring the impact of bronchial thermoplasty on airway smooth muscle using polarization sensitive optical coherence tomography

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Patients with severe asthma experience a proliferation of airway smooth muscle (ASM) and excessive bronchoconstriction. Bronchial thermoplasty (BT) is a new therapeutic modality involving the delivery of radiofrequency energy to the airway to reduce ASM mass with the goal of decreasing excessive bronchoconstriction. In this study we evaluated the potential of polarization sensitive optical coherence tomography (PS-OCT) to monitor the impact of BT on ASM in a canine preclinical model. Due to the birefringent nature of ASM, PS-OCT enhances the contrast of ASM in the airway lumen structure. Imaging is performed at a speed of 50 frames per second with an axial resolution of 9 um in air. Imaging was performed in animals exposed to sulfur mustard as well as methyl isocyanate, and controls, with substantial injury induced changes evident. Utilization of the OCT images directed sectioning of the airways at appropriate locations in order to capture regions with greatest pathology. Comparison between OCT images and histology sections will be presented.
The effect of low level laser therapy on ventilator-induced lung injury in mice
Margit V. Szabari, Alyssa J. Miller, Lida P Hariri M.D., Michael R. Hamblin, Guido Musch, Helene Stroh, Melissa J. Suter, Massachusetts General Hospital (United States)

Although mechanical ventilation (MV) is necessary to support gas exchange in critically ill patients, it can contribute to the development of lung injury and multiple organ dysfunction. It is known that high tidal volume (VT) MV can cause ventilator-induced lung injury (VILI) in healthy lungs and increase the mortality of patients with Acute Respiratory Distress Syndrome. Low level laser therapy (LLLT) has demonstrated to have anti-inflammatory effects. We investigated whether LLLT could alleviate inflammation from injurious MV in mice.

Adult mice were assigned to 2 groups: VILI+LLLT group (3 h of injurious MV: Vt=25-30 ml/kg, respiratory rate (RR)=50/min, positive end-expiratory pressure (PEEP)=0 cmH2O, followed by 3 h of protective MV: VT=9 ml/kg, RR=140/min, PEEP=2 cmH2O) and VILI+no LLLT group. LLLT was applied during the first 30 min of the MV (810 nm LED system, 5 J/cm2, 1 cm above the chest). Respiratory impedance was measured in vivo with forced oscillation technique and lung mechanics were calculated by fitting the constant phase model. At the end of the MV, bronchoalveolar lavage (BAL) was performed and inflammatory cells counted. Lungs were removed en bloc and fixed for histological evaluation.

We hypothesized that LLLT can reduce lung injury and inflammation from VILI. This therapy could be translated into clinical practice, where it can potentially improve outcomes in patients requiring mechanical ventilation in the operating room or in the intensive care units.

Imaging of mucus clearance in the airways of living spontaneously breathing mice by optical coherence microscopy
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Mucus transport is essential to remove inhaled particles and pathogens from the lung. Impaired removal of mucus often results in worsening of lung diseases. To understand the mechanisms of mucus transport and to monitor the impact of therapeutic strategies, it is essential to visualize airways and mucus in living animals without disturbing transport processes by intubation or surgically opening the airways.

We developed a custom-built optical coherence microscope (OCM) providing a lateral and axial resolution of approximately 1.5 μm with a field of view of 2 mm at up to 150 images/s. Images of the intact trachea and its mucus transport were recorded in anesthetized spontaneously breathing mice. NaCl solution (0.9% and 7%) or Lipopolysaccharide were applied intranasally.

OCM resolved detailed structure of the trachea and enabled measuring the airway surface liquid (ASL) thickness through the tracheal wall. Without stimulation, the amount of ASL was only a few μm above the epithelium and remained constant. After intranasal application of 30 μl saline at different concentrations, an early fast cough-like fluid removal with velocities higher than 1 mm/s was observed that removed a high amount of liquid. The ASL thickness increased transiently and quickly returned to levels before stimulation. In contrast to saline, application of Lipopolysaccharide induced substantial mucus release and an additional slow mucus transport by ciliary beating (around 100 μm/s) towards the larynx was observed.

In conclusion, OCM is appropriate unique tool to study mechanisms of mucus transport in the airways and effects of therapeutic interventions in living animals.

OCT-based three-dimensional, three vector component imaging of cilia-driven fluid flow in animal models of ciliated respiratory epithelium
Brendan K. Huang, Kevin C. Zhou, Ute A. Gamm, Vineet Bhandari, Mustafa K. Khokha M.D., Michael A. Choma M.D., Yale Univ. (United States)

One critical barrier to the robust study of cilia-driven fluid flow is the lack of methods for acquiring three-dimensional (3D) images of three vector component (3C) measurements of flow velocities. A 3DC map of cilia-driven fluid flow quantifies the flow speed along three Cartesian axes (i.e. three Cartesian vector components v_x, v_y, v_z) at each point in three-dimensional space. Our correlation-based methods were demonstrated in Xenopus embryo skin and ex vivo mouse trachea. Second, we developed a new approach to particle tracking velocimetry that generates 2D2.5C (2.5C: v_x, |v_y|, v_z) velocity fields from single-plane 2D image acquisitions. We demonstrated this particle streak velocimetry method in calibrated flow phantoms as well as in Xenopus embryos and ex vivo mouse trachea. Additionally, we have preliminary results extending particle streak velocimetry to 3DC in calibrated flow phantoms with ongoing work in Xenopus embryos.

Comprehensive imaging of mucociliary clearance, pH, and rheology
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Cystic fibrosis (CF) is an autosomal recessive genetic disorder that is characterized by impaired mucociliary clearance (MCC), leading to lung function loss through airway obstructions and increased incidence of infection. The fundamental defect in CF is known to be a mutation in the gene encoding the anion channel known as the cystic fibrosis transmembrane conductance regulator (CFTR), though the pathogenic relationship linking the basic ionic transport defect and mucus transport failure remains elusive. One recent hypothesis implicates the lack of bicarbonate transport in CF epithelia, which is thought to thwart maturation of mucus produced by secretory cells, resulting in highly viscous and tethered mucus strands that resist clearance. Cell culture models such as human bronchial epithelial (HBE) cells express the key features of CF disease, including ion transport, viscosity, and MCC defects, and have thus served as key platforms to probe the mechanisms of CF and to investigate possible treatment strategies.
We have developed a multimodality optical instrument that comprehensively characterizes mucociliary clearance, pH, and fluid rheology properties in HBE CF culture models in vitro, allowing the correlations between these parameters to be interrogated. We utilize micro-optical coherence tomography (OCT), a 1-µm axial resolution imaging modality developed in our laboratories to characterize the moving parts of the MCC apparatus, including ciliary beat frequency, mucus volume, and flow rate. We have also integrated a co-aligned scanning dual-excitation fluorescence module, designed for the ratiometric pH sensing dye BCECF. The diffusion of the BCECF dye in the mucus also reveals its fluid rheology properties by configuring the excitation laser scan to perform fluorescence recovery after photobleaching (FRAP).

We present combined OCT/pH/FRAP imaging results from HBE cultures that conjunctively characterize their mucus transport, ion transport, and fluid rheology properties, thus capturing the critical aspects of CF disease that are manifested by the in vitro model. This tool will be useful for studying the pathogenic mechanisms of CF and to investigate the effects of candidate treatment compounds.

9691-41, Session 10
Towards all-optical quantification of force- and power-based performance metrics in cilia-driven fluid flow physiology

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In pulmonary ciliary physiology, most tissue-level measures of performance focus on flow velocity. However, as with the heart, fluid transport performance requires an understanding of force and power generation under various loading conditions. Here, we present our initial work in quantifying shear force and net power dissipation from OCT-based cilia-driven fluid flow velocimetry. Typical measurements of force require invasive contact with the ciliated surface, while measurements of power rely on metabolic consumption that reflect energy consumption not just from cilia, but from the entirety of cellular processes. We will present two different approaches to non-contact, all-optical shear force and power dissipation physiology. First, we developed a lumped-parameter model of flow driven by a ciliated surface. The lumped-parameter model yields semi-quantitative, Ohm's law-type relationships (F=U*R and P=U*F) between flow velocity (U), shear force (F), viscous resistance (R), and power dissipation (P). This model allows a lumped (spatially averaged) approach to evaluate force and power performance under viscous loading, an approach we demonstrated using ciliated Xenopus embryos. Second, we numerically estimate shear force and power dissipation using flow velocity fields acquired using OCT. Specifically, the velocity gradient tensor estimated from the flow velocity field contains the required information to estimate both shear force and net power dissipation. We have preliminary data using this numerical approach in Xenopus. Our results support the feasibility of an all-optical approach to estimating mesoscopic measures of force and power in ciliary physiology.

9691-42, Session 10
Endoscopic optical coherence microscopy for imaging the upper airways in humans

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We present endoscopic optical coherence microscope (eOCM) for imaging the nasal mucosa including the concha nasalis in humans. Our eOCM device is based on a supercontinuum light source, a custom made 400 nm spectrometer, and a custom made high resolution GRIN endoscope. We achieved a lateral resolution below 1 µm and a axial resolution better than 1.7 µm in air. The imaging speed reached up to 150 B-scans per second. Additionally, an irrigation channel and a chip-on-the-tip RGB camera were integrated. The semi-contact endoscope has an outer diameter of 3 mm and is connected via a novel compact MEMS scanner to light source and spectrometer.

In healthy human volunteers, the epithelium and the subepithelial tissue including mucus glands and blood vessels of concha nasalis were visible. The beating of cilia was visualized in living humans and the ciliary beat frequency could be determined by a contrast based algorithm.
eOCM has a great potential for imaging functional changes of the upper airways in patients. The new eOCM provides the technical means for clinical diagnosis different diseases like ciliary dyskinesia and cystic fibroses.

9691-43, Session 11
Assessing idiopathic pulmonary fibrosis with bronchoscopic optical coherence tomography

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Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal form of fibrotic lung disease, with a significantly worse prognosis than other forms of pulmonary fibrosis (3-year survival rate of 50%). Distinguishing IPF from other fibrotic diseases is essential to patient care because it stratifies prognosis and therapeutic decision-making. However, making the diagnosis often requires invasive, high-risk surgical procedures to look for microscopic features not seen on chest CT, such as characteristic cystic honeycombing in the peripheral lung. Optical coherence tomography (OCT) provides rapid 3D visualization of large tissue volumes with microscopic resolutions well beyond the capabilities of CT. We aim to determine whether bronchoscopic OCT can provide a low-risk, non-surgical method for IPF diagnosis. We have developed bronchoscopic OCT catheters that access the peripheral lung and conducted in vivo peripheral lung imaging in patients, including those with pulmonary fibrosis. We also conducted bronchoscopic OCT in ex vivo lung from pulmonary fibrosis patients, including IPF, to determine if OCT could successfully visualize features of IPF through the peripheral airways. Our results demonstrate that OCT is able to visualize characteristic features of IPF through the airway, including microscopic honeycombing (< 1 mm diameter) not visible by CT, dense peripheral fibrosis, and spatial disease heterogeneity. We also found that OCT has potential to distinguish mimickers of IPF honeycombing, such as traction bronchiectasis and emphysema, from true honeycombing. These findings support the potential of bronchoscopic OCT as a minimally-invasive method for in vivo IPF diagnosis. However, future clinical studies are needed to validate these findings.

9691-44, Session 11
Rapid multispectral imaging endoscopy system for real-time mapping of the mucosa blood supply in the lung

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We developed a rapid multispectral imaging endoscopy system for real-time mapping of mucosa blood supply in the bronchial tree. For fast mapping, the system is designed to acquire images in 18 spectral bands spanning 400–760 nm by combining a customized light source with six triple-band filters and a standard color CCD camera. A new method is developed to calibrate the overlapping spectral responses of the three imaging channels (red, green, blue) in the color CCD camera. Imaging speed of 15 spectral image cubes/second is achieved. A spectral analysis algorithm
based on a linear matrix inversion approach is developed. The algorithm is implemented with graphics processing unit (GPU) programming and has been used to map the mucosa optical properties. The safety and efficacy of the developed device and method are evaluated through in-vivo lung patient measurements. The accuracy of the results is validated using point spectroscopy measurements and analysis. With a medium performance GPU (GeForce GTX 470), we demonstrated that the device and method developed for spectral analysis can produce, in 2 seconds, quantitative images (200x200 resolution) of the hemoglobin concentration and the hemoglobin oxygen saturation index with accuracy comparable to that obtained from spectroscopy measurements. We envision that with the advance of GPU processors, the proposed device and method would provide real-time mapping of mucosa’s blood supply. A clinical trial to test the utility of the system for improving early lung cancer detection is warranted.

9691-45, Session 11

Using polarization-sensitive optical coherence tomography to identify tumor stromal fibrosis and increase tumor biopsy yield

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Tissue biopsy is the principal method used to diagnose tumors in a variety of organ systems. It is essential to maximize tumor yield in biopsy specimens for both clinical diagnostic and research purposes. This is particularly important in tumors where additional tissue is needed for molecular analysis to identify patients who would benefit from mutation-specific targeted therapy, such as in lung carcinomas. Inadvertent sampling of fibrotic stroma within tumor nodules contaminates biopsies, decreases tumor yield, and can impede diagnosis. The ability to assess tumor composition and guide biopsy site selection in real time is likely to improve diagnostic yield. Polarization sensitive OCT (PS-OCT) measures birefringence in organized tissues, such as collagen, and could be used to distinguish tumor from fibrosis. In this study, PS-OCT was obtained in 65 lung nodule samples from surgical resection specimens containing varying ratios of tumor and fibrosis. PS-OCT was obtained with either a custom-built helical scanning catheter (0.8 or 1.6mm in diameter) or a dual-axis bench-top scanner. Strong birefringence was observed in nodules containing dense fibrosis, with no birefringence in adjacent regions of tumor. Tumors admixed with early, loosely-organized collagen demonstrated mild-to-moderate birefringence, and tumors with little collagen content showed little to no birefringent signal. PS-OCT provides significant insights into tumor nodule composition, and has potential to differentiate tumor from stromal fibrosis during biopsy site selection to increase diagnostic tumor yield.

9691-46, Session 11

Exploiting the relationship between birefringence and force to measure airway smooth muscle contraction with PS-OCT

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The ability to observe airway dynamics is fundamental to forming a complete understanding of pulmonary diseases such as asthma. We have previously demonstrated that Optical Coherence Tomography (OCT) can be used to observe structural changes in the airway during bronchoconstriction, but standard OCT lacks the contrast to discriminate airway smooth muscle (ASM) bands- ASM being responsible for generating the force that drives airway constriction- from the surrounding tissue. Since ASM in general exhibits a greater degree of birefringence than the surrounding tissue, a potential solution to this problem lies in the implementation of polarization sensitivity (PS) to the OCT system. By modifying the OCT system so that it is sensitive to the birefringence of tissue under inspection, we can visualize the ASM with much greater clarity and definition. In this presentation we show that the force of contraction can be indirectly measured by an associated increase in the birefringence signal of the ASM. We validate this approach by attaching segments of swine trachea to an isometric force transducer and stimulating contraction, while simultaneously measuring the exerted force and imaging the segment with PS-OCT. We then show how our results may be used to extrapolate the force of contraction of closed airways in absence of additional measurement devices. We apply this technique to assess ASM contractility volumetrically and in vivo, in both asthmatic and non-asthmatic human volunteers.

9691-47, Session 12

Multimodal in vivo imaging of lung cancer and its microenvironment

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Despite significant advances in targeted therapies for lung cancer, nearly all patients develop drug resistance within 6-12 months and prognosis remains poor. Developing drug resistance is a progressive process that involves tumor cells and their microenvironment. We hypothesize that microenvironment factors alter tumor growth and response to targeted therapy. We conducted in vitro studies in human EGFR-mutant lung carcinoma cells, and demonstrated that factors secreted from lung fibroblasts results in increased tumor cell survival during targeted therapy with EGFR inhibitor, gefitinib. We also demonstrated that increased environment stiffness results in increased tumor survival during gefitinib therapy. In order to test our hypothesis in vivo, we developed a multimodal optical imaging protocol for preclinical intravital imaging in mouse models to assess tumor and its microenvironment over time. We have successfully conducted multimodal imaging of dorsal skinfold chamber (DSC) window mice implanted with GFP-labeled human EGFR mutant lung carcinoma cells and visualized changes in tumor development and microenvironment facets over time. Multimodal imaging included structural OCT to assess tumor viability and necrosis, polarization-sensitive OCT to measure tissue birefringence for collagen/fibroblast detection, and Doppler OCT to assess tumor vasculature. Confocal imaging was also performed for high-resolution visualization of EGFR-mutant lung cancer cells labeled with GFP, and was coregistered with OCT. Our results demonstrated that stromal support and vascular growth are essential to tumor progression. Multimodal imaging is a useful tool to assess tumor and its microenvironment over time.

9691-48, Session 12

Optical coherence tomography imaging to analyze biofilm thickness from distal to proximal regions of endotracheal tubes

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Ventilator-associated pneumonia (VAP) is a nosocomial lung infection that has been linked to the presence of biofilm in endotracheal tubes of critical care patients. Biofilm development can start as early as one day post intubation, and progressively accumulate as time progresses. The longer a patient is intubated, the higher the risk of contracting VAP. Prior methods to evaluate biofilm in endotracheal tubes are difficult and cannot be performed in-vivo or on a repeated basis. Optical Coherence Tomography (OCT), a non ionizing tomographic imaging modality, has the capabilities to assess biofilm formation non-invasively. The goal of this study is to use OCT to evaluate differences in biofilm accumulation from the proximal to the distal tip of the endotracheal tube. Biofilm localization investigation will enable insights into the development and progression of biofilm and factors influencing the relationship between biofilm accumulation and the risk of developing VAP. In this study, we imaged 20cm segments of extubated endotracheal tubes from critical care patients using a side viewing rotational OCT probe. Tubes were collected from patients at variable times, dependent on their own treatment and illnesses. The OCT data was then processed using a MATLAB algorithm to calculate the percent occlusion of the tube caused by the biofilm location within the tube. Ultimately, the goal will be to determine if OCT can be a tool to visualize biofilm development and potential interventions to reduce the incidence of VAP.

9691-49, Session 12
Correction of motion artifacts in OCT-AFI data collected in airways
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Optical coherence tomography (OCT) provides in vivo imaging with near-histologic resolution of tissue morphology. OCT has been successfully employed in clinical practice in non-pulmonary fields of medicine such as ophthalmology and cardiology. Studies suggest that OCT has the potential to be a powerful tool for the detection and localization of malignant and non-malignant pulmonary diseases. The combination of OCT with autofluorescence imaging (AFI) provides valuable information about the structural and metabolic state of tissues. Successful application of OCT or OCT-AFI to the field of pulmonary medicine requires overcoming several challenges. This work address those associated with motion: cardiac cycle, breathing and non-uniform rotation distortion (NURD) artifacts. Mechanically rotated endoscopic probes often suffer from image degradation due to NURD. In addition cardiac and breathing motion artifacts may be present in-vivo that are not seen ex-vivo. These motion artifacts can be problematic in OCT-AFI systems with slower acquisition rates and have been observed to generate identifiable prominent artifacts which make confident interpretation of observed structures (blood vessels, etc.) difficult. Understanding and correcting motion artifact could improve the image quality and interpretation. In this work, the motion artifacts in pulmonary OCT-AFI data sets are estimated in both AFI and OCT images using a locally adaptive registration algorithm that can be used to correct/reduce such artifacts. Performance of the algorithm is evaluated on images of a NURD phantom and on in-vivo OCT-AFI datasets of peripheral lung airways.

9691-50, Session 12
Ultra-small 3D printed micro-lens and mirror assembly for endoscopic assessment of the airway
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Miniaturization of OCT probes for endoscopic applications presents significant design and fabrication challenges. Common approaches include the use of GRIN lenses; ball lenses; and carefully controlled lengths of GRIN fiber. Recent developments in 3D printing have enabled the fabrication of smooth, transparent constructs on the scale of tens to hundreds of microns. Commercialized by Nanoscribe (Germany), the technology has the potential to be used to fabricate micro-lenses appropriate for use with OCT systems. We have developed a 3D printed lens and reflector assembly, designed as part of an endoscopic probe for imaging the airway lumen. Measuring only 0.6 x 1.3 mm (diameter x length), the IP-S construct consists of a small dome that collimates the light as it enters, and a cylindrical body that contains a diagonal air gap orientated at 48 degrees to redirect the light beam using total internal reflection. It is mounted on a micromotor (1 x 3.5 mm), which rotates the lens to radially scan the beam. A length of Kapton tubing is used to hold the lens and single-mode optical fiber cleaved endface at a controlled distance.

To assess the performance of the 3D printed micro-lens and reflector assembly, we also constructed a more traditional probe comprising a GRIN lens focusing light onto a 0.7 mm, tilted mirror mounted on the end of a micromotor, and in both cases attached to a 1300nm MEMS-VCEL swept-source light source. We present a characterization of the performance of both probes, and show results acquired with ex vivo airway.
Basophils activation test as a new implementation of optics in medicine

Anna Skotny, Wroclaw Medical Univ. (Poland); Barbara Kmiecik, Wroclaw Univ. of Technology (Poland)

Medicine strives for non-invasive, fast, and accurate diagnostic methods. Flow cytometry (FCM) as the optics-based research technique meets those expectation and adopting it as standard diagnostic method could revolutionize nowadays allergology. About 30-40% of people suffers from different types of allergies and the improvement of existing diagnostic methods is essential.

Basophils activation tests (BAT), based on FCM, might be the solution. Since BAT are conducted in vitro the risk of causing anaphylaxis or other dangerous complications is eliminated. Method is time-efficient and enables to diagnose patient with good accuracy and selectivity.

There is still much to be done in area of new markers used in identification protocols. Existing protocols aren't ideal. Conducted research allow to conclude about potential of anti-CCR3 as an great identification marker. Conducted comparative research about protocols such as CCR3+, CRTH2+/CD3-/CD203c+ and CD123+/HLA-DR- shall be presented. It would allow to found most stable and accurate protocol of identification as well as impulse to look for new markers that still can be found[1].

However, due to lack of well-established procedure standards, at the moment the method is considered inappropriate for routine diagnostics. Properly selected activation markers and identification protocols makes BAT test considered as an excellent complementary diagnostic method. In conclusion, making BAT test a standard in allergy diagnostics could largely improve patients' comfort and safety.

References

9692-1, Session 1

Comparing irradiation parameters on disinfecting enterococcus faecalis in root canal disinfection

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Although bacteria can immigrate through deep dentin tubules, lack of penetration for chemical irrigants is a major problem for the endodontic treatment. Laser-assisted-disinfection is a new but precise and important era in endodontics because light can penetrate to the depths through dentinal tubules and also effective even in branches and accessory canals. Selecting the appropriate laser and defining the optimum parameters are major purposes for the right treatment.

The sterilizing capability of two different lasers, Er:YAG and Thulium Fiber Laser were explored in our study. Different laser powers were selected according to our pre-studies in root canal treatment, and each combination of parameter were tested on E.faecalis bacteria. There were also non-treated control groups for each irradiation group. Effect of each laser power was compared with its own control group.

The results of treated and non-treated groups were compared for this part of the study. Both laser-treated groups were significantly different from the control groups (Mann-U Whitney, significance p 0.05). While comparing Er:YAG Laser with a power of 0.50 W to EDTA Control Group, laser caused a 71.08% reduction in root canal. On the other hand, thulium fiber laser with the same power. caused only 60.09% reduction. When we increased laser power of Thulium Fiber Laser to 1W, a reduction of 98.18% was observed. Thus showed that disinfection effect is directly proportional to laser power and the values and increasing power makes laser a promising method in disinfection.

9692-2, Session 1

405-nm diode laser, alone and in conjunction with a Fenton’s reaction system in the endodontic root canal disinfection

Giuseppe Lagori, Jean Paul Rocca D.D.S., Carlo Fornaini M.D., Univ. de Nice Sophia Antipolis (France); Elisabetta Merigo D.D.S., Univ. de Nice Sophia Antipolis (France)

One of the most important challenges in endodontics is represented by the achievement of the complete disinfection of root canals. Traditionally, the technique involves, in addition to mechanical preparation, canals disinfection with chemical agents such as sodium hypochlorite, hydrogen peroxide and chlorexidine, alone or in combination, even if some bacterial species are particularly resistant to the eradication. Taking into account as term of reference the Enterococcus Faecalis (EF), in this preliminary study we tested the bactericidal effectiveness of two different methods, 405 nm laser and Fenton’s reagent (Hydrogen peroxide + Fe++ ions), alone and in conjunction with each other (photo-Fenton reaction). In a micro-titre we put 0.1 ml of EF bacterial 1 Mc Farland standard concentration solution and subsequently were added increasing concentrations of sodium hypochlorite or Fenton’s reagent. A 0.1 ml of EF Mc Farland solution was added with scalar concentrations of sodium hypochlorite or Fenton’s reagent. Irradiation with 405 nm laser light at increasing values of power density, alone and in conjunction with the described agents, was performed to verify the bactericidal action following the minimum inhibition concentration (MIC) technique. The photo-Fenton reaction showed the ability to eradicare EF: this result in terms of bactericidal efficacy needs to be confirmed in in vitro and in vivo tests, possibly representing a new opportunity to disinfect root canals with lower toxicity and greater safety.

9692-3, Session 1

A digital moiré interferometric analysis on the effect of nanoparticle conditioning on the mechanical behavior of dentin

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Background

Dentin is a calcified and hydrated biological composite that forms the major bulk of tooth structure. Understanding the biomechanical response of dentin structure to forces is crucial to develop treatment strategies to restore the loss of mechanical integrity associated with dentin loss during dental treatment procedures. Digital Moiré Interferometry (DMI) is an optical interferometry based technique which provides a whole field information in real-time with high sensitivity. They can be applied to study the biomechanical response in dental hard tissues under clinically realistic loading conditions, distinctly different to traditional mechanical testing.

Objectives

The aim of this study is to conduct a DMI analysis to evaluate (1) the degree and pattern of microstrain distributions in dentin after root canal preparation, and (2) the influence of nanoparticle based conditioning on the mechanical microstrain distribution in dentin, qualitatively.

Materials and Methods

Fifteen extracted human upper anterior teeth were prepared to slab shaped facio-lingual section of dentin with #15 (n=3), #30 (n=6) and #50 (n=6) canal sizes and treated with/without nanoparticles conditioning. A high frequency grating of 2400 lines/mm were replicated on the specimens which were subsequently loaded compressively from 10 to 50N at the cervical regions of tooth. Moiré fringes were analyzed to determine the in-plane deformation in dentin that perpendicular (U-field) and parallel to the dentinal tubules (V-field).

Results and Conclusion

The strain distribution gradually increased in the direction perpendicular to the dentinal tubules, while it remained constant in the direction parallel to the tubules during compressive loadings. The root dentin, which was conditioned with the nanoparticles solution, displayed markedly less microstrain increase with loads. The current photomechanical experiment highlighted the significance of nanoparticle treatment to improve the mechanical integrity of dentin.

9692-4, Session 2

Microsecond enamel ablation with 10.6 µm CO2 laser radiation

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Application of a laser device in dental surgery allows removal of tissue with higher precision, which results with minimal loss of healthy dental tissue. In this study we use a RF discharge excited CO2 laser operating at 10.6 µm. The wavelength of 10.6 µm overlaps with a phosphate band (PO3-4) absorption in dental hard tissue hence the CO2 laser radiation has been selected as a potential source for modification of the tissue. This research describes an in-depth analysis of single pulse laser ablation. To determine the parameters that are best suited for the ablation of hard dental tissue without thermal cracking, a range of pulse lengths (10-200 µs), and fluences (0-250 J/cm2) are tested. In addition, different laser focusing approaches are investigated to select the most beneficial way of delivering laser radiation to the surface (divergent/convergent beam). Furthermore initial results presenting multiple
pulse tissue removal are shown.

To ensure that any of these processes do not increase the temperature above the critical threshold and cause the necrosis of the tissue a set of thermocouples was placed into the pulpal chambers. Both continuous and intermittent laser radiation were investigated with and without application of a water spray to cool down the ablation site and the adjacent area.

Results show that the temperature can be kept below the critical threshold either by using water spray or by decreasing the repetition rate. We demonstrate that CO2 laser pulses with pulse lengths in the regime of 10 µs can provide precise enamel tissue removal without introducing any unwanted thermal damage.

9692-5, Session 2

A new sealed RF-excited CO2 laser for hard tissue ablation operating at 9.4-µm with pulse duration of 26 µs

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Several studies over the past 20 years have identified that carbon dioxide lasers operating at wavelengths between 9.3 and 9.6-µm with pulse durations near 20-µs are ideal for hard tissue ablation. Those wavelengths are coincident with the peak absorption of the mineral phase and the pulse duration is close to the thermal relaxation time of the deposited energy of a few microseconds to minimize peripheral thermal damage and long enough to minimize plasma shielding effects to allow efficient ablation at practical rates. The desired pulse duration near 20-µs has been difficult to achieve since it is too long for TEA lasers and too short for RF-excited lasers for efficient operation. Recently, Coherent Inc. (Santa Clara, CA) developed the J5-V laser for microvia drilling which can produce laser pulses greater than 100 mJ in energy at 9.4-µm with a pulse duration of 26-µs and it can achieve pulse repetition rates of 3 KHz. We report the first results using this laser to ablate enamel and dentin. The onset of plasma shielding does not occur until the fluence exceeds 100 J/cm² allowing efficient ablation at rates exceeding 50-µm per pulse. This laser is ideally suited for the selective ablation of carious lesions.

9692-6, Session 2

Enhancing caries resistance with a short-pulsed CO2 9.3 µm laser: a laboratory study

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The objective of this laboratory study was to test whether irradiation with a new 9.3µm microsecond short-pulsed CO2-laser enhances enamel caries resistance with and without additional fluoride applications. 101 human enamel samples were divided into 7 groups. Each group was treated with different laser parameters (Carbon-dioxide laser, wavelength 9.3µm, 43Hz pulse-repetition rate, pulse duration between 37s to 77s (1.5µJ/pulse to 2.9µJ/pulse). Using a pH-cycling model and cross-sectional microhardness testing determined the mean relative mineral loss delta Z (ΔZ) for each group. The pH-cycling was performed with or without additional fluoride. The CO2 9.3µm short-pulsed laser energy rendered enamel caries resistant with and without additional fluoride use.

9692-7, Session 2

Er:YAG laser metal and ceramic bracket debonding

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The Er:YAG laser (wavelength 2.94 µm) has been proved as the drilling device for hard dental tissues and carries removal in the past. In our study this laser radiation was used for the brackets debonding. For the experiment three groups of metal and ceramic brackets with two adhesive materials were examined. The amount of enamel loss and residual resin on teeth has been evaluated. During the treatment the only brackets were irradiated by Er:YAG laser and water cooling was implemented. Temperature rise inside the tooth (measured by special thermometer in the place of pulp) as well as temperature value on the tooth surface (checked by IR sensitive pyroelectric camera) was observed giving the possibility to find the value of optimal laser radiation mean power as well as the interaction time for the most rapid and safe debonding. The dose of Er:YAG laser was: energy of 270 mJ with repetition rate 6 Hz - mean power 1.6 W (250 us long pulses), action ~ 140 s. The enamel surface was than evaluated in scanning electron microscope (SEM).

9692-8, Session 3

Long-wave infrared thermophotometric imaging of early demineralization in dental hard tissue

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Dental caries is identified as the most prevalent chronic disease and the leading cause of tooth loss worldwide. Existing clinical diagnostic methods, such as visual/tactile inspections and X-ray radiography, suffer from poor sensitivity and specificity in detecting the early stages of caries formation, when the process can still be stopped or even reversed. In this paper, we present a clinically and commercially viable thermophotometric imaging (TPI) technology capable of detecting early enamel caries using an inexpensive long-wavelength infrared (LWIR, 8-14µm) camera. The system utilizes the enhanced light absorption property of early caries as the source of diagnostic contrast. Application of LWIR detectors in dental TPI is novel and beneficial due to the enhanced light absorption of enamel in the LWIR band, suppressing the masking effect of direct thermal radiation from subsurface regions. Moreover, the peak of the black body radiation for an object at room temperature lies in the LWIR band, providing approximately 20 times more photon flux to the detector than that in the mid-wavelength infrared band (3-5µm) used in the existing TPI systems. To further exploit the advantages of LWIR detection, theoretical modeling of subsurface light absorption in an optically excited turbid medium is studied by solving the associated coupled diffuse-photon-density and thermal-wave field problem. The performance of the system is evaluated through experiments carried out on non-biological samples as well as on extracted teeth with natural and artificially-induced caries. Results suggest the capability of the developed inexpensive system in resolving early enamel demineralization.

9692-9, Session 3

A system for simultaneous near-infrared reflectance and transillumination imaging of occlusal lesions

Jacob C. Simon, Cynthia L. Darling, Daniel Fried, Univ. of
Clinicians need technologies to improve the diagnosis of questionable occlusal carious lesions (QOCs) and determine if decay has penetrated to the underlying dentin. Assessing lesion depth from near-infrared (NIR) images holds great potential due to the high transparency of enamel and stain to NIR light at $\lambda \approx 1300-1700\text{-nm}$, which allows direct visualization and quantified measurements of enamel demineralization. Unfortunately, NIR reflectance measurements alone are limited in utility for approximating occlusal lesion depth beyond $>200$-$\mu$m due to light attenuation from the lesion body. Previous studies sought to combine NIR reflectance and transillumination measurements taken at $\lambda \approx 1300$-$\mu$m alone in order to estimate QOC depth and severity. The objective of this study was to quantify the change in lesion contrast and size measured from multispectral NIR reflectance and transillumination images of natural occlusal carious lesions with increasing lesion depth and severity in order to determine the optimal multimodal wavelength combinations for estimating QOC depth. Extracted teeth with varying amounts of natural occlusal decay were measured using a multispectral-multimodal NIR imaging system at prominent wavelengths within the $\lambda \approx 1300-1700$-$\text{nm}$ spectral region. Image analysis software was used to calculate lesion contrast and area values between sound and carious enamel regions. Optical measurements were then compared with histological measurements of lesion depth acquired from transverse microradiography (TMR) and polarized light microscopy (PLM).

Structured changes in dentin lesions after remineralization with PS-OCT, thermal and near-infrared reflectance imaging

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

Accurate detection and measurement of the highly mineralized surface layer that forms on caries lesions is important for diagnosis of the lesion activity. Previous studies have demonstrated that optical imaging methods can be used to measure the degree of remineralization on enamel lesions. The purpose of this study was to determine if PS-OCT, thermal and near-IR reflectance imaging could be used to assess the remineralization process in simulated dentin lesions. Artificial bovine (n=30) dentin lesions were prepared by immersion in a demineralization solution for either 8 and 24 hours and they were subsequently placed in an acidic remineralization solution for up to 12-days. The thickness of the surface layer and the integrated reflectivity of the subsurface lesion were measured using PS-OCT. The samples were dehydrated using an air spray for 30 seconds and imaged using thermal camera and InGaAs cameras. The area enclosed by the time-temperature curve, $Q_I$, from thermal imaging decreased significantly with longer periods of remineralization ($P<0.05$). However, near-IR reflectance intensity differences, $I_I$, before and after dehydration failed to show any significant relationship with the degree of remineralization. This study shows that PS-OCT and thermal imaging can be used for the assessment of the remineralization of dentin lesions.

Comparative study for labial frenectomy: diode laser 980 nm vs Nd:YAG laser

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Labial frenulums are anatomical structures that, occasionally, can assume inadequate size or location and may lead to functional and aesthetic limitations requiring a surgical treatment called frenectomy. The aim of this study was to compare the effect of two laser wavelengths on the degree of post-operative pain experienced by paediatric patients following a frenectomy. Sixteen females and 14 males were included in the study: 16 patients underwent to frenectomy using a diode laser 980 nm, 14 patients were treated with Nd:YAG laser for frenectomy. The VAS scores of pain was evaluated through the Faces Pain Scale revised on days 3 and 10 from the operation.

No differences in terms of bleeding were recorded between the two laser wavelengths ($p>0.05$): all the operations recorded an optimum degree of haemostasis.

All the patients in both groups recorded a low pain score.

The inflammation score at postoperative day 3 was significantly lower in the group of patients treated with Nd:YAG laser when compared to the diode group. No differences were recorded at day 10 between the two techniques.

The results obtained for bleeding control and haemostasis confirmed that the two techniques are similar and comparable. In addition, no differences in patient compliance was recorded in the two groups of patients.

All the patients in both groups experienced low post-operative pain, reporting a score of below 4. In particular, the patients treated with Nd:YAG laser had significantly less immediate (day 3) post-operative pain and functional complications compared to diode surgery.

Blue diode laser: a new approach in oral surgery?

Carlo Fornaini M.D., Elisabetta Merigo D.D.S., Stefano Selleri, Annamaria Cucinotta, Univ. degli Studi di Parma (Italy)

In 1962 the first laser with a semiconductor as active medium was realized and it had soon a great diffusion also in medicine and, later, in dentistry tank to its great advantages.
The two main wavelengths up today used in oral surgery are 810 nm and 980 nm even if, recently, also other wavelengths emitting in the absorption spectrum of the water have been proposed.

Moreover, even if lasers emitting in the infrared portion are the most used in dentistry, also others in the visible portion were described as effective (Argon, KTP).

The use of 405nm diode laser seems to be very interesting; in fact, several authors have reported about its antimicrobial power, alone and in association to yellow cromophores according to the PDT technique, others have underlined its biomodulating properties, and possibility to polymerize composite resins and to activate the bleaching agents.

The aim of this study is to evaluate the effectiveness of this wavelength in the oral soft tissue surgery.

After a series of “ex vivo” tests performed on animal models to compare this laser to the others commonly used in dentistry, some “in vivo” tests have been performed on human subjects.

The results have demonstrated that 405nm diode laser may be successfully used, also at very low energy, without side effects and excessive thermal elevation.

9692-14, Session PSun
Evaluation of gingival thickness and biotype using optical coherence tomography
Chiaki Maeda, National Ctr. for Geriatrics and Gerontology (Japan); Junji Tagami, Tokyo Medical and Dental Univ. (Japan); Yasunori Sumi D.D.S., National Ctr. for Geriatrics and Gerontology (Japan)

The anatomical characteristics of the gingival thickness for evaluating the gingival biotype influence the planning and prognosis of dental treatment. Therefore, the precise and careful examination of the gingival thickness is necessary to guide the dental treatment.

Many studies have been reported on measuring the gingival thickness for evaluating the gingival biotype. Of them, probe visibility is most popular and the clinical golden standard method to discriminate the gingival thickness and biotype. However, this method is prone to subjective interpretation and has slight invasiveness. An objective and non-invasive method for measuring the gingival thickness is necessary.

Optical coherence tomography (OCT) is a new method of biomedical imaging that can generate high-resolution and cross-sectional images of microstructures. OCT has been widely used as a diagnostic tool in clinical trials and animal studies, due to the nature of noninvasive scanning, high-speed imaging, high spatial resolution and no extraneous agent needed.

Few studies of the gingival thickness for evaluating the gingival biotype using OCT images have been published in the literature. We have applied newly developed swept-source optical coherence tomography (SS-OCT, Yoshida Dental Co., Ltd.) for measuring the labial gingival thickness for evaluating the gingival biotype.

The purpose of this study is to evaluate labial gingival thickness using the OCT system, and to discuss the application of our new OCT system for gingival thickness for evaluating the gingival biotype.

9692-15, Session PSun
Non-destructive inspection methods for metal-ceramic restoration using swept-source optical coherence tomography
Chiaki Maeda, National Ctr. for Geriatrics and Gerontology (Japan); Junji Tagami, Tokyo Medical and Dental Univ. (Japan); Yasunori Sumi D.D.S., National Ctr. for Geriatrics and Gerontology (Japan)

Metal-ceramic restorations are widely used in dentistry with a high degree of general success. However, ceramic materials have the potential to fracture due to their internal defects and brittle nature.

A reduction in the internal defect rate is much needed for the long-term function and stability of prostheses.

Quality control is an important aspect of metal-ceramic restorations and processing from the standpoint of providing them with acceptable clinical value, and assuring the safety of products.

Non-destructive inspection is an extensive group of analysis techniques used in science and industry to evaluate the properties of a material, component or system without causing damage. However, only a few applications of non-destructive inspection to dental materials have been reported.

The purpose of this study was to present the first internal examination of metal-ceramic restorations using a new advanced-type OCT (Optical coherence tomography) scanner, and to discuss the application of the new OCT system for non-destructive inspection of metal-ceramic restorations.

SS-OCT images of the 119 metal-ceramic restorations were obtained in order to investigate their porosity.

Internal defects (one or more voids of ≥20 μm) were observed in 5.3% of all teeth in this study.

It is concluded that OCT can detect nonvisible internal structures in metal-ceramic restorations, a finding not reported to date. OCT may, therefore, be an appropriate method for detecting interior defects in metal-ceramic restorations nondestructively.
Three-dimensional quantification of dental plaque using swept-source optical coherence tomography

Yoshihiro Heshiki, National Ctr. for Geriatrics and Gerontology (Japan)

The major causes of tooth loss are caries and periodontal disease. The pathogenic factor in these diseases is dental plaque. There are many methods in the literature for measuring or quantifying dental plaque, however, most of them have problems of poor reproducibility and lack of true objectivity. An objective method for the evaluation of dental plaque is required in clinical practice.

The purpose of this in vivo study was to investigate swept-source optical coherence tomography (SS-OCT) as a new tool for evaluating three-dimensional dental plaque more objectively. The National Center for Geriatrics and Gerontology has developed a new SS-OCT system jointly with Santec Corporation of Aichi, Japan. We evaluated dental plaque in 25 patients using SS-OCT at the Division of Oral and Dental Surgery, National Centre for Geriatrics and Gerontology. Three-dimensional data provided by SS-OCT were analyzed using analysis software (Avizo6.1). Three-dimensional data on dental plaque were clearly observed with SS-OCT, and it was possible to calculate the area and volume of dental plaque. This method can acquire consistent data, according to the present invention that automatically extract a dental plaque region in an image with no involvement of human evaluation by using a computer.

It is possible to obtain clear three-dimensional images of dental plaque with SS-OCT. This method has the potential for objective quantitative screening of dental plaque. It is concluded that SS-OCT is an objective method for detecting area, volume and three-dimensional images of dental plaque.

Hard-tissue drilling by short-pulse CO2 laser with controllable pulse-tail energy

Kazuyuki Uno, Tatsufumi Sasaki, Takuya Yamamoto, 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, and is very important for laser dentistry. A short-pulse CO2 laser can produce excavation of hard tissue (dentine and enamel) without carbonization. We developed a short-pulse CO2 laser pumped by a longitudinally excitation scheme. The laser pulse waveform of our CO2 laser has a spike pulse with a pulse width of about 100 ns and an energy of several mJ, and a pulse tail with a controllable energy (about 0 – 50 mJ). Therefore, in this work, we investigated the hard-tissue drilling characteristics (the depth, the surface diameter, and carbonization) depended on the pulse-tail energy and the fluence.

In the longitudinally excited CO2 laser, the laser tube consisted of a 45-cm-long alumina ceramic pipe with an inner diameter of 9 mm, a ZnSe output coupler (RR85%), and a high-reflection mirror with (r20 m). The laser pulse waveform was controlled by an excitation circuit, an input energy and medium gas. Several types of laser pulse waveforms (for example, the same spike-pulse energy of 1 mJ and the different pulse-tail energy of 7 mJ, 12 mJ or 21 mJ) were focused with a ZnSe lens with a focal length of 5 cm. In the 1 shot irradiation, higher pulse-tail energy produced the deeper ablation depth. The spike pulse gives ablation process and the pulse tail gives heat process. High pulse tail energy improves a processing efficiency but may gives carbonization.
9692-21, Session PSun

Structural changes in the irradiated dentin with Nd:YAG and Er:YAG lasers for cervical hypersensitivity treatment and their influence on the microtensile resistance in resin-dentin interface

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This study aimed to evaluate, in vitro, the structural changes in dentin surfaces irradiated with Er:YAG (2940 nm, 90 mJ, 2 Hz, 300 s), spot diameter 0.9 mm, 60 s/cm2, using the handpiece at 6 cm of distance to surface) and Nd:YAG (1064 nm, 1 W, 10 Hz, 300 s, optical fiber diameter 300 7m, 60 s/cm2, using the handpiece at 2 mm of distance to surface) lasers to the treatment of cervical hypersensitivity and the respective bond strength compromising of resin composite restorations over these surfaces. 45 bovine teeth were selected, and removed the enamel portion of the buccal surface for laser irradiation and restorative procedure. Samples were divided into three groups: G1: control, only fluoride therapy; G2: irradiated with Er:YAG laser; G3: irradiated with Nd:YAG laser. Samples were submitted to optical coherence tomography analysis and subsequently they were restored with resin composite and sectioned into sticks for microtensile tests of achievement. ANOVA analysis of variance for the maximum force (N) and the 50 mJ laser groups showed no statistically significant differences, regardless of the adhesive system used.

9692-22, Session PSun

Evaluation of microshear bond strength of composite to enamel of dental adhesive systems associated with Er,Cr:YSGG laser

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The aim of this in vitro study was to evaluate the microshear bond strength (MSBS) of resin composite to enamel etching by Er,Cr:YSGG laser with the use of two different adhesives systems. Fifty freshly extracted human molars halves were embedded in acrylic resin. The outer surface of the enamel specimens was then ground flat with water-cooled sandpaper of decreasing grit (400, 600) in order to produce a clinical relevant and standardize smear layer. The enamel specimens (n=10) were randomly assigned into six groups according to etching treatment and adhesive system. A two-step self-etching primer system (Clearfil SE Bond) and a universal adhesive used as an etch-and-rinse adhesive (Adaptra Single Bond Universal) were applied to the nonirradiated enamel surface according to manufacturer’s instructions, as control groups (G1 and G2, respectively). For the other groups, enamel surfaces were irradiated with the Er,Cr:YSGG laser with 0.5 W, 75 mJ and 66 J/cm2 (G3 and G4) and 1.25 W, 50 mJ and 44 J/cm2 (G5 and G6). Irradiation was performed under air (50%) and water (50%) cooling. The same adhesives were applied on Er,Cr:YSGG laser-irradiated enamel. Mean MSBS ± sd (MPa) for each group was 16.30 ± 3.03, 17.87 ± 5.83, 12.23 ± 2.02, 9.88 ± 2.26, 15.94 ± 1.98, 17.62 ± 2.10, respectively. Data were analyzed [ANOVA, Tukey post-hoc, a<0.05]. The control groups and the 50 mJ laser groups showed no statistically significant differences, regardless of the adhesive system used.

9692-23, Session PSun

Evaluation of the erosive action of juices on dental enamels by laser fluorescence and x-rays microfluorescence

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The aim of this study was to analyze the effect of industrialized fruit juices plus soy extract on the surface of dental enamel in order to establish the erosive potential of these solutions. The sample consisted of 70 bovine incisors whose crowns were prepared and randomly allocated into 7 groups (G1-Ades Grape; G2- Ades Apple; G3 - Solly’s Grape, G4 - Solly’s Apple, G5 - More Vita Grape G6 - More Vita Apple, G7 - Control, n=10 per group). The crowns were subjected to the action of juices plus soy extracts for 15 days. The pH values and Knoop microhardness were recorded and the samples were evaluated using X-rays microfluorescence (XRF) and laser fluorescence (FL.). Parametric test (Student t test, ANOVA, Tukey test) and nonparametric test (Wilcoxon and Kruskal - Wallis ) were used for statistical purposes. The medium pH for the juices for the first moment (p<1.14) and after 15 days (p<3.56) were below the level considered critical for the development of dental erosions (p<5.5). There was a decrease of about 49 % in the average microhardness values measured in groups of teeth exposed to the juices in the before / after comparison (p<0.05). The analysis by XRF showed a decrease of approximately 7 % Calcium and 4 % phosphorus present on the surface of the bovine crowns and laser fluorescence showed the final values of calcium and phosphorous were maintained below the borderline. The dental enamel suffers a real demineralization when it is exposed to the action of industrialized fruit juices and soy extract.

9692-24, Session PSun

Optical coherence tomography investigations of ceramic Lumineers

Luana O. Fernandes, Natalia D. R. L. Graça, Luciana S. A. de Melo, Claudio H. V. Silva, Anderson S. L. Gomes, Univ. Federal de Pernambuco (Brazil)

Lumineers are reduced veneer laminates with thickness ranging from 0.2 to 0.8mm. They are used as alternative for high quality aesthetic dental solutions without the need of preparation or minimal intervention to the tooth surface. However, the only means of adaptation and evaluation of clinical integrity of the laminates is limited to visual inspection. The objective of this study was to use the Optical Coherence Tomography (OCT) technique working in spectral domain to analyze in vivo the integrity and adaptation of 14 lumineers, 180 days after cementation. It was possible to observe in the obtained images the efficacy of OCT to provide the identification of various types of undesirable alterations, from the presence of bubbles in the cement line to the presence of stress areas in the laminate. Moreover, it was possible to measure the size of these bubbles and define its location. It was concluded that the OCT is an effective and promising method for clinical assessment of lumineers.
9692-25, Session PSun

**Monitoring the gingival regeneration after aesthetic surgery with optical coherence tomography**

Luana O. Fernandes, Natalia D. R. L. Graça, Luciana S. A. de Melo, Claudio H. V. Silva, Anderson S. L. Gomes, Univ. Federal de Pernambuco (Brazil)

The objective of this study was to use Optical Coherence Tomography (OCT) working in spectral domain to monitor the process of tissue reparation in patients undergoing periodontal plastic surgery, observing the gingival behavior over a period of 60 days from 42 sites on the vestibular face of the anterior teeth. The images obtained by OCT analysis (Swept Source OCT at 1325 nm, Thorlabs, New Jersey, USA), allowed identifying and differentiating relevant dental and periodontal anatomic regions and thus follow the gingival healing process. The images obtained before surgery showed the gingival health of the periodontal region and the existing intimate contact between the gum and tooth. It was possible to visualize the gingival behavior 15 days after periodontal surgery, in which the gum was still in the process of healing and re-establishment of the position of the tooth. On day 60 it was noticed the full gingival recovery, as well as the presence of new gingival sulcus, since this period corresponds to sufficient time to ensure the neof ormation of the junctional epithelium (14 days) and maturation of connective tissue (45-60 days). Moreover, it was possible to observe the change of the gingival profile of a higher to a lower tissue thickness, which interferes directly on the success, prognosis and treatment of prosthetic implant. Thus it can be said that despite some technical limitations, the OCT is an efficient method for gingival evaluation.

9692-26, Session PSun

**A comparative study of shear bond strength of orthodontic bracket after acid-etched and Er:YAG treatment on enamel surface**

Juliana C. Leao, Claudia C. B. O. Mota, Patricia F. Cassimiro-Silva, Anderson S. L. Gomes, Univ. Federal de Pernambuco (Brazil)

The purpose of this study was to evaluate the shear bond strength (SBS) of teeth prepared for orthodontic bracket bonding with 37% phosphoric acid conditioning and Er:YAG laser conditioning. Forty bovine incisors were randomly divided into two groups. In Group I, the teeth were conditioned with 37% phosphoric acid and then central incisor stainless steel brackets were bonded with Transbond XT; in Group II the teeth were irradiated with the Er:YAG and bonding with Transbond XT. The samples were stored in water at 37 °C for 24 hours and thermocycling for 500 cycles. Subsequently, the shear bond strength tests were performed and the mean SBS ± sd (MPa) for each group was calculated. The adhesive remnant index (ARI) was determined. Optical Tomography Coherence was used to evaluate the surface of enamel before and after the bond test as an alternative nondestructive technique. Student’s t-test was used to compare the mean between the groups. Mean SBS for acid-etched enamel, group I, was higher than for laser-etched enamel, Group II. Adhesion to dental hard tissues after Er:YAG laser etching was inferior to obtained after conventional acid etching, but exceeded the strength of 6 to 8 MPa that is believed to be clinically sufficient.

9692-27, Session PSun

**Selective removal of dental composite with a diode-pumped Er:YAG laser**

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

Selective removal of dental composite with high precision is best accomplished using lasers operating at high pulse repetition rates focused to a small spot size. Conventional flash-lamp pumped Er:YAG lasers are poorly suited for this purpose, but new diode-pumped Er:YAG lasers have become available operating at high pulse repetition rates. The purpose of this study was to compare the ablation rates and selectivity of sound and demineralized enamel and dentin for a 30 W diode-pumped Er:YAG laser operating with a pulse duration of 30-50-µs and evaluate it’s suitability for the selective removal of composite from tooth surfaces. The depth of ablation and changes in surface morphology were assessed using digital microscopy, optical coherence tomography and surface profilometry.

9692-28, Session PSun

**Enhancement of OCT images with PVS**

Hobin J. Kang, Cynthia L. Darling, Daniel Fried, Univ. of California, San Francisco (United States)

Several studies have shown that optical coherence tomography (OCT) can be used to measure the remaining enamel thickness and detect the location of subsurface lesions hidden under the sound enamel. Moreover studies have shown that high refractive index liquids can be used to improve the visibility of subsurface lesions in OCT images. In this study, we demonstrate that polyvinyl siloxane (PVS) impression materials, which are routinely used in dentistry, can be used to enhance the detection of dentinal lesions on tooth occlusal surfaces. Lesion presence was confirmed with polarized light microscopy and microradiography.

9692-29, Session PSun

**Selective removal of esthetic composite restorations with spectral guided laser ablation**

Ivana Yi, Kenneth H. Chan, Michal Staninec, Cynthia L. Darling, Daniel Fried, Univ. of California, San Francisco (United States)

Dental composites are used for a wide range of applications such as fillings for cavities, adhesives for orthodontic brackets, and fillings for gaps (diastemases) between teeth for minor reshaping of teeth in full or partial crowns. Anterior restorations are used to replace missing, diseased and unsightly tooth structure for both appearance and function. When these restorations must be replaced, they are difficult to remove mechanically without causing excessive removal or damage to enamel because dental composites are color matched for esthetic reasons. Previous studies have shown that a CO2 laser has high ablation selectivity and are well suited for removal of composite on occlusal surfaces while minimizing healthy tissue loss. A spectral feedback guidance system may be used to discriminate between dental composite and dental hard tissue for selective ablation of composite material. The removals of composite restorations filling diastemases are more challenging due to the esthetic concern for anterior teeth. The objective of this study is to determine if composite spanning a diastema between anterior teeth can be removed by spectral guided laser ablation at clinically relevant rates with minimal damage to peripheral healthy tissue and higher selectivity by a laser than with a high speed dental hand-piece.
Analysis of smooth surface structural changes on primary and permanent teeth exposed to acidulated phosphate fluoride in simulated enamel lesion with PS-OCT

Wilson Tam, Robert C. Lee, Brent P. Lin, Cynthia L. Darling, Daniel Fried, Univ. of California, San Francisco (United States)

Detecting demineralized enamel with current lesion assessment techniques, involving radiographic imaging and visual examination by a clinician, is a difficult task. With current assessment methods, the lesions detected usually have progressed too extensively, resulting in restorative measures as the main treatment option. Previous studies indicated that near-infrared (NIR) based imaging systems, such as polarization-sensitive optical coherence tomography (PS-OCT) and NIR reflectance, and quantitative light fluorescence (QLF) can detect early-demineralized enamel. Studies have also illustrated that fluoride strengthens the enamel structure and decreases the effect of acid attack; therefore, decreasing the likelihood to develop caries. This study will be comparing images taken with PS-OCT (~1310nm), on extracted primary (n=20) and permanent (n=20) teeth to determine the imaging system’s capability to evaluate the structural changes on the smooth surface of the enamel (buccal or lingual surface). The prepared teeth will have a topical application of acidulated phosphate fluoride (APF) and will be exposed to a simulated enamel lesion. The artificial enamel lesion consists of immersing the prepared teeth in a demineralization solution (pH 4.5) for 48 hours and subsequently transferred to an acidic remineralization solution (pH 4.8) for 10 days.

Evaluation of the respective thresholds for surface modification and increased acid resistance after CO2 laser irradiation using PS-OCT and digital microscopy

Jin Wan Kim, Kenneth H. Chan, Cynthia L. Darling, Daniel Fried, Univ. of California, San Francisco (United States)

Several studies have demonstrated that pulsed CO2 laser irradiation can render enamel more resistant to acid dissolution. However, it is not clear that resistance can be imparted without physical modification of the enamel surface. At laser intensities below the threshold for ablation, carbonated hydroxyapatite is converted to the purer phase hydroxyapatite with improved acid resistance. However, even subablative fluence may result in physical and optical modification of the surface that increases the reflectivity and adversely affects the appearance of the enamel. The purpose of this study was to determine if it is possible to render the enamel surface resistant to acid dissolution with minimal increase to the enamel reflectivity. An RF-exited CO2 laser operating at 9.3-µm with pulse duration of 26-µs and pulse repetition rates of 100-1000 Hz was used to irradiate bovine enamel samples. The laser beam had a Gaussian spatial beam profile and a diameter of 1.4 mm and it was scanned across the specimens in one direction producing a continuously varying fluence distribution along the long axis of the sample window. The specimens were subjected to an acid challenge after irradiation and the resulting surface changes, demineralization and erosion were evaluated using polarization sensitive OCT (PS-OCT) and digital microscopy.
OMAG angiograms in different layers of DR patients. The contour line of MAs were observed in both superficial and middle retinal layers based on network visions that were less affected by hemorrhage and leakage. The great agreement with FA. Meanwhile, OMAG gave more distinct vascular section view. En face OMAG images of different macular diseases showed with different colors. Flow and structure images were combined for cross-

was used to obtain 2-dimensional angiograms of different layers coded correlation method. The 3D angiography was segmented into 3 layers in used to extract the blood flow and removed the bulk motion by 2D cross-

tracking system. OMAG algorithm based on complex differentiation was used to generate images by montage scanning protocol based on the

deviations of ~ 6%. Therefore the system should be sensitive enough to average variation for total flow measurements is sufficiently low to detect deviations of ~ 6%. Therefore the system should be sensitive enough to detect retinal blood flow alterations in glaucomatous patients in the future.

9693-2, Session 1
Wide field OCT based microangiography in living human eye
Qinqin Zhang, Chieh-Li Chen, Zhongdi Chu, Anqi Zhang, Univ. of Washington (United States); Lin An, Mary Durbin, Utkarsh Sharma, Cari Zeiss Meditec, Inc. (United States); Philip J. Rosenfeld, Bascom Palmer Eye Institute (United States); Ruikang K. Wang, Univ. of Washington (United States)

To investigate the application of optical microangiography (OMAG) in living human eye. Patients with different macular diseases were recruited, including diabetic retinopathy (DR), geographic atrophy (GA), retinitis pigmentosa (RP), and venous occlusion, et al. Wide field OCT angiography images can be generated by montage scanning protocol based on the tracking system. OMAG algorithm based on complex differentiation was used to extract the blood flow and removed the bulk motion by 2D cross-correlation method. The 3D angiography was segmented into 3 layers in the retina and 2 layers in the choroid. The en-face maximum projection was used to obtain 2-dimensional angiograms of different layers coded with different colors. Flow and structure images were combined for cross-sectional view. En face OMAG images of different macular diseases showed a great agreement with FA. Meanwhile, OMAG gave more distinct vascular network visions that were less affected by hemorrhage and leakage. The MAs were observed in both superficial and middle retinal layers based on OMAG angiograms in different layers of DR patients. The contour line of FAZ was extracted as well, which can be quantitative the retinal diseases. For GA patient, the damage of RPE layer enhanced the penetration of light and enabled the acquisition of choriocapillaries and choroidal vessels. The wide field OMAG angiogram enabled the capability of capturing the entire geographic atrophy. OMAG provides depth-resolved information and detailed vascular images of DR and GA patients, providing a better visualization of vascular network compared to FA.

9693-3, Session 1
Accurate presentation of choroidal neovascularization in patients using feature space OCT micro-angiography
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Choroidal neovascularization (CNV) involves the growth of abnormal blood vessels originating from choroid. CNV is a major cause of vision loss and impairment involved in a number of retinal pathologies such as age-

related macular degeneration and high myopia. Fluorescein angiography (FA) and indocyanine green angiography (ICGA) have been the golden standard for detecting neovascularization within retina and choroid in clinic. However, none of these methods is depth resolved and both FA and ICGA require intravenous dye injection. Optical coherence tomography micro-

angiography (OMAG) is a novel imaging modality providing visualization of three dimensional blood vessel networks, without a need of exogenous contrast agents. The current OMAG algorithm is however prone to the noise that arises from static background, for example the nerve fiber layer and retinal pigment epithelium (RPE). In the diagnosis of CNV cases, the new blood vessels arising from choroid are breaking through the Bruch’s membrane under the RPE or directly through the RPE, depending upon type I or type II CNVs. In the OMAG angiographic results, the artifacts of RPE strongly affect the interpretation of the pathological developed vessels.

In this work, we present a newly developed feature space optical microangiography (fsOMAG) method and examines the performance of fsOMAG in CNV cases. Demonstrated through in-vivo human posterior eye imaging of CNV subjects, the fsOMAG algorithm is shown to effectively eliminate the artifacts of static tissue background in the subretinal space of OCT micro-angiography, resulting in better visualization of the pathological neovascularization when compared with the current OMAG approach.

9693-4, Session 1
Retinal axial and transversal flow quantification using Doppler OCT with noise-bias correction
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In Doppler optical coherence tomography (OCT) the flow of moving
particles is determined from phase differences between repeated A-lines from the same location in the tissue. However, besides flow also noise contributes to these phase differences which is problematic for accurate flow quantification. Here, a maximum a posteriori probability (MAP) estimator for the estimation of flow velocities in the human retina from in vivo Doppler OCT measurements is presented. The MAP estimator combines models from previous studies which describe the effect of shot noise, repositioning errors (RE) and flow on the OCT signal in order to isolate the contribution of the flow and to determine its velocities in retinal capillaries. The MAP estimator was compared to a direct calculation of the flow velocities from phase differences without taking noise influences into account. It was found that the MAP estimator significantly reduces bias which was otherwise caused by shot noise and RE in the direct calculation. Purely scattering layers were examined to find local variations in the noise level caused by the RE. An varying noise floor, induced by the scan pattern, was found which led to artifacts in the direct calculation but was removed with the MAP estimator. The MAP estimator is thus more robust than the direct calculation method and the considered noise sources cannot be neglected for flow quantification in the human retina. This method is potentially interesting for studying retinal pathology such as age-related macular degeneration.

9693-5, Session 2

4D microscope-integrated OCT improves accuracy of ophthalmic surgical maneuvers

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Ophthalmic surgeons manipulate micron-scale tissues using stereopsis through an operating microscope and instrument shadowing for depth perception. While ophthalmic microsurgery has benefitted from rapid advances in instrumentation and techniques, the basic principles of the stereo operating microscope have not changed since the 1930’s. Optical Coherence Tomography (OCT) has revolutionized ophthalmic imaging and is now the gold standard for preoperative and postoperative evaluation of most retinal and many corneal procedures. We and others have developed initial microscope-integrated OCT (MI OCT) systems for concurrent OCT and operating microscope imaging, but these are limited to 2D real-time imaging and require offline post-processing for 3D rendering and visualization. Our previously presented 4D MI OCT system can record and display the 3D surgical field stereoscopically through the microscope oculars using a dual-channel heads-up display (HUD) at up to 10 micron-scale volumes per second. In this work, we show that 4D MI OCT guidance improves the accuracy of depth-based microsurgical maneuvers (with statistical significance) in mock surgery trials in a wet lab environment. Additionally, 4D MI OCT was successfully performed in 38/45 (84%) posterior and 14/14 (100%) anterior eye human surgeries, and revealed previously unrecognized lesions that were invisible through the operating microscope. These lesions, such as residual and potentially damaging retinal deformation during pathologic membrane peeling, were visualized in real-time by the surgeon. Our integrated system provides an enhanced 4D surgical visualization platform that can improve current ophthalmic surgical practice and may help develop and refine future microsurgical techniques.

9693-6, Session 2

Imaging of fibrotic lesions in neovascular age related macular degeneration by polarization sensitive OCT

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With the advent of combined anti-vascular endothelial growth factor / platelet-derived growth factor (anti-VEGF/PDGF) therapies, imaging and quantification of fibrotic lesions are of increasing interest in follow-up studies of neovascular age related macular degeneration (nAMD). Presently used technologies like color fundus photography, intensity based optical coherence tomography (OCT), or fluorescein angiography have some shortcomings like lack of 3D information, lack of specificity, or invasiveness. Polarization sensitive (PS) OCT is a functional extension of OCT that draws advantage from measuring the polarization state of backscattered light. Therefore, PS-OCT can be used to generate tissue specific contrast and to provide quantitative information. PS-OCT can differentiate between polarization preserving, birefringent, and depolarizing tissue and thus be used to segment and quantify retinal tissues and lesions. Fibrotic tissue secondary to nAMD consists of highly organized collagen fibers and therefore shows form birefringence which can be measured and imaged by PS-OCT. Therefore, PS-OCT has a great potential for analysis of nAMD lesions.

In this study, we imaged 15 eyes of patients with nAMD who had developed fibrotic lesions. Patients were imaged by PS-OCT (spectral domain, 70 kA-scans/s, integrated retinal tracker) and conventional technologies (color fundus photography, fluorescein angiography, intensity based OCT). From the PS-OCT data sets, cross sectional and en-face projection images were derived, using various contrast modalities (intensity, retardation, axis orientation, degree of polarization uniformity). The PS-OCT images showed a good correlation with conventional images, with the added advantage of providing 3D information.

9693-7, Session 2

long working distance optical coherence tomography for pediatric imaging

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Conventional optical coherence tomography (OCT) systems have working distances of about 25 mm, and require cooperative subjects to immobilize and fixate on a target. Handheld OCT probes have also been demonstrated for successful imaging of pre-term infants and neonates up to ~1 year old. However, no technology yet exists for OCT in young children due to their lack of attention and inherent fear of large objects close to their face. In this work, we demonstrate a prototype retinal swept-source OCT system with a long working distance (from the last optical element to the subject’s eye) to facilitate pediatric imaging. To reduce the footprint and weight of the system compared to the conventional 4f scheme, a novel 2f scanning configuration was implemented to achieve a working distance of 348mm with a +/- 8° scanning angle prior to cornea. Employing two custom-designed lenses, the system design resolution was nearly diffraction limited through a -8D to +5D refractive corrections. A fixation target displayed on a LCD monitor and an iris camera were used to facilitate alignment and...
Simultaneous hand-held contact color fundus and SD-OCT imaging for pediatric retinal diseases

Marco Ruggeri, Victor M. Hernandez, Carolina De Freitas, Nidhi Relhan, Juan Silgado, Fabricie Manss, Jean-Marie A. Parel, Bascom Palmer Eye Institute (United States)

Hand-held wide-field contact color fundus photography is currently the standard method to acquire diagnostic images of children during examination under anesthesia and in the neonatal intensive care unit. The recent development of portable non-contact hand-held OCT retinal imaging systems has proved that OCT is of tremendous help to complement fundus photography in the management of pediatric patients. Currently, there is no commercial or research system that combines color wide-field digital fundus and OCT imaging in a contact-fashion. The contact of the probe with the cornea has the advantages of reducing motion experienced by the photographer during the imaging and providing fundus and OCT images with wider field of view that includes the periphery of the retina. In this study we produce proof of concept for a contact-type hand-held unit for simultaneous color fundus and OCT live view of the retina of pediatric patients. The front piece of the hand-held unit consists of a contact ophthalmoscopy lens integrating a circular light guide that was recovered from a digital fundus camera for pediatric imaging. The custom-made rear piece consists of the optics to: 1) fold the visible aerial image of the fundus generated by the ophthalmoscopy lens on a miniaturized level board digital color camera; 2) conjugate the eye pupil to the galvanometric scanning mirrors of an OCT delivery system. Wide-field color fundus and OCT images were simultaneously obtained in an eye model and sequentially obtained on the eye of a conscious 25 year-old human subject with healthy retina.

Need for technologies for advancement in delivering community-based eye care service (Keynote Presentation)

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No Abstract Available
Corneal imaging devices have played an important role in the diagnosis of corneal diseases which are among the leading causes of visual impairment and blindness. However, the spatial information of cellular or subcellular components in cornea could not be achieved in situ via current imaging devices. Noninvasive Micro optical coherence tomography (MOCT) with a resolution of $2.5\mu m \times 2.5\mu m \times 1.3\mu m$ in air is able to provide three dimensional view of biological tissues both in situ and in real time. The purpose of this study is to evaluate the performance of MOCT in detecting cellular structures of cornea. Eyes of normal adult rats and swine were enucleated immediately after euthanasia and were placed into a container with the corneal side up. Image acquisition of central cornea was performed ex vivo subsequently. Cellular biological structures were detectable from images captured by MOCT. Corneal layers were clearly differentiated with high scattering interfaces between adjacent layers on the cross-sectional MOCT images. Morphological features including hexagonally shaped endothelial cells, dendritic keratocytes with a large body and several processes, and needle-like or linear collagen fibers were clearly presented on the en face MOCT images. Both microstructural similarities and differences between rats’ and porcine corneas were investigated according to images captured by MOCT. Our results indicate that MOCT is able to visualize microstructures of cornea at a cellular level and it may become a useful tool for the understanding and diagnosis of corneal diseases.

Fluorescent scanning laser ophthalmoscopy for cellular resolution in vivo mouse retinal imaging: benefits and drawbacks of implementing adaptive optics

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Scanning Laser Ophthalmoscopy (SLO) is a very important imaging tool in ophthalmology research. By combining with Adaptive Optics (AO) technique, AO-SLO can correct for ocular aberrations resulting in cellular level resolution, allowing longitudinal studies of single cells morphology in the living eyes. The numerical aperture (NA) sets the optical resolution that can be achieved in the “classical” imaging systems. Mouse eye has more than twice NA of the human eye, thus offering theoretically higher resolution. However, in most SLO based imaging systems the imaging beam size at mouse pupil sets the NA of that instrument, while most of the AO-SLO systems use almost the full NA of the mouse eye. In this report, we first simulated the theoretical resolution that can be achieved in vivo for different imaging beam sizes (different NA), assuming two cases: no aberrations and aberrations based on published mouse ocular waveform data. Then we imaged mouse retinas with our custom build SLO system using different beam sizes to compare these results with theory. Further experiments include comparison of the SLO and AO-SLO systems for imaging different depth of field resolution. Further experiments include comparison of the SLO and AO-SLO systems for imaging different depth of field resolution. Further experiments include comparison of the SLO and AO-SLO systems for imaging different depth of field resolution. Further experiments include comparison of the SLO and AO-SLO systems for imaging different depth of field resolution. Further experiments include comparison of the SLO and AO-SLO systems for imaging different depth of field resolution.
OCT. The OCT system provided high resolution (3 μm) and high speed (up to 500 frames/s) imaging of mouse retina. An animal holder equipped with custom designed ear bar and bite bar was used to reduce bulk motion due to breath and heartbeat. Residual eye movement in OCT images was removed by accurate image registration. Dynamic OCT intensity-variance revealed robust IODs predominantly observed from photoreceptor outer segments immediately (<10ms) after the stimulation delivery. We are currently pursuing OCT phase-variance processing to verify IOS responses from inner retina. Further IOS study with mouse models will pave the way toward using IOS imaging as a new method for eye disease detection and treatment evaluation.

9693-17, Session 5

Registration of orthogonally oriented wide-field of view OCT volumes using orientation-aware optical flow and retina segmentation

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Patient motion artifacts are an important source of data irregularities in OCT imaging. With longer duration OCT scans – as is needed for large wide field of view scans or increased scan density – motion artifacts become increasingly problematic. Strategies to mitigate these motion artifacts are then necessary to ensure OCT data integrity. A popular strategy for reducing motion artifacts in OCT images is to capture two orthogonally oriented volumetric scans containing uncorrelated motion and subsequently reconstructing a motion-free volume by combining information from both datasets. While many different variations of this registration approach have been proposed, even the most recent methods might not be suitable for wide FOV OCT scans which can be lacking in features away from the optic nerve head or arcades. To address this problem, we propose a two-stage motion correction algorithm for wide FOV OCT volumes. In the first step, X and Y axes motion is corrected by registering OCT summed voxel projections (SVPs). To achieve this, we introduce a method based on a custom variation of the dense optical flow technique which is aware of the motion free orientation of the scan. Secondly, a depth (Z axis) correction approach based on the segmentation of the retinal layer boundaries in each B-scan using graph-theory and dynamic programming is applied. This motion correction method was applied to wide field retinal OCT volumes (approximately 80° FOV) of 3 subjects with substantial reduction in motion artifacts.

9693-19, Session 5

Fully-automated segmentation of cone photoreceptors in split detector adaptive optics scanning light ophthalmoscope images

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Quantitative analysis of the photoreceptor mosaic within the retina is potentially useful for diagnosis, prognosis, and treatment of many ocular and neurodegenerative diseases. The adaptive optics scanning light ophthalmoscope (AOSLO) has made high resolution visualization of human photoreceptor mosaics in vivo possible, and the more recent non-confocal split detector AOSLO (SD-AOSLO) allows for improved visualization of photoreceptor cones over standard AOSLO systems. However, quantitative analysis of SD-AOSLO images currently requires the time-consuming manual marking of all cones within an image, which bottlenecks the rate at which data from SD-AOSLO can be utilized. There are currently no published methods for the segmentation of SD-AOSLO. In this work, we present a fully-automated method for the detection of cones in SD-AOSLO images of healthy eyes. The two step algorithm works by first applying an adaptive Fourier domain based filter to remove unnecessary information, then detecting cones by utilizing a priori information about their appearance due to the split detector set up. The algorithm is validated against the current gold standard of manual segmentation, in SD-AOSLO images from a wide variety of locations within the retina. The results show that our algorithm performs with a high degree of sensitivity and specificity in comparison to manual grading.

9693-18, Session 5

Length-adjusted graph cuts for automatic segmentation of pathological features in OCT images

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Optical coherence tomography (OCT) is used for diagnosis, management, and prognosis of ocular diseases. Our previous popular automatic segmentation method, graph theory and dynamic programming (GTDP), relied on Dijkstra’s algorithm to find the shortest path through a graph, corresponding to a single layer boundary in a retinal image. Dijkstra based GTDP was useful for segmentation of a variety of anatomic and pathological features in retinal images. However, for some diseased retinas, such as those with pseudodrusen or macular holes, the shortest path does not always correctly represent pathological layer deformation. This is because, the shortest path segmentation methods often ignore features in the diseased retina that appear to change rapidly or go in the reverse direction of the search. We demonstrate the utility of a new metric in graph search to address this issue, the exponentially adjusted mean arc length. Our proposed method is an adaptation and extension of the normalized cuts technique for OCT segmentation. We addressed computational issues in implementing a path-length adjusted graph search and challenges with respect to removing cycles in our segmented path. While applications of this metric are general, in this first report, we demonstrate its application for segmentation of the vitreous/retina boundary in patients with full thickness macular holes imaged by Spectralis spectral domain OCT. We show that our new retinal layer segmentation method outperforms the Dijkstra’s shortest path based GTDP, when compared to expert manual grading. Thus, we expect it to become a standard component of future graph-based automated segmentation methods.

9693-20, Session 5

Anterior-segment polarization-sensitive OCT with efficient polarimetric speckle reduction

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PS-OCT has been developed for visualization of birefringent tissues in various applications. For quantitative and statistical analysis using PS-OCT, however, it has been known that measured phase retardation is biased
because of asymmetric statistical distribution. We develop a novel method that estimates Jones matrix of the sample using its maximum likelihood estimator. We validate our method using quarter waveplates and a glass plate under low signal-to-noise ratio. The double-pass phase retardation was asymptotically unbiased and close to the true value by increasing number of pixels over ~30 pixels, indicating the efficacy of our method. The method is applied to images of the anterior eye segment as a spatial filter, and shows superior performance in polarimetric speckle reduction of local retardation images compared to coherent Jones-matrix averaging filter that was previously developed by our group.

Our method is not limited to the estimation of Jones matrix and phase retardation, but can be used to calculate the randomness of the Jones matrix. It is an extension of degree of polarization uniformity to the target Jones matrix, and is useful for contrast enhancement in the iris pigment epithelium, ciliary body, and choroid. We demonstrate the application of our method to the image processing of the normal anterior eye segment and poorly functioning filtering bleb after trabeculectomy.

9693-21, Session 5
Quantitative polarization and flow evaluation of choroid and sclera by multifunctional Jones matrix optical coherence tomography
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Quantitative evaluation of structure, birefringence, polarization uniformity and flow properties of normal and myopic eyes are performed by using Jones-matrix based multifunctional optical coherence tomography (J-M OCT).

For fully objective evaluation, an automatic segmentation algorithm for the choroid and sclera is developed. This algorithm is based on not only the scattering OCT but also birefringence, polarization uniformity, and flow information. And hence, it can segment these tissues with more anatomical rationality.

Choroidal DOPU decreases with age (r = -0.9, P = 0.015). It is also negatively correlated with refractive error (r = -0.84, P = 0.044). Choroidal flow (interframe time correlation of OCT subtracted from unity) showed lower value (lower flow) with more highly myopic eyes (r = 0.95, P = 0.026). The scleral birefringence showed lower value with more highly myopic eye (r = 0.84, P = 0.046). It was reported that the scleral birefringence was positively correlated with its mechanical stiffness [5]. Therefore, this result indicates that the scleral stiffness is low for myopia.

It is noteworthy that refractive error is significantly correlated with all the optical parameters including the birefringence (r = 0.85, P = 0.039), DOPU (r = -0.84, P = 0.044), and cmOCA (r = 0.95, P = 0.0026) of the choroid. On the other hand, the refractive error is not well correlated with the choroidal thickness. It would suggest a higher sensitivity of these optical parameters to myopia than the choroidal thickness.

9693-22, Session 5
Estimating a structural bottle neck for eye-brain transfer of visual information from 3D-volumes of the optic nerve head from a commercial OCT device
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The aim of this project is to investigate the possibility of using OCT optic nerve head 3D information captured with Topcon OCT 2000 for detection of the shortest distance between the inner limit of the retina and the central limit of the pigment epithelium. 3D volumes are captured with the Topcon OCT 2000. Primary intensity information is exported to a Cartesian coordinate system in MATLAB. A reference point is arbitrarily set in the center of the optic nerve head. The intensity information around the reference point is then transformed to polar coordinates. Altogether, 500 radial cross sections of the intensity information of the optic nerve head around the reference point is then exported to a custom made interactive software for manual localization of points of interest in an image. A trained operator then scans through the 500 radial scans and identifies the coordinates of the central limit of the pigment epithelium. The coordinates for each radius are imported into Matlab. The surface of the inner limit of the retina is automatically located: First, the 3D volume images are filtered using a small Gaussian kernel to reduce noise. For each column of the image stack, the first voxel whose filtered intensity value exceeds a given threshold is then identified. Together, the obtained voxel coordinates delineate the inner limit of the retina.

Finally, Pigment epithelum - Inner limit of retina Minimal Distance (PIMD) is calculated for each radius. The average PIMD for the 500 radii is defined as PIMD(0-2Pi).

9693-23, Session 5
Analysis of the variation in OCT measurements of a structural bottle neck for eye-brain transfer of visual information from 3D-volumes of the optic nerve head, PIMD(0-2Pi)
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The aim of this study was to estimate the precision of clinical measurements of PIMD (0-2Pi). Patients clinically diagnosed with early phase glaucoma were prospectively measured at two occasions, at least 1 week apart. At each occasion, three 3D volumes of the optic nerve head was captured with the Topcon OCT 2000. The 3D intensity information was exported to Matlab and PIMD (0-2Pi) was estimated semiautomatically in three iterations for each image. The PIMD (0-2Pi) estimates were analyzed with an analysis of variance with a model assuming random variation of patients, occasions within patients, 3D volumes captured within occasions, and iterations of PIMD (0-2Pi) estimates within 3D volumes captured. A primary analysis of measured data indicated that the relationship among variances was S25:0:150:1. The large variation among patients reflects variation in anatomy of patients and is not relevant for follow up of individual patients. In follow up of patients, the significance of the variability of PIMD (0-2Pi) among 3D captures depends on the number of 3D captures averaged. The low variance for iterations imply that one semiautomatic estimation of PIMD (0-2Pi) is sufficient for patient follow up. Considering five 3D captures of the optic nerve head and 1 semiautomatic estimation of PIMD (0-2Pi) in each 3D capture, it was found that the variation coefficient for a 0.2 change from baseline PIMD (0-2Pi) in one occasion to the next was 0.15. It is concluded that PIMD (0-2Pi) has the potential to be an efficient morphometric variable for follow up of glaucoma.

9693-24, Session 6
Eye motion corrected OCT imaging with Lissajous scan pattern
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Multimodal ophthalmic imaging using swept source spectrally encoded scanning laser ophthalmoscopy and optical coherence tomography

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Scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) benefit clinical diagnostic imaging in ophthalmology by enabling in vivo noninvasive en face and volumetric visualization of retinal structures, respectively. Spectrally encoding methods enable confocal imaging through fiber optics and reduces system complexity. Previous applications in ophthalmic imaging include spectrally encoded confocal scanning laser ophthalmoscopy (SECSLO) and a combined SECSLO-OCT system for image guidance, tracking, and registration. However, spectrally encoded imaging suffers from speckle noise because each spectrally encoded channel is effectively monochromatic. Here, we demonstrate in vivo human retinal imaging using a swept source spectrally encoded scanning laser ophthalmoscopy and OCT (S-S:SECSLO-OCT) at 1060 nm. S-S:SECSLO-OCT uses a shared 100 kHz Axsun swept source, shared scanner and imaging optics, and are detected simultaneously on a shared, dual channel high-speed detector. The acquired pattern images are stacked, pixels sorted according to intensity, and a Lissajous scan pattern is well corresponds to CF. Motion correction was applied and compared with color fundus photo (CF). Finally, en face projection image of Lissajous scan is well corresponds to CF and volumetric three-dimensional OCT intensity image was obtained.

In-vivo, real-time cross-sectional images of retina using a GPU enhanced master slave optical coherence tomography system

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In our previous reports we demonstrated a novel Fourier domain optical coherence tomography method, Master Slave optical coherence tomography (MS-OCT), that does not require resampling of data and can deliver en-face images from several depths simultaneously. While ideally suited for delivering information from a selected depth, the MS-OCT has been so far inferior to the conventional FFT based OCT in terms of time of producing cross-section images. Here, we demonstrate that by taking advantage of the parallel processing capabilities offered by the MS-OCT method, cross-sectional OCT images of the human retina can be produced in real-time by assembling several T-scans from different depths. We analyze the conditions that ensure a real-time B-scan imaging operation, and demonstrate in-vivo real-time images from human fovea and the optic nerve, of comparable resolution and sensitivity to those produced using the traditional Fourier domain based method.

Parallel scanning light ophthalmoscope for retinal imaging

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Scanning laser ophthalmoscopy is a confocal imaging technique that allows high-contrast imaging of retinal structures. Rapid, involuntary eye movements during image acquisition are known to cause artefacts and high-speed imaging of the retina is crucial to avoid them. To reach higher imaging speeds we propose to illuminate the retina with multiple parallel lines simultaneously within the whole field of view (FOV) instead of a single focused line that is raster-scanned. These multiple line patterns were generated with a digital micro-mirror device (DMD) and by shifting the line pattern, the whole FOV is scanned. The back-scattered light from the retinal layers is collected via a beam-splitter and imaged onto an area camera. After every pattern from the sequence is projected, the final image is generated by combining these back-reflected illumination patterns. Image processing is used to remove the background and out-of-focus light. Acquired pattern images are stacked, pixels sorted according to intensity and finally bottom layer of the stack is subtracted from the top layer to produce confocal image. The obtained confocal images are rich in structure, showing the small blood vessels around the macular avascular zone and the bow tie of Henle’s fiber layer in the fovea. In the optic nerve head images the large arteries/veins, optic cup rim and cup itself are visualized. Images have good contrast and lateral resolution with a 10°?10° FOV. The initial results are promising for the development of high-speed retinal imaging using spatial light modulators such as the DMD.
These dynamic tissue responses were measured non-invasively with a µ-spatially-focused (<1ms) air-pulse delivery system. Scale deformations were induced at the apex of the corneal tissue using a 20% dextran solution every 5 minutes for one hour, followed by 0.9% saline every 5 minutes for 1 hour, then decreased sharply after Dextran application (thickness: –46% [–3.15/6.82 µm]; RR: –24% [–0.7/2.88 ms⁻¹]; GV: –19% [–0.6/3.2 m/s]). Corneal thickness and corneal stiffness (RR) were well correlated (R² = .66). Corneal biomechanical properties are highly correlated with tissue hydration over a wide range of corneal thickness and these changes in corneal stiffness are quantifiable using OCE.

9693-30, Session 7

Imaging of Keratoconic and normal human cornea with a Brillouin imaging system

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Keratoconus is a degenerative disorder of the eye characterized by human cornea thinning and morphological change to a more conical shape. Current diagnosis of this disease relies on topographic imaging of the cornea. Early and differential diagnosis is difficult. In keratoconus, mechanical properties are found to be compromised. A clinically available invasive technique capable of measuring the mechanical properties of the cornea is of significant importance for understanding the mechanism of keratoconus development and improve detection and intervention in keratoconus.

The capability of Brillouin imaging to detect local longitudinal modulus in human cornea has been demonstrated previously. We report our non-contact, non-invasive, clinically viable Brillouin imaging system engineered to evaluate mechanical properties human cornea in vivo. The system takes advantage of a highly dispersive 2-stage virtually imaged phased array (VIPA) to detect weak Brillouin scattering signal from biological samples. With a 15-mW light beam from a 780-nm single-wavelength laser source, the system is able to detect Brillouin frequency shift of a single point in human cornea less than 0.3 second, at a 57m/307m lateral/axial resolution. Sensitivity of the system was quantified to be ~10 MHz. A-scans at different sample locations on a human cornea with a motorized human interface. We imaged both normal and keratoconic human corneas with this system. Whereas no significantly difference were observed outside keratoconic cones compared with normal cornea, a highly statistically significantly decrease was found in the cone regions.

9693-28, Session 6

Enhancing sensitivity of high resolution optical coherence tomography using an optional spectrally encoded extended source

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High-resolution optical coherence tomography (OCT) is of critical importance to disease diagnosis because it is capable of providing detailed microstructural information of the biological tissues. However, a compromise usually has to be made between its spatial resolutions and sensitivity due to the suboptimal spectral response of the system components, such as the linear camera, the dispersion grating, and the focusing lenses, etc. In this study, we demonstrate an OCT system that achieves both high spatial resolutions and enhanced sensitivity through utilizing a spectrally encoded source. The system achieves a lateral resolution of 3.1 µm and an axial resolution of 2.3 µm in air; when with a simple dispersive prism placed in the infinity space of the sample arm optics, the illumination beam on the sample is transformed into a line source with a visual angle of 10.3 mrad. Such an extended source technique allows a -4 times larger maximum permissible exposure (MPE) than its point source counterpart, which thus improves the system sensitivity by ~6dB. In addition, the dispersive prism can be conveniently switched to a reflector. Such flexibility helps increase the penetration depth of the system without increasing the complexity of the current point source devices. We conducted experiments to characterize the system’s imaging capability using the human fingertip in vivo and the swine eye optic nerve disc ex vivo. The higher penetration depth of such a system over the conventional point source OCT system is also demonstrated in these two tissues.
linking (RGX) has been proposed as an alternative to UV-A Riboflavin collagen cross-linking (UV-CXL) for treatment of keratoconus. However, the effects of RGX on the biomechanical properties of the cornea are not as well understood as UV-CXL. In this work, we demonstrate the feasibility of quantifying the viscoelasticity of the rabbit cornea before and after RGX using a noncontact method of phase-stabilized swept source optical coherence elastography (PhS-SSOCE) and finite element modeling (FEM). Viscoelastic FE models of the corneas were constructed to simulate the elastic wave propagation based on the OCE measurements. In addition, the effect of the fluid-structure interface (FSI) between the corneal posterior surface and aqueous humor on the elastic wave group velocity was also investigated. The effect of the FSI was first validated by OCE measurements and FEM simulations on contact lenses, and the OCE and FEM results were in good agreement. The Young’s modulus of the rabbit cornea before RGX was assessed as E=80 kPa, and the shear viscosity was ?=0.40 Pâ·s at intraocular pressure (IOP) of 15 mmHg. After RGX, the Young’s modulus increased to E=112 kPa and shear viscosity was ?=0.37 Pâ·s. Both the corneal OCE experiments and the FE simulations also demonstrated that the FSI significantly reduced the group velocity of the elastic wave, and thus, should be considered when determining the biomechanical properties of the cornea.

9693-32, Session 7
Morphological characterization of keratoconus-affected human cornea provided by SHG imaging and correlation analysis
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Keratoconus is an ophthalmic disease in which the cornea acquires an abnormal conical shape that prevents the correct focusing on the retina and thus cause visual impairment. An early diagnosis would be very helpful to prevent the disease progression.

In several previous works, it was observed a different organization of the lamellae immediately below Bowman’s membrane in keratoconic corneas with respect to healthy corneas. This study is intended to investigate this anomaly by the use of Second Harmonic Generation (SHG) microscopy in a configuration of clinical relevance.

We focused our attention on the altered organization of collagen lamellae in the first 30 microns stroma layer below Bowman’s membrane in human keratoconic and healthy corneas. Backward-SHG imaging was performed to acquire image stacks of central cornea portions with sagittal optical sectioning geometry, finding that in healthy corneas the lamellae are in average more inclined with respect to Bowman’s membrane than in keratoconic ones. Then, both Backward-SHG and Forward-SHG image stacks were acquired using “en face” optical sectioning geometry, demonstrating that it is possible to identify the inclined lamellae below Bowman’s membrane also with an optical scheme that can be adopted in vivo. Further, the inclination of corneal lamellae below Bowman’s membrane was parametrized by means of a correlation analysis on images acquired subsequently in a single stack. Such method provided good discrimination capabilities in both sagittal and “en face” sectioned samples, demonstrating that this method could be used for analyzing SHG images and diagnose keratoconus both ex vivo and in vivo.

9693-33, Session 7
OCT-based profiler for automating ocular surface prosthetic fitting
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The use of a Prosthetic Replacement of the Ocular Surface Environment (PROSE) device is a revolutionary treatment for military patients that have lost their eyelids due to 3rd degree facial burns and for civilians who suffer from a host of corneal diseases. However, custom manual fitting is often a protracted painful, inexact process that requires multiple fitting sessions. Training for new practitioners is a long process. Automated methods to measure the complete corneal and scleral topology would provide a valuable tool for both clinicians and PROSE device manufacturers and would help streamline the fitting process.

PSI has developed an ocular anterior-segment profiler based on Optical Coherence Tomography (OCT), which provides a 3D measure of the surface of the sclera and cornea. This device will provide topography data that will be used to expedite and improve the fabrication process for PROSE devices. OCT has been used to image portions of the cornea and sclera and to measure surface topology for smaller contact lenses [1-3]. However, current state-of-the-art anterior eye OCT systems can only scan about 16 mm of the eye’s anterior surface, which is not sufficient for covering the sclera around the cornea. In addition, there is no systematic method for scanning and aligning/stitching the full scleral/corneal surface and commercial segmentation software is not optimized for the PROSE application.

Although preliminary, our results demonstrate the capability of PSI’s approach to generate accurate surface plots over relatively large areas of the eye, which is not currently possible with any other existing platform. Testing the technology on human volunteers is currently underway at Boston Foundation for Sight.

9693-34, Session 7
Setup for analysis of optical and geometrical property changes in ex vivo crystalline lenses during simulated accommodation modified with fs-laser pulses for presbyopia treatment
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The decrease of flexibility of the crystalline lens due to its continual growth also implies a decrease of accommodation amplitude, which is called presbyopia. During femtosecond (fs)-lentotomy, fs-laser pulses induce micro cuts inside the lens tissue in order to increase its deformability. To analyze the impact of fs-laser treatment on the accommodating crystalline lens in detail, a laboratory setup is necessary to perform ex vivo measurements during simulated accommodation close to the in vivo situation.

The crystalline lens geometry is detected with a two-sided spectral domain optical coherence tomography system and the equatorial lens diameter is determined by a camera with a telecentric, infinity corrected objective. The optical crystalline lens properties are examined with a scanning laser ray tracing system. Human or porcine lenses can be mounted via its ciliary body in a lens stretching device to simulate different accommodation states while monitoring the occurring forces. The lenses are inserted into solution and can be treated with fs-laser pulses while situated in the measuring setup.
The setup is evaluate geometrical and optical property changes due to the fs-lentotomy treatment in ex vivo crystalline lenses during simulated accommodation. Hence, it can be used to support the basic understanding of fs-lentotomy.

9693-35, Session 8

A novel automated instrument designed to determine photosensitivity thresholds

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As there is no clinically available instrument to systematically and reliably determine the photosensitivity thresholds of patients with dry eyes, blepharospasms, migraines, traumatic brain injuries, and genetic disorders such as Achromatopsia, retinitis pigmentosa and other retinal dysfunctions, such as Achromatopsia, retinitis pigmentosa and other retinal dysfunctions, a novel instrument was developed. This instrument is a computer-controlled optoelectronic system designed to determine the photosensitivity thresholds of patients with dry eyes, blepharospasms, migraines, traumatic brain injuries, and genetic disorders such as Achromatopsia, retinitis pigmentosa and other retinal dysfunctions. During human psychophysics experiments, we demonstrated that subjects suffering from Achromatopsia experienced lower photosensitivity thresholds than normal subjects. Hence, the system can safely and reliably determine the photosensitivity thresholds of healthy and light-sensitive subjects by detecting and quantifying the individual differences. Future studies will be performed with this system to determine the photosensitivity threshold differences between normal subjects and subjects suffering from other conditions that affect light sensitivity.

9693-36, Session 8

Age dependent sensitivity of two-photon isomerization of rhodopsin chromophores in the human retina

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Light sensation relies on photoisomerization of chromophores in rod and cone photoreceptor cells. Spectral sensitivity of these photoreceptor cells in the retina is determined by the absorption spectra of their pigments, which covers a range from 400 nm to above 700 nm. Regardless the mechanisms leading to visual pigment isomerization, light sensation is triggered every time visual pigment molecules change their conformation. Thus, two-photon absorption (TPA) should produce the same result (visual sensation) as single photon absorption of light. This observation was positively verified and published by our group. During human psychophysics experiments, we found that humans can perceive light in the infrared (IR) range as colors that mark half of the wavelength of the applied laser beam. Other experiments and theoretical research, such as mouse electrophysiology, biochemical studies of TPA in rhodopsin or molecular modeling studies, confirmed that visual sensation can be triggered by TPA. There are few publications describing human near infrared (NIR) perception and no formal proposals to use this phenomenon to improve ophthalmic diagnosis and monitor treatment. Here we report that the use of novel instrumentation revealed that the sensitivity threshold for NIR vision depends on age.

9693-38, Session 8

Pupillary responses of healthy subjects to chromatic light stimuli at incremental intensities at central and peripheral visual field locations

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Purpose: To determine the pupillary responses (PR) of healthy participants to chromatic light stimuli presented at increasing intensities in central and peripheral locations in a 30 degree visual field (VF).

Methods: A multifocal chromatic pupillometer (MCP) was used to record pupil responses (PR) of 17 healthy subjects and 5 Best Vitelliform macular dystrophy patients. Blue and red light stimuli (peak 485 nm and 620 nm, respectively) were presented at light intensities of 400 and 1000 cd/m2, respectively at 76 different points in a 16.2 degree VF. The PR of patients were compared with their findings on Humphrey’s 24-2 perimetry, optical coherence tomography and the PR obtained from healthy subjects.

Results: Patients demonstrated reduced percentage of pupillary contraction and slower maximal contraction velocity, more than two standard errors (SE) away from the mean of healthy subjects in response to red light in majority of VF locations. In response to blue light, the percentage of pupillary contraction was lower (by over two SE) compared with normal controls only in central locations. The latency of maximal contraction velocity was shorter in patients compared with healthy subjects in response to both colors.

Conclusions: This study demonstrated the advantage of using MCP-based objective VF to assess central scotoma in macular degeneration. Our finding also suggests that chromatic perimetry may differentiate between PR mediated by cones and rods, and can specifically detect defects in macular cones. Different parameters of PR such as latency of maximal contraction velocity may shed light on the pathophysiology of different blinding diseases.

9693-37, Session 8

Chromatic multifocal pupillometer for objective perimetry patients with macular degeneration

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Purpose: To objectively assess visual field (VF) defects and retinal cell function in healthy subjects and patients with macular degeneration using a chromatic multifocal pupillometer.

Methods: A multifocal chromatic pupillometer (MCP) was used to record pupillary responses (PR) of 17 healthy subjects and 5 Best vitelliform macular dystrophy patients. Blue and red light stimuli (peak 485 nm and 620 nm, respectively) were presented at light intensities of 400 and 1000 cd/m2, respectively at 76 different points in a 16.2 degree VF. The PR of patients were compared with their findings on Humphrey’s 24-2 perimetry, optical coherence tomography and the PR obtained from healthy subjects.

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Conclusions: This study demonstrated the advantage of using MCP-based objective VF to assess central scotoma in macular degeneration. Our finding also suggests that chromatic perimetry may differentiate between PR mediated by cones and rods, and can specifically detect defects in macular cones. Different parameters of PR such as latency of maximal contraction velocity may shed light on the pathophysiology of different blinding diseases.
9693-39, Session 8

Simultaneous refraction measurement and OCT axial biometry of the eye during accommodation

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The purpose of this project is to design and evaluate a system that will enable objective assessment of the optical accommodative response in real-time while acquiring axial biometric information. The system combines three sub-systems which were integrated and mounted on a joystick x-y-z adjustable modified slit-lamp base to facilitate alignment and data acquisition: (1) a Shack-Hartmann wavefront sensor for dynamic refraction measurement, provided software calculates sphere, cylinder and axis values, (2) an extended-depth Optical Coherence Tomography (OCT) system using an optical switch records high-resolution cross-sectional images across the length of the eye, from which, dynamic axial biometry (corneal thickness, anterior chamber depth, crystalline lens thickness and vitreous depth) can be extracted, and (3) a modified dual-channel accommodation stimulus unit based on the Badal optometer for providing a step change in accommodative stimulus. The prototype system is capable of taking simultaneous measurements of both the optical and the mechanical response of lens accommodation. These measurements can provide insight into correlating changes in lens shape with changes in lens power and ocular refraction and ultimately provide a more comprehensive understanding of accommodation, presbyopia and an objective assessment of presbyopia correction techniques.

9693-40, Session 8

Optical extended depth of focus lens design for children myopia control

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The mechanism that causes to the progress of children myopia is not completely clear yet but the predominant recent speculation is that it is related to hyperopic blurring in the periphery of the retina. In our article we discuss a proof of concept to an innovative optical design that can control the focal plane of any lens and to extend its depth of focus and as a result to cause the focal plane to conjugate with the fovea also in its peripheral region (note that the trials made so far to solve the problem did not use extended depth of focus lenses) and therefore to assist in resolving the children myopia control problem. In our article we focused on the optical design with respect to all others articles that suggested optical elements that can do myopic shift but didn’t show simulations results to prove it.

9693-41, Session 9

Non-invasive detection of laser-induced retinal injury through the vitreous using dynamic light scattering

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Laser radiation entering the eye has the potential of damaging the retina. As an inflammatory response, the proteins can rush to the lesion site created by laser exposure. We explore the hypothesis if these proteins can be detected non-invasively. In this preliminary study, we developed a new brief-case size dynamic light scattering instrument to detect these proteins in-vivo in the rabbit vitreous. The results were validated with bio-chemical analysis.

9693-42, Session 9

Developing a one-second automatic glaucoma treatment using trans-scleral laser trabeculoplasty (LTP) without a gonioscopy lens

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Developing a one-second automatic glaucoma treatment using trans-scleral laser trabeculoplasty (LTP) without a gonioscopy lens

Purpose: Developing an LTP device for delivering multiple simultaneous trans-scleral applications of low energy laser irradiation to the trabecular meshwork (TM) for reducing Intraocular Pressure (IOP).

Methods: Concept proof: A randomized, masked, controlled one was performed on open angle glaucoma patients. The control group underwent conventional SLT (100 laser spots through a gonioscope for 360 degrees directly on the TM). The trial group underwent irradiation by the same laser at the same irradiation parameters on the sclera overlying the TM. Topical glaucoma therapy was not changed during the 12 months trial.

Feasibility trial: Using optimized laser parameters, 60 discrete applications were administered on similar locations of patients’ sclera.

Results: Concept proof: Trans-scleral applications (N=15), IOP decrease from 20.21 mmHg before treatment to 16.00 (22.7%) at one year. The corresponding numbers for the control group (n=15), were 21.14 mmHg and 14.30 (23.4%). There was no statistical difference between the two groups in IOP reduction. The complications rate was significantly higher in the control group. Trial 2: IOP was reduced from an of 25.3 mmHg to 19.3 (23.7%) in the 11 patients.

Conclusions: Laser coherency, lost in tissue transmission, is not required for the therapeutic effect. The new method will possibly enable treatment of angle closure glaucoma as well as simultaneous applications of all laser spots to the sclera. When used conjointly with target acquisition, will make feasible an automatic glaucoma treatment in less than one second.
Heat shock protein expression as guidance for the therapeutic window of retinal laser therapy

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Sub-visible and non-damaging retinal laser therapy are effective treatments for central serous retinopathy and diabetic macular edema. Unlike conventional photocoagulation, the laser-induced temperature rise does not exceed the damage threshold of the retina in non-damaging treatment, thus avoiding adverse effects. The mechanism leading to the therapeutic effect of macular laser therapy in general, and non-damaging treatment in particular, is not well understood. Activation of heat shock response to laser-induced hyperthermia is one possibility. In this study, we examine heat shock protein 70 (Hsp70) expression in the retinal pigment epithelium (RPE) by whole-mount immunohistochemistry after laser therapy at various pulse energies in pigmented rabbits.

A titration algorithm for the PASCAL laser, Endpoint Management, relates the experimental pulse energies to barely visible lesions by adjusting power and pulse duration. 577 nm wavelength and 200 7m retinal spot size are used in this study. Seven hours following laser treatment, the eye is enucleated for analysis. Scanning electron microscopy, histology and viability/cytotoxicity staining are used to confirm the retinal damage threshold of 40% of titration energy. We show Hsp70 expression in the RPE at non-damaging energy settings of 25% and 30% of titration energy, indicating cellular response to hyperthermia, which may be responsible for therapeutic effect. Hsp70 expression is also seen at the single cell-wide perimeter of damaging lesions, as expected based on a computational model of laser heating. Relatively narrow window of non-damaging activation of cellular response to laser irradiation and variability with pigmentation highlight the importance of careful titration in each patient.

Towards real time speckle controlled retinal photocoagulation

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Photocoagulation is a laser treatment widely used for the therapy of several retinal diseases. Intra- and inter-individual variations of the ocular transmission, light scattering and the retinal absorption makes it impossible to achieve a uniform effective exposure and hence a uniform damage throughout the therapy. A real-time monitoring and control of the induced damage is highly requested. Here, an approach to realize a real time optical feedback using dynamic speckle analysis is presented.

A 532 nm continuous wave Nd:YAG laser is used for coagulation. This process is monitored by dynamic speckle investigation. While coagulation, speckle are produced by a coherent object illumination using a 830 nm diode laser and analyzed by a CMOS camera with a frame rate up to 6kHz. Regarding the trend of using very short exposure times in the ms time range it is obvious that a control system needs to determine whether the desired damage is achieved to shut down the system in a fraction of this time. Here we use a fast and simple adaption of the generalized difference algorithm. This algorithm runs on a FPGA and is able to calculate a feedback value which is correlated to the thermally and coagulation induced tissue motion and thus the achieved damage.

For different spot sizes (50-200 ?m) and different exposure times (50-500 ms) the algorithm shows the ability to discriminate between certain categories of retinal pigmen epithelial damage ex-vivo in enucleated porcine eyes.

Effect of laser polarization and pulse energy on therapeutic, femtosecond laser-induced second harmonic generation in corneal tissue

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Some of the most commonly performed surgical operations in the world, including laser-assisted in-situ keratomileusis (LASIK), lens replacement (e.g. cataract surgery), and keratoplasty (cornea transplant), now employ therapeutic infrared femtosecond lasers (FSLs) for their extreme precision, low energy delivered into tissue and advanced ablation characteristics. Although the widely exploited applications of FSLs in medical therapeutics offer significant benefits, FSLs must generate very high intensities in order to achieve optical breakdown, the predominant tissue ablative mechanism, which can also stimulate nonlinear optical effects such as harmonic generation, an effect that generates coherent visible and UV light in the case of second- (SHG) and third-harmonic generation (THG), respectively. In order to improve the understanding of HG in corneal tissue, the effect of FSL polarization and pulse energy were investigated. FSL stimulated SHG intensity in corneal tissue was measured as the laser polarization was rotated 360 degrees. Further, the pulse energy at the SHG wavelength were measured for single FSL pulses as the pulse energy at the fundamental wavelength was varied through a range of clinically relevant values. The results of this study revealed SHG intensity oscillated with laser polarization, having a variation greater than 20%. This relationship seems to due to the intrinsic anisotropy of collagen fibril hyperpolarizability, not related to tissue birefringence. SHG pulse energy measurements showed an increase in SHG pulse energy with increasing FSL pulse energy, however conversion efficiency decreased. This may be related to the dynamic relationship between optical breakdown leading to tissue destruction and HG evolution.

Investigating the effect of photodynamic therapy on Pseudomonas aeruginosa keratitis isolates

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Keratitis associated with Pseudomonas aeruginosa is difficult to manage. Treatment includes antibiotic eye drops, however, some strains of Pseudomonas aeruginosa are resistant. Current research efforts are focused on finding alternative and adjunct therapies to treat multi-drug resistant bacteria. One promising alternate technique is photodynamic therapy (PDT). The purpose of this study was to evaluate the effect of riboflavin- and rose bengal-mediated PDT on Pseudomonas aeruginosa keratitis isolates in vitro. Two isolates (S+U- and S-U+) of Pseudomonas aeruginosa were derived from keratitis patients and exposed to five experimental groups: (1) Control (dark, UV-A irradiation, 525nm irradiation); (2) 0.3% riboflavin (dark, UV-A irradiation); and (3) 0.1% rose bengal, (4) 0.05% rose bengal and (5) 0.01% rose bengal (dark, 525nm irradiation). Three days after treatment, in dark conditions of all concentration of riboflavin and rose bengal showed no inhibition in both S+U- and S-U+ strains of Pseudomonas aeruginosa. In 0.1%
and 0.05% rose bengal irradiated groups, for both S+U- and S-U+ strains, there was complete inhibition of bacterial growth in the central 50mm zone corresponding to the diameter of the green light source. These in vitro results suggest that rose bengal photodynamic therapy may be an effective adjunct treatment for Pseudomonas aeruginosa keratitis.

9693-47, Session 9

Two-photon fluorescence microscopy for determination of the riboflavin concentration in the anterior corneal stroma when using the Dresden Protocol

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Patients suffering from keratoconus develop a cone-shaped deformation of the cornea. In order to stop or to slow down the progression of this deformation, corneal crosslinking can be performed. In clinical routine, crosslinking is achieved by applying the Dresden protocol. The first part of this protocol describes the removal of the epithelium and an application of riboflavin to the surface of the cornea for 30 minutes. During this time period, diffusion leads to a spreading of riboflavin inside the stromal region. So far, no accurate determination of the riboflavin concentration in different corneal depth could be achieved. In our study, we used the two-photon fluorescence signal of riboflavin in order to determine its concentration in different corneal depths. Signal acquisition has been performed during 2-stacks through the riboflavin treated corneas with a multiphoton microscope. In order to compensate signal losses due to scattering and absorption, corneas had been saturated with riboflavin after the first multiphoton measurement. After a steady state was reached, the two-photon signal of the saturated corneas was measured again. A pairwise division of the intensity values in different corneal depths (fluorescence intensity after 30 minutes of treatment / fluorescence intensity of saturated cornea) led to real riboflavin concentrations. Experimentally obtained data are supported by the theory of diffusion. Different diffusion coefficients could be determined when using different dextran solutions (increasing dextran concentration led to a decreasing diffusion coefficient). Thus, fluorescence signal analysis allows the determination of riboflavin content inside stromal tissue when applying the Dresden Protocol.

9693-48, Session 10

Imaging human retinal pigment epithelium cells using adaptive optics optical coherence tomography

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Retinal pigment epithelium (RPE) cells are vital to health of the outer retina, but are often compromised in ageing and major ocular diseases that lead to blindness. Early manifestation of RPE disruption occurs at the cellular level, but while biomarkers at this scale hold considerable promise, RPE cells have proven extremely challenging to image in the living human eye. We present a novel method based on OCT equipped with adaptive optics (AO) that overcomes the associated technical obstacles. The method takes advantage of 3D resolution of AO-OCT, but more critically sub-cellular segmentation and registration algorithms that we have developed and now permit averaging of RPE images without loss of RPE mosaic information. With this method we have observed the RPE mosaic in every subject and retinal location imaged to date (six eyes at 3° and 7° temporal to the fovea) and have quantified RPE packing geometry in terms of cell density, cone-to-RPE ratio, and number of nearest neighbors using Voronoi and power spectra analyses. RPE cell density (cells/mm2) showed no significant difference between 3° (5777±133) and 7° (5797±233). In contrast, cone-to-RPE ratio was significantly higher at 3° (3.19±0.32:l) than 7° (1.89±0.31:l). Voronoi analysis also showed most RPE cells have six nearest neighbors, which was significantly larger than the next two most prevalent associations: five and seven. Averaged across the six subjects, prevalence of cells with six neighbors was 49.1±3.5% at 3°, and 51.0±3.5% at 7°. These results are consistent with histology and in vivo studies using other imaging modalities.

9693-49, Session 10

Computational adaptive optics of the human retina

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It is well known that patient-specific ocular aberrations limit imaging resolution in the human retina. Previously, hardware adaptive optics (HAO) has been employed to measure and correct these aberrations to acquire high-resolution images of various retinal structures. While the resulting aberration-corrected images are of great clinical importance, clinical use has not been widespread due to the cost and complexity of HAO systems. We present a technique termed computational adaptive optics (CAO) for aberration correction in the living human retina without the use of hardware adaptive optics components. In CAO, complex interferometric data acquired using optical coherence tomography (OCT) is manipulated in post-processing to adjust the phase of the optical wavefront. In this way, the aberated wavefront can be corrected. We summarize recent results in this technology for retinal imaging, including aberration-corrected imaging in multiple retinal layers and practical considerations such as phase stability and image optimization.

9693-50, Session 10

Compact adaptive optics fundus camera/ optical coherence tomography system for high resolution retinal imaging

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We present a new compact multi-modal imaging prototype that combines an adaptive optics (AO) fundus camera with AO-optical coherence tomography (OCT) in a single instrument. The prototype allows acquiring AO fundus images with a field of view of 4°x4° and with a frame rate of 10 fps. The exposure time of a single image is 10 ms. This short exposure time results in nearly motion artifact free high resolution images of the fundus. The AO-OCT mode allows acquiring volumetric data of the retina at 200kHz A-scan rate. OCT data is acquired within a field of view of 2°x2° located at the central part of the AO fundus image. The typical recording time of volume data takes 0.8 seconds. The performance of the new system is tested in healthy volunteers and patients with retinal diseases.
9693-51, Session 10

Axial analysis of cones and adjacent retinal structures using AOSLO

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We imaged the retina using the Indiana Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO). Our system uses two deformable mirrors to provide en face, high-resolution images of retinal structures at a 28 Hz frame rate. The wavelength of the sensor light was 850 nm and the imaging wavelength was 820 nm at 50 and 120 7W respectively. The confocal pinhole was located in a position conjugate with the retina allowed us to segment one retina plane. Two different confocal apertures of 75 7m and 100 7m (1.5 and 2 times the Airy disk size) were used to provide different amounts of confocal or scattered light. The imaging area was 1.4 x 1.2 deg which corresponds roughly to 400 x 350 7m.

Using the large stroke deformable mirror, which provides the focusing capability of the confocal system, we imaged the same location at different planes. We moved from superficial layers to the retinal pigmented epithelium in 0.3 D increments. The range of adjustments included the subjectively best overall image, and focal planes anterior and posterior to this.

We imaged 10 subjects at approximately 7.5 deg temporal from the fovea. A video of individual frames was taken, and the individual frames were warped, aligned, and averaged. We measured 10 bright and 10 dim cones for each subject at the 10 depths, with brightness groupings based subjectively on the most superficial location. The function for amount of light reflected differed for the two groups of cones. Reflectivity varied as a function of depth.

9693-52, Session 10

Parafocal retinal cone mosaic imaging in children with ultra-compact switchable SLO/OCT handheld probe

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In vivo photoreceptor imaging has enhanced the way vision scientists and ophthalmologists understand the retinal structure, function, and etiology of numerous retinal pathologies. However, the complexity and large footprint of current systems capable of resolving photoreceptors has limited imaging to patients who are able to sit in an upright position and fixate for several minutes. Unfortunately, this excludes an important fraction of patients including bedridden patients, small children, and infants. Here, we show that our dual-modality, high-resolution handheld probe with a weight of only 94 g is capable of visualizing photoreceptors in supine children. Our device utilizes a microelectromechanical systems (MEMS) scanner and a novel telescope design to achieve over an order of magnitude reduction in size compared to similar systems. The probe has a 7° field of view and a lateral resolution of 8 μm. The optical coherence tomography (OCT) system has an axial resolution of 7 μm and a sensitivity of 101 dB. High definition scanning laser ophthalmoscopy (SLO) and OCT images were acquired from children ranging from 14 months to 12 years of age with and without pathology during examination under anesthesia in the operating room. Parafocal cone imaging was shown using the SLO arm of this device without adaptive optics using a 3° FOV for the first time in children under 4 years old.

This work lays the foundation for pediatric research, which will improve understanding of retinal development, maldevelopment and early onset of diseases at the cellular level during the beginning stages of human growth.

9693-53, Session 10

Retinal photoreceptor imaging with high-speed line-field parallel spectral domain OCT

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We present retinal photoreceptor imaging with a line-field parallel spectral domain OCT modality, utilizing a commercially available 2D CMOS detector array operating at and imaging speed of 500 B-scans/s. Our results demonstrate the first in vivo structural and functional retinal assessment with a line-field OCT setup providing sufficient sensitivity, lateral and axial resolution and 3D acquisition rates in order to resolve individual photoreceptor cells. The setup comprises a Michelson interferometer illuminated by a broadband light source, where a line-focus is formed via a cylindrical lens and the back-propagated light from sample and reference arm is detected by a 2D array after passing a diffraction grating. The spot size of the line-focus on the retina is 57μm, which corresponds to a PSF of 50?m and an oversampling factor of 3.6 at the detector plane, respectively.

A full 3D stack was recorded in only 0.8 s. We show representative en face images, tomograms and phase-difference maps of cone photoreceptors with a lateral FOV close to 2°. The high-speed capability and the phase stability due to parallel illumination and detection may potentially lead to novel structural and functional diagnostic tools on a cellular and microvascular imaging level. Furthermore, the presented system enables competitive imaging results as compared to respective point scanning modalities and facilitates utilizing software based digital aberration correction algorithms for achieving 3D isotropic resolution across the full FOV.

9693-54, Session PSsun

Objective straylight assessment of the human eye with a novel device

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Forward scattered light from the anterior segment of the human eye can be measured by Shack-Hartmann (SH) wavefront aberrometers with limited visual angle. We propose a novel Point Spread Function (PSF) reconstruction algorithm based on SH measurements with a novel measurement devise to overcome these limitations.

In our optical setup we used a Digital Mirror Device as variable field stop, which is conventionally a pinhole suppressing scatter and reflections. Images with 21 different stop diameters were captured and from each image the average subaperture image intensity and the average intensity of the pupil were computed. The 21 intensities represent integral values of the pupil image, which corresponds to a PSF of the visual angle. A generalized form of the Stiles-Holladay-approximation is fitted to the PSF resulting in a stray light parameter Log(IS). Addinally the transmission loss of eye is computed. For the proof of principle, a study on 13 healthy young volunteers was carried out. Scatter filters were positioned in front of the volunteer’s eye during C-Quant and scatter measurements to generate straylight emulating scatter in the lens. The straylight parameter is compared to the C-Quant measurement parameter Log(ISC) and scatter density of the filters SDF with a partial correlation.

LOG(IS) shows significant correlation with the SDF and Log(ISC). The correlation is more prominent between LOG(IS) combined with the transmission loss and the SDF and Log(ISC).
Our novel measurement and reconstruction technique allow for objective stray light analysis of visual angles up to 4 degrees.

9693-55, Session PSun

Computer assisted quantification of choroidal neovascularization

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Choroidal Neovascularization (CNV) is the uncontrolled growth of vessels into the retina, and is a characteristic of wet Age Related Macular Degeneration (AMD) and Diabetic Retinopathy. The pathology is commonly modeled with a photothermal lesion induced by a surgical photocoagulation laser. To better understand the progression of the acute inflammatory response in the CNV model, we used a Heidelberg Spectralis HRA+OCT system for multimodal fundus imaging and Optical Coherence Tomography (OCT) imaging of the lesions in vivo. Although the features seen in morphology of interest such as the Nerve Fiber Layer (NFL) and Retinal Pigment Epithelial Layer (RPE) are often at the limits of resolution, using OCT for structural information without an invasive biopsy is preferable. Because of the biological and technical complications, the lesions can be hard to image or visualize; therefore we developed an image processing algorithm that can detect and enhance the features in the image. Steerable filters were designed for semi-automated analysis of retinal layers segmentation and detection of lesion borders. The steerable filters encode the directionality and magnitude of the edges to provide a grader a more quantifiable and methodical method of determining disruptions to retinal layer morphology. The algorithm provides perceptual enhancement for images where the SNR falls below 25DB and therefore reduces inter-grader variation. The advantage of the proposed algorithm design, is that it is fast and efficient enough to be embedded into an imaging system for real time processing, while allowing flexibility to be customized and extended for automated segmentation.

9693-56, Session PSun

Preliminary studies on sunglasses lenses UV protection degradation by using an automated prototype

Leonardo M. Mariano Gomes, Artur D. Loureiro, Liliane Ventura, Univ. de São Paulo (Brazil)

Sunglasses may assure ultraviolet (UV) safeness when adequate UV filters protection are used. There is evidence that ultraviolet protection of sunglasses can degrade with exposure to the sun, but such an experiment has never been done. The Brazilian standard for sunglasses (NBR15111/2013) establishes artificial aging test for lenses in solar simulator for 50 hours of exposure for evaluating changes in transmittance of the visible spectrum. However, no sunglasses standards take into account UV spectral transmittance changes. Thus, this project aims expose the lenses for 24 months using a prototype, which consists of a panel with cover, housing 100 lenses arranged in the vertical position to the ground, fixed on a rotating axis, which will be irradiated by the sun from sunrise until sunset. The lid opens and turns the panel towards the sun automatically, so that the lens will always be facing the sun. Humidity, dust, time and UV index sensors, as well as a video camera are part of the system. The exposure time and UV index will be recorded and automatic opening or closing the lid may also be controlled by a PC using online software. The tests are being conducted for a month already and previously to irradiation, spectroscopy was performed and then will be repeated every 30 days of exposure. After one month spectral changes have been observed. These non-negligible changes obtained in short exposure time are promising data, justifying an investigation with longer exposure time.

9693-57, Session PSun

In vivo measurement of intraocular distances in human eyes by using Fourier domain low-coherence interferometry

Liang Feng, Northeastern Univ. at Qinhuangdao (China); Qinghua Li, Northeastern Univ. (China); Zhenhe Ma, Yi Wang, Northeastern Univ. at Qinhuangdao (China)

We introduce a system for rapidly measuring the intraocular distances of human eyes in vivo with high sensitivity by using Fourier domain low-coherence interferometry. A focal point displacement unit is used to rapidly adjust the focal point of a probe beam, and a multiple references unit is employed to extend the depth range up to the human eye length. The system is experimentally verified by measuring the anterior segment length, vitreous chamber depth, and axial eye length in human eyes in vivo. The results are in good agreement with those obtained using the IOL Master (Carl Zeiss Meditec Inc.) and Lenstar LS 900 (Haag-Streit AG).

9693-58, Session PSun

Optical design of a novel instrument that uses the Hartmann-Shack sensor and Zernike polynomials to measure and simulate customized refraction correction surgery outcomes and patient satisfaction

Fatima M. M. Yasuoka, Instituto de Física de São Carlos (Brazil) and BR Labs. Tecnologia Optica e Fotonica Ltda. (Brazil); Luciana de Matos, Instituto de Física de São Carlos (Brazil); Antonio F. R. Cremasco, Miriam Numajiri, Rafael Marcato, Wavetek Technologies Ltda. (Brazil); Otavio G. Oliveira, Mediphacos Ltda. (Brazil); Jbarcs C. Castro Neto, Vanderlei S. Bagnato, Instituto de Física de São Carlos (Brazil); Luis Albert V. Carvalho, Wavetek Technologies Ltda. (Brazil)

An optical system that conjugates the patient’s pupil to the plane of a Hartmann-Shack (HS) wavefront sensor was simulated using software of optical design and mounted on the optical bench. This optical bench prototype is composed by mechanical eye device, beam splitter, eye illumination system, three positive lenses, three plane mirrors, mirrored prism, movable mirror, wavefront sensor and camera CCD. The mechanical eye device is used to simulate aberrations of the eye. From this device the rays are emitted and directed to the optical system, travelling by the beam splitter, where part of the rays fall on the camera CCD. The other part passing in the optical system and finally reach the sensor. Several eye models based on in vivo typical eye aberrations were constructed with varying parameters of anterior corneal surface, anterior and posterior lens surface, and axial distances using the optical design software Zemax. The CAD outcomes of each HS images for each of these cases were saved, and these images are processed using in-house techniques from our laboratory. A Badal system was designed to compensate myopia or hyperopia and an accommodation system was also designed. The simulated and true images for low order aberrations (LOA) were compared using the (x,y) centroid coordinates to assure that the optical system was constructed precisely in order to match the simulated system.

Afterwards a simulated version of retinal images were constructed to show how these typical eyes would perceive an optotype positioned 20 ft away. Certain personalized corrections were allowed by eye doctors based on different Zernike polynomial values and the optical images were rendered to the new parameters. Optical images of how that eye would see with and without corrections of certain aberrations were generated and allowed
which aberrations can correct and in what degree. The patient can then “personalize” the correction to their own satisfaction.

Simulated images of the accommodation picture will be presented with different degrees of aberration elimination, which may be chosen by the patient. We believe this new approach to wavefront sensing, optical design and software are a promising change in paradigm towards the betterment of the current patient-physician relationship.

6963-59, Session PSun
Assessing the elasticity change of cataract lens with OCE
Chen Wu, Chih-Hao Liu, Manmohan Singh, Zhaolong Han, Jiasong Li, Raksha Ragunathan, Univ. of Houston (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States)

Cataract is one of the most common degenerative diseases that cause blindness. Careful quantification of lens biomechanical properties can greatly assist in early detection as well as selection of personalized treatment procedures. In this study, we use phase-sensitive optical coherence elastography (OCE) system to assess the influence of the cold cataract on the changes of biomechanical properties of the porcine crystalline lens in vitro. Relaxation rates of air puff induced elastic waves were measured on the same crystalline lens before and after the induction of the cold cataract. Obtained results demonstrate that the relaxation rate increased indicating that the elastic properties of the porcine lens have increased after the induction of cold cataract. Therefore, this non-contact method has a potential for quantitative assessment of lens biomechanical properties both ex vivo and in vivo.

6963-60, Session PSun
Improvement of glaucoma diagnosis with 3D depth measurement on stereo retinal images using novel instrumentation and algorithm for focus stacking: preliminary results on model and in vivo eyes
Luis Albert V. Carvalho, Wavetek Technologies Ltda. (Brazil) and Univ. de São Paulo (Brazil)

Modern day eye care professionals in developing countries feel an urgent need for cost effective instrumentation for the early diagnosis of Glaucoma, a leading cause of blindness. In a previous project [1] we have developed a low cost and promising 3D retinal camera. Nevertheless, a great challenge in 3D reconstruction is the cross correlation process, largely influenced by the depth of field (DOF). To improve precision we have applied here a new focus stacking algorithm to in vivo and model eyes. A matlab algorithm was implemented to pre-process video files and select specific image sequences with distinct focus characteristics. In-focus regions of each image were detected automatically via Fourier analysis and the final image pair was generated. Our stacking algorithm was tested on a model of each image were detected automatically via Fourier analysis and the final image pair was generated. Our stacking algorithm was tested on a model eye.


9693-61, Session PSun
Effects of short term changes in the blood glucose level on the autofluorescence lifetime of the human retina in healthy volunteers
Matthias Klemm, Technische Univ. Ilmenau (Germany); Edgar Nagel, Ophthalmic Practice Ankermedicin (Germany); Dietrich Schweitzer, Universitätsklinikum Jena (Germany); Stefan Schramm, Jens Haueisen, Technische Univ. Ilmenau (Germany)

Purpose: Fluorescence lifetime imaging ophthalmoscopy (FLIO) provides in vivo metabolic mapping of the ocular fundus. Changes in FLIO have been found in e.g. diabetes patients. The influence of short term metabolic changes caused by blood glucose level changes is unknown. Aim of this work is the detection of short-term changes in fundus autofluorescence lifetime during an oral glucose tolerance test.

Methods: FLIO was performed in 10 healthy volunteers (29±4 years, fasting for 12h) using a scanning laser ophthalmoscope (30° fundus, 34µm resolution, excitation with 473nm diode laser with 70 ps pulses at 80 MHz repetition rate, detection in two spectral channels 500-560nm (ch1) and 560-720nm (ch2) using the time-correlated single photon counting method). The blood glucose level (BGL) was measured by an Accu-Chek® Aviva self-monitoring device. Before and after a glucose drink (300ml solution, containing 75g of glucose (Accu-Chek® Dextrose O.G.T.), BGL and FLIO were measured every 15min. The FLIMX software package was applied to compute the average fluorescence lifetime ? on the inner ring of the ETDRS grid using a modified 3-exponential approach.

Results: The results are mean ± standard deviation over all volunteers in ch1. Baseline measurement: BGL: 5.3±0.4 mmol/l, ?m: 147±13ps. A significant reduction (?=5%; Wilcoxon rank-sum test) in ?m is detected after 30min (BGL: 8.4±1.1 mmol/l, ?m: 140±13ps) and after 90min (BGL: 6.3±1.4 mmol/l, ?m: 139±13ps). Results of ch2 show smaller deviations in the fluorescence lifetimes over time.

Conclusions: FLIO measurements should control for BGL, at least patients should be fasting.

9693-62, Session PSun
Imaging choroidal neovascularization in the mouse retina using optical coherence tomography angiography
Jang Ryul Park, Yongjoo Kim, KAIST (Korea, Democratic Peoples Republic of); Hye Kyong Hong, Gun Hwi Lee, Seoul National Univ. Bundang Hospital (Korea, Republic of); Sang Jun Park, Seoul National Univ. Bundang (Korea, Republic of); Jaeryung Kim, KAIST (Korea, Republic of); Yoonha Hwang, Pilhan Kim, Gou Young Koh, KAIST (Korea, Democratic Peoples Republic of); Se Joon Woo, Kyu Hyung Park, Seoul National Univ. Bundang Hospital (Korea, Republic of); Wang-Yuhl Oh, KAIST (Korea, Democratic Peoples Republic of)

Understanding the pathogenesis of retinal diseases is greatly facilitated by utilizing small animal models because of their ease of maintenance and availability. For investigating pathophysiology and drug development in the rodent models, it is essential to visualize structure and vasculature of retinal tissue to monitor disease progression. Optical Coherence Tomography (OCT) is a highly promising imaging technique in ophthalmology that provides 3D structural information and vasculature information of retina noninvasively.

In the present study, we developed a rodent retina OCT system and imaged
3D structure and angiography of normal and choroidal neovascularization (CNV)-induced retina in mice and rats. A lab-built high-speed OCT using wavelength-swept laser (repetition rate of 230 kHz) centered at 1050 nm allowed enough imaging penetration depth for visualizing choroid layer. The OCT scanned a 1.7 x 1.7-mm area in mice, a 3.5 x 3.5-mm area in rats and sampled 1024 x 1024 x 3 A-scans. For an angiographic cross-sectional image, a complex differential variance image from 3 consecutive B-scans at each vertical position was generated. Motion compensation algorithm was used to minimize bulk motion noise. For visualizing CNV clearly, the inner retinal layer, the outer retinal layer and choroid layer were segmented. The inner retina shows retinal vasculature and the CNV vessels are identified in the outer retina. En-face OCT angiography and cross-sectional angiography showed volumes and locations of CNV.

9693-63, Session PSun
A comparison study of Riboflavin/UV-A and Rose-Bengal/Green light cross-linking of the rabbit corneas using optical coherence elastography
Jiasong Li, Mannmohan Singh, Zhaolong Han, Srilatha Vantipalli, Chih-Hao Liu, Chen Wu, Raksha Raghunathan, Tina Kazemi, Univ. of Houston (United States); Michael D. Twu, The Univ. of Alabama at Birmingham (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States)

The biomechanical properties of the cornea are critical factors which determine its health and subsequent visual acuity. Keratoconus is a structural degeneration of the cornea which can diminish visual acuity. Riboflavin/UV-A corneal collagen cross-linking (UV-CXL) is an emerging treatment which increases the stiffness of the cornea and improves its ability to resist further degeneration. While UV-CXL has shown great promise, there are concerns associated with the UV irradiation, such as keratocyte cytotoxicity. Rose-bengal/green light corneal collagen cross-linking (RGX) has been proposed as an alternative to UV-CXL. Because of the high absorbance of the rose-bengal dye at green wavelengths, the treatment time is significantly shorter than with UV-CXL. Moreover, because green light is used in lieu of UV irradiation, there are no cytotoxic side-effects. In this study, noncontact optical coherence elastography (OCE) was used to compare the outcomes of UV-CXL and RGX treatment in the rabbit cornea. Low-amplitude (micrometer scale) elastic waves were induced by a focused air-pulse loading system. The elastic wave propagation was then imaged by a home-built phase-stabilized swept source OCT (PhS-SSOCE) system. The depth-resolved micro-scale phase-velocity distribution in the cornea was used to reveal the depth-wise heterogeneity before and after both cross-linking techniques. Our results show that UV-CXL and RGX increased the stiffness of the corneas by -54% and -5% while reducing the viscosity by -42% and -17%, respectively. The depth-wise phase velocities showed that UV-CXL affected the anterior -1/3 of the corneas, while RGX only affected the anterior -1/7 of the corneas.

9693-64, Session PSun
Quantitative assessment of rat corneal thickness and morphology during stem cell therapy by high-speed optical coherence tomography
Cerine Lal, James McGrath, Hrebesh M. Subhash, Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Optical Coherence Tomography (OCT) is a non-invasive 3-dimensional optical imaging modality that enables high resolution cross sectional imaging in biological tissues and materials. Its high axial and lateral resolution combined with high sensitivity, imaging depth and wide field of view makes it suitable for wide variety of high resolution medical imaging applications at clinically relevant speed. With the advent of swept source lasers, the imaging speed of OCT has increased considerably in recent years. OCT has been used in ophthalmology to study dynamic changes occurring in the cornea and iris, thereby providing physiological and pathological changes that occur within the anterior segment structures such as in glaucoma, during refractive surgery, lamellar keratoplasty and corneal diseases. In this study, we quantitatively assess the changes in corneal morphology and thickness in the anterior segment of the eye during wound healing process in a rat corneal burn model followed by stem cell therapy using a high speed swept source OCT. We demonstrate the feasibility of mapping changes in corneal morphology and corneal thickness.

9693-65, Session PSun
Mimicking cataract-induced visual dysfunction by means of protein denaturation in egg albumen
Biagio Mandraccchia, Andrea Finizio, Pietro Ferraro, Istituto di Scienze applicata e Sistemi Intelligenti (Italy)

Cataract is the world’s leading cause of blindness. According to World Health Organization (WHO), it is responsible for the 51% of the estimated 39 million cases of blindness occurring worldwide. The most common symptoms of cataracts are glared and blurred vision. Usually, people with cataract have trouble seeing or reading at distance or in low light and also their color perception is altered. Cataract is a sneaky disease as it is usually a very slow but progressive process, which creates adaptation so that patients find it difficult to recognize and for doctors it can be very difficult to explain and give comprehensive answers to the patients’ symptoms. Cataract is a disease associated with aging and with photo-oxidative denaturation (and cross-linking) of lens crystallins and other proteins. We built and tested an optic device that uses egg albumen to mimic the optical degradation of the crystalline related cataracts and that is able to visualize how the cataract impairs vision. At best of our knowledge it is the first experimental system developed at this aim. This can be a valuable tool, which can be of help in education for students in medical sciences as well as to provide a method to illustrate the patients how their vision is affected by cataract progression process.

9693-66, Session PSun
Objective chromatic perimetry using a multifocal pupillometer
Ygal Rotenstreich, Ron Chibel, Soad Haj Yahia, Daniel Ben-Ner, Mohamad Mahajna, The Chaim Sheba Medical Ctr. (Israel); Asaf Achiron, Wolfson Medical Ctr. (Israel); Yakir Berchenko, Bernice Oberman, Ofra Kalter-Leibovici, Laurence Freedman, The Gertner Institute (Israel); Ifat Sher-Rosenthal, The Chaim Sheba Medical Ctr. (Israel)

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in response to the red light between different test point locations was significantly higher in RP patients (range: 0.16-0.47) than in healthy subjects (range: 0.02-0.16, p < 0.0001). The MCP detected abnormal VF with high accuracy, with an area under the curve of 0.96 using Whitney–Wilcoxon test.

CONCLUSIONS: Defects in pupil contraction were substantially more profound in response to blue than to red light in RP patients. Patients with less severe VF defects showed higher pupil responses especially for the red light stimuli, correlating with preservation of cone function. This study demonstrated the feasibility of using MCP-based perimeter for objectively assessing VF defects and retinal function in patients with retinal degeneration. This method may be used to distinguish between the damaged cells underlying the VF defect.

9693-67, Session PSun

Tilt and decentration tolerance of intraocular lenses: measurements with an improved mechanical model eye

Luukas Traxler, Bernd Reutterer, Natascha Bayer, Fachhochschule Technikum Wien (Austria); Elisabet Rank, Sylvia Krause, Erik Beckert, Fraunhofer-Institut für Angewandte Optik und Feinmechanik (Germany); Andreas Drauschke, Fachhochschule Technikum Wien (Austria)

Cataract, a clouding of the crystalline eye lens, is the leading cause of blindness. It can effectively be treated by cataract surgery, where the clouded lens is replaced by an artificial Intraocular Lens (IOL). Postoperative healing processes can cause a displacement of the IOL, which further leads to the fact that the quality of vision is deteriorated. Studies show that the imaging quality of high-sophisticated IOL designs is more sensitive to lens displacements than simpler designs, like e.g. spherical IOLs. The effects of IOL displacements are not well represented and tested within the current IOL test standard ISO 11979-2. This fact implies the development of new test standards for novel and more sophisticated IOL designs. In this paper we present a mechanical eye-model, which extends the current standard in three main aspects: First, the eye-model is very close to the physiology of the human eye. Second, electromechanic drives allow an automatic and precise simulation of postoperative lens tilts and decentrations and finally additional to the ISO 11979-2 standard conform MTF analysis, in the proposed setup also wavefront aberrations are measured. The latter reveals discrete image aberrations caused by lens displacements. This new measurement setup allows to objectively analyze the displacement tolerance of various IOL designs. The functionality of this novel setup is demonstrated by measurements of two different IOL designs, namely using a conventional spherical design and an aspheric IOL design. The measurement results are verified with numerical simulations, which reveal a proper design of the proposed measurement method.

9693-68, Session PSun

Basic studies on laser-assisted phacoemulsification using diode-pumped Er:YAG laser

Florian Hausladen, Holger Wurm, Karl Stock, Univ. Ulm (Germany)

The aim of this study was to determine the potential of a novel diode-pumped Er:YAG laser for phacoemulsification in basic experimental studies. An appropriate experimental setup was created, including a translation stage for the sample movement, a sample holder, a water spray for sample humidification and a surgical microscope for observation. The analysis of the laser cuts and histological sections was done by light microscopy. As samples porcine eye lenses cured by formalin were used. In ablation experiments with different spot diameters and radiant powers and a constant repetition rate \( f = 200 \text{ Hz} \) the maximum ablation depths of \((4.346 \pm 0.044) \text{ mm} \) are reached at \((\Phi = 480 \mu \text{m}, f = 24.15 \text{ W})\). The average ablation efficiency is \(0.241 \text{ mm}^3/\text{J} \). The maximum extent of thermal damage is \((0.171 \pm 0.024) \text{ mm} \).

Using a sapphire cylinder with a diameter of \(412 \mu \text{m} \) in direct tissue contact with water spray the ablation depth reaches \((1.017 \pm 0.074) \text{ mm} \) at \(4.93 \text{ W} \) with an efficiency of \(0.286 \text{ mm}^3/\text{J} \). In case of under water treatment, the achievable ablation depth significantly decreases to 31.3 % at 4.91 W caused by the missing tracking possibility. Experiments using a manually guided sapphire fiber \((\Phi = 425 \mu \text{m}) \) show an efficient tissue ablation at an average power of \( f = 5 \text{ W} \). The observed decrease of ablation efficiency was nearly eliminated by manually fiber tracking.

In conclusion, the investigations show that the diode-pumped Er:YAG laser has a great potential for cataract surgery.

9693-69, Session PSun

Correlation of blood flow and functional changes in the rat retina during acute ocular pressure elevation

Bingyao Tan, Akshay Gurdita, Kirsten Carter, Vivian Choh, Univ. of Waterloo (Canada); Karen M. Joos, Vanderbilt Univ. (United States); Kostadinka Bizheva, Univ. of Waterloo (Canada)

Glaucoma causes progressive damage to the retinal morphology, blood perfusion and the retinal ganglion cells functional response, and eventually leads to blindness. In this study we explored the correlation between transient changes in the retinal blood flow and the functional response of the retina to a flash stimulus, associated with acute elevation of the intraocular pressure (IOP). The changes were measured with a combined, research-grade, high-resolution OCT+ERG system in a rat model of glaucoma. A loop procedure was used to elevate the IOP in steps from baseline (10 mmHg) to 80 mmHg and the IOP was kept constant for ~20 min at each step. Volumetric morphological and Doppler OCT data, as well as full field ERG recordings with single-flash, white light stimulus were acquired for each IOP value. Results from our study show that while the retinal blood flow decreases progressively with elevated IOP, the magnitudes of the ERG a-wave and b-wave increase progressively until the IOP reaches ~40 mmHg and then monotonically decrease to a 0 for IOP = 80 mmHg.

9693-70, Session PSun

Public sunglasses tester: acknowledging population of UV protection

Fernando Lahoz, Liliane Ventura, Univ. de São Paulo (Brazil)

Sunglasses lenses are crucial to assure the protection of human eyes from excessive solar exposure, which may cause several eye diseases such as cataract, pterygium, photokeratitis and others. With the assured reduction of the ozone layer, more UV radiation reaches our eyes and, for that reason, protection measurements should be taken even more seriously. This project aims to develop a reliable and accurate system to test the transmittance of lenses for each frequency of the visible and UV spectrum and establish a protection level for the lens according to its category. The system is based on the principles of spectroscopy, in which a source of light is diffracted through a prism or a diffraction grating and the results are collected and analyzed by a specific sensor. In this case, it was used a linear CCD sensor with response under the UV spectrum. A diffraction grating that is able to diffract waves between the wavelengths of 280 to 400 nm was also used. The lenses are placed in-between the diffraction grating and the source of light, which is a combination of several lamps, in order to reach an emission spectrum similar to the sun. The data collected by the CCD sensor is...
transmitted to a Raspberry Pi through a serial port and it is displayed in a
7-inches screen. The final user can visually track, through a graphic,
the amount of UV radiation that is absorbed by the lens for each wavelength
of the UV spectrum.

9693-71, Session PSun

Comparison of performance of some common Hartmann-Shack centroid
estimation methods
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Madras (India); Abbas Ommani, Univ. of Waterloo
(Canada); Ritambhar Burman, Jadavpur Univ. (India);
Damber Thapa, Univ. of Illinois at Chicago (United States);
Natalie Hutchings, Vasudevan Lakshminarayanan, Univ. of
Waterloo (Canada)

The accuracy of the estimation of optical aberrations by measuring the
distorted wave front using a Hartmann-Shack wave front sensor (HSWS)
is mainly dependent upon the measurement accuracy of the centroid of
the focal spot. The most commonly used methods for centroid detection
such as the brightest spot centroid; first moment centroid; weighted center
of gravity and intensity weighted center of gravity, are generally applied
on the entire individual sub-apertures of the lens let array. However, these
processes of centroid estimation are sensitive to the influence of reflections,
scattered light, and noise; especially in the case where the signal spot area
is smaller compared to the whole sub-aperture area. In this paper, we give
a comparison of performance of the commonly used centroiding methods
on estimation of optical aberrations, with and without the use of some
pre-processing steps (thresholding, Gaussian smoothing and adaptive
windowing). As an example we use the aberrations of the human eye model.
This is done using the raw data collected from a custom made ophthalmic
aberrometer and a model eye to emulate myopic and hyper-metropic
defocus values up to 2 Diopters. We show that the use of any simple
centroiding algorithm is sufficient in the case of ophthalmic applications
for estimating aberrations within the typical clinically acceptable limits of a
quarter Diopter margins, when certain pre-processing steps to reduce the
impact of external factors are used.

9693-72, Session PSun

Visible-light optical coherence
tomography measures oxygen metabolism
in a rat model of retinopathy of
prematurity
Brian T. Soetikno, Ji Yi, Ronil Shah, Wenzhong Liu, Patryk
Purta, Hao F. Zhang, Amani A. Fawzi M.D., Northwestern
Univ. (United States)

Using visible-light optical coherence tomography (vis-OCT), for the
first time, we explored oxygen metabolism of the inner retina in a rat
model of the blinding childhood disease: retinopathy of prematurity
(ROP). Using our custom-built vis-OCT system, we measured the oxygen
saturation of hemoglobin within inner retinal blood vessels in rats with
50/10 oxygen-induced retinopathy (OIR) on postnatal day 18. We then
coupled this information with blood flow measurements made on the same
imaging system, utilizing the scanning pattern from dual-circle Doppler
OCT. Altogether, the measurements were combined to derive the inner
retinal oxygen delivery (irDO2) and metabolic rate of oxygen (irMRO2).
Measurements were compared between two groups: age-matched
room-air controls and rats with 50/10 OIR. We found a 61% decrease in
the irDO2 in the OIR group, which we attribute to a significant decrease
in retinal vascular density within the superficial and deep retinal vascular
capillary networks. We also measured a 59% decrease in the irMRO2, which
we attributed to decreased neuronal oxygen utilization, and, therefore,
correlated our measurements with a reduced retinal thickness in the OIR
group. Our study provides insight into the disease mechanisms of ROP and
OIR and reveals the potential of vis-OCT as a valuable clinical and research
tool in ophthalmology.
Conference 9694:
Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXV
Saturday - Sunday 13–14 February 2016
Part of Proceedings of SPIE Vol. 9694 Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXV

9694-1, Session 1
Mechanistic studies on a sequential PDT protocol (Invited Paper)
David H. Kessel, Wayne State Univ. School of Medicine (United States)

In prior reports, we demonstrated that a low (~-LD10-20) PDT dose resulting in selective lysosomal photodamage markedly promoted photokilling by subsequent mitochondrial photodamage. An important clue concerning the nature of this effect was discovered when we found that the effect was absent in a cell line depleted of the autophagy-related protein ATG5. It is known that calpain can cleave ATG5 to yield a pro-apoptotic fragment. Release of calcium from photodamaged lysosomes was also demonstrated. Maximum photokilling occurred when lysosomal photodamage came first; otherwise effects were only additive. Since Photofrin targets both mitochondria and lysosomes, we considered the possibility that this agent could be used for either phase of the sequential PDT process. An experimental trial confirmed this supposition. These results, together with an analysis of the role of autophagy in photokilling provide further insights into means for enhancing death pathways after PDT.

9694-2, Session 1
Spatiotemporally synchronized cancer combination therapy using photo-activated nanoparticle drug delivery systems (Invited Paper)
Tayyaba Hasan, Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States)

This talk will introduce a new nanotechnology platform for cancer combination therapy that utilizes near infrared light activation not only for photodynamic damage but also as an extrinsic mechanism to initiate release of complimentary drugs to suppress dynamic bursts in molecular signaling networks that promote tumor cell survival and treatment escape. The goal is to achieve co-delivery with concomitant activity of photodynamic, molecular inhibitor and chemotherapeutic agents, selectively within the tumor. This approach overcomes challenges in achieving synergistic interactions using sequential drug delivery. Conventional drug delivery is compromised by the differential pharmacokinetics of individual agents and potentially antagonistic effects—such as vascular shutdown by one agent that limits delivery of the second. Here, photodynamic damage—which efficiently kills drug-resistant cells via damage of common proteins involved in drug-resistance (such as anti-apoptosis factors and drug-efflux transporters)—is synchronized spatially and temporally with the photoinitiated release of complimentary agents—to enable full interaction amongst the individual therapies. This spatiotemporal synchronization offers new prospects for exploiting time-sensitive synergistic interactions. Specific implementations of these concepts will be presented in preclinical models of cancer. Strategies to enable molecular-targeting of cancer cells via site-specific attachment of targeting moieties to the outer lipid shell of these nanovehicles will also be discussed. If successful in humans, this new paradigm for synchronized, tumor-focused combination therapy will ultimately supersede the present use of chronic drug injection by increasing efficacy per cycle whilst reducing systemic exposure to toxic drugs.

9694-3, Session 1
Designing PDT-based combinations to overcome chemoresistance in heterocellular 3D tumor models
Imran Rizvi, Brigham and Women’s Hospital (United States) and Harvard Medical School (United States); Emma A. Briars, Massachusetts General Hospital (United States) and Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States); Anne-Laure Bulin, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States); Sriram R. Anbil, Wellman Ctr. for Photomedicine (United States) and Howard Hughes Medical Institute (United States); Daniela Vecchio, Ahmed Alkhatteeb, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States); William R. Hanna, Jonathan P. Celli, Univ. of Massachusetts Boston (United States); Tayyaba Hasan, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States)

A major barrier to treating advanced-stage cancers is heterogeneity in the responsiveness of metastatic disease to conventional therapies leading to resistance and treatment failure. Photodynamic therapy (PDT) has been shown to synergize with conventional agents and to overcome the evasion pathways that cause resistance. Developing PDT-based combinations that target resistant tumor populations and cooperate mechanistically with conventional agents is an increasingly promising approach to improve therapeutic efficacy while minimizing toxicity, particularly in complex disease sites. Identifying the molecular, cellular, and microenvironmental cues that lead to heterogeneity and treatment resistance is critical to developing strategies to target unresponsive regions of stubborn disease. Cell-based research platforms that integrate key microenvironmental cues are emerging as increasingly important tools to improve the translational efficiency of new agents, and to design combination regimens. Among the challenges associated with developing and scaling complex cell-based screening platforms is the need to integrate, and balance, biological relevance with appropriate, high-content imaging routines that provide meaningful quantitative readouts of therapeutic response. The benefits and challenges associated with deriving meaningful insights from complex cell-based models will be presented, with a particular emphasis on overcoming chemoresistance mediated by physical stress and communication with stromal partners (e.g. tumor endothelial cells, which are emerging as dynamic regulators of treatment resistance) using PDT-based combinations.

9694-4, Session 2
Defining a path for critical dosimetry measures and surrogate tools that can facilitate clinical success
Brian W. Pogue, Scott C. Davis, Stephen C. Kanick, Thayer School of Engineering at Dartmouth (United States); Edward V. Maytin M.D., Cleveland Clinic Lerner Research Institute (United States); Stephen P. Pereira, Univ. College London Hospitals (United Kingdom); Aikilan Palanisami,
Photodynamic therapy can be a highly complex treatment with more than one parameter to control, or in some cases it is easily implemented with little control other than prescribed drug and light values. The role of measured dosimetry as related to clinical adoption has not been as successful as it could have been, and part of this may be from the conflicting goals of advocating for as many measurements as possible for accurate control, versus companies and clinical adopters advocating for as few measurements as possible, to keep it simple. An organized approach to dosimetry selection is required, which shifts from mechanistic measurements in pre-clinical and early phase I trials, towards just those essential dose limiting measurements and a focus on possible surrogate measures in phase II/III trials. This essential and surrogate approach to dosimetry should help successful adoption of clinical PDT if successful. The examples of essential dosimetry points and surrogate dosimetry tools which might be implemented in phase II and higher trials are discussed for solid tissue PDT with verteporfin and skin lesion treatment with aminolevulinic acid.

A feasibility study of singlet oxygen explicit dosimetry (SOED) of PDT by an intercomparison with a singlet oxygen luminescence dosimetry (SOLD) system

Michele M. Kim, Univ. of Pennsylvania School of Medicine (United States); Rozhin Penjweini, The Univ. of Pennsylvania Health System (United States); Nathan R. Gemmell, Robert H. Hadfield, Univ. of Glasgow (United Kingdom); Israel Veilleux, Univ. of Toronto (Canada) and Princess Margaret Hospital (Canada); Brian C. Wilson, Princess Margaret Hospital (Canada) and Univ. of Toronto (Canada) and Univ. Health Network (Canada); Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

An explicit dosimetry model has been developed to calculate the apparent reacted IO2 concentration ([IO2]rx) in an in-vivo model. In the model, a macroscopic quantity, g, is introduced to account for oxygen perfusion to the medium during PDT. In this study, the SOED model is extended for PDT treatment in phantom conditions where vasculature is not present; the oxygen perfusion is achieved through the air-phantom interface instead. The solution of the SOED model is obtained by solving the coupled photochemical rate equations incorporating oxygen perfusion through the air-liquid interface. Experiments in phantoms were performed for two photosensitizers (PS), e.g., Rose Bengal (RB) and Photofrin, using SOED and SOLD measurements to determine both the instantaneous [IO2] as well as cumulative [IO2]rx concentrations, where [IO2]rx is the time integral of [IO2] divided by singlet oxygen life time. The PS concentrations varied between 10 and 100 μM for RB and between 200 and 400 μM for Photofrin, respectively. The [IO2]rx generation for Photofrin was also examined in optical phantoms with different absorption and scattering coefficients. The result showed that different fluence rates had generated different amount [IO2]rx that can be confirmed by both SOED and SOLD measurements. The resulting magnitude of [IO2] were compared between SOED and SOLD. In addition, we have explored the difference of spatial distribution of [IO2]rx generated by 523 nm and 630 nm light in-vivo for Photofrin based on SOED and optical properties measured for the two wavelengths in-vivo.

Therapeutic enhancement of verteporfin-mediated photodynamic therapy with PI3K signaling pathway targeted therapy

Bin Chen, Univ. of the Sciences in Philadelphia (United States)

Photodynamic therapy (PDT) induces cell injury and death through generation of reactive oxygen species (ROS) after light activation. Verteporfin is a photosensitizer that has been approved for the treatment of age-related macular degeneration and is under investigation for cancer therapy. Being primarily localized in mitochondria, verteporfin-mediated PDT induced a rapid apoptotic cell death in SVEC mouse endothelial cells by activating mitochondria-initiated cell death pathways. However, activation of phosphatidylinositol 3-kinase (PI3K) signaling pathway was also observed after verteporfin-PDT. Activation of PI3K signaling was related to cell regrowth after PDT. Thus, it is hypothesized that therapeutic outcome of verteporfin-PDT can be enhanced by targeting PDT-induced pro-survival PI3K signaling pathway. As the most frequently mutated pathway in human cancers, PI3K signaling pathway plays an important role in cell survival and more than twenty PI3K targeted agents are currently under clinical trials for cancer treatments. In this presentation, we will summarize our recent work on using different PI3K inhibitors to enhance verteporfin-PDT outcomes. Our results indicate that targeting pro-survival PI3K signaling pathway is an effective approach for enhancing tumor response to verteporfin-PDT.

Quantification of pancreas tumor interstitial pressure, matrix components and stiffness, as related to verteporfin perfusion

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Pancreas tumors are known to have high stiffness from extracellular matrix components and a resulting high intratumoral pressure. The origins of the high pressure and the key contributing factors which dominate this are still debated, but one of the known outcomes from this is a hypoperfusion of the tumor interior, and a resulting lack of efficacy for both acute and chronic therapies for pancreas cancer. In this presentation, we will summarize our recent work on using different PI3K inhibitors to enhance verteporfin-PDT outcomes. Our results indicate that targeting pro-survival PI3K signaling pathway is an effective approach for enhancing tumor response to verteporfin-PDT.
Photodynamic tumor cell and microvessel damage with simultaneous inhibition of multiple molecular signaling escape pathways

Bryan Q. Spring, R. Bryan Sears, Lei Z. Zheng, Zhiming Mai, Massachusetts General Hospital (United States); Reika Watanabe, Univ. of California, San Diego (United States); Margaret E. Sherwood, David A. Schoenfeld, Massachusetts General Hospital (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States); Stephen P. Pereira, Univ. College London (United Kingdom); Elizabeth Villa, Univ. of California, San Diego (United States); Tayyaba Hasan, Massachusetts General Hospital (United States)

We introduce photoactivatable multi-inhibitor nanoliposomes (PMILs) for photodynamic tumor cell and microvessel damage in synchrony with photo-initiation of tumor-confined, multikinase inhibitor release. The PMIL is a biodegradable delivery system comprised of a nanoliposome carrying a photoactivatable chromophore (benzoporphyrin derivative monoacid A, BPD) in its bilayer. A multikinase inhibitor-loaded PEG-PLGA nanoparticle is encapsulated within the liposome, which acts a barrier to nanoparticle erosion and drug release. Following intravenous PMIL administration, near infrared irradiation of tumors triggers photodynamic therapy and initiates tumor-confined drug release from the nanoparticle. This talk presents promising preclinical data in mouse models of pancreatic cancer utilizing this concept to suppress the VEGF and MET signaling pathways—both critical to cancer progression, metastasis and treatment escape. A single PMIL treatment using low doses of a multikinase inhibitor (cabozantinib, XL184) achieves sustained tumor reduction and suppresses metastatic escape, whereas combination therapy by co-administration of the individual agents has significantly reduced efficacy. The PMIL concept is amenable to a number of molecular inhibitors and offers new prospects for spatiotemporal synchronization of combination therapies whilst reducing systemic drug exposure and associated toxicities.

Activation of photodynamic therapy in vitro with Cerenkov luminescence generated from Yttrium-90

Brad A. Hartl, Univ. of California, Davis (United States); Henry Hirschberg, Univ. of California, Irvine (United States); Laura Marcu, Simon R. Cherry, Univ. of California, Davis (United States)

Translation of photodynamic therapy to the clinical setting has primarily been limited to easily accessible and/or superficial diseases where traditional light delivery can be performed noninvasively. Cerenkov luminescence, as generated from medically relevant radionuclides, has been suggested as a means to deliver light to deeper tissues noninvasively in order to overcome this depth limitation. We report on the use of Cerenkov luminescence generated from Yttrium-90 as a means to active the photodynamic therapy process in monolayer tumor cell cultures. The current study investigates the utility of Cerenkov luminescence for activating both the clinically relevant aminolevulinic acid at 1.0 mM and also the more efficient photosensitizer TPPS2a at 1.2 µM. Cells were incubated with aminolevulinic acid for 6 hours prior to radionuclide addition, as well as additional daily treatments for three days. TPPS2a was delivered as a single treatment with an 18 hour incubation time before radionuclide addition. Experiments were completed for both C6 glioma cells and MDA-MB-231 breast tumor cells. Although aminolevulinic acid proved ineffective for generating a therapeutic effect at any activity for either cell line, TPPS2a produced at least a 20% therapeutic effect at activities ranging from 6 to 60 µCi/well for the C6 cell line. Current results demonstrate that it may be possible to generate a therapeutic effect in vivo using Cerenkov luminescence to activate the photodynamic therapy process with clinically relevant photosensitizers.
investigate the complex dependency of treatment efficacy on dose rate and fractionation schedule, which further help to inform the power and duty cycle specifications of the device. To leverage PpIX tumor imaging under the same cost and resource constraints we have tested a simple smartphone-based imaging fluorescence device that successfully shows tumor contrast and quantitative PpIX imaging. This is integrated with a phone App we developed to assist users in PDT dosimetry calculations as we move towards a simple-to-use smartphone-controlled PDT device that incorporates quantitative imaging of lesion size and photosensitizer photobleaching for treatment monitoring.

9694-12, Session 3

Determination of the low concentration correction in the macroscopic singlet oxygen model for PDT

Michele M. Kim, Univ. of Pennsylvania School of Medicine (United States); Rozhin Penjweini, Perelman Ctr. for Advanced Medicine (United States); Jarod C. Finlay, Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

The macroscopic singlet oxygen model has been used for singlet oxygen explicit dosimetry in photodynamic therapy (PDT). The photophysical parameters for commonly used sensitizers, HPPH, BPD, and Photofrin, have been investigated in pre-clinical studies using mouse models. So far, studies have involved optimizing fitting algorithms to obtain the some of the photophysical parameters (? , ? , g) and the threshold singlet oxygen dose ([(102)x,sh]), while other parameters such as the low concentration correction, ?, has been kept as a constant. In this study, using photobleaching measurements of mice in vivo, the value of ? was also optimized and fit to better describe experimental data. Furthermore, the value of the specific photobleaching ratio (7?) was also fine-tuned using the photobleaching results. Based on literature values of ?, ? for photosensitizers can be uniquely determined using the additional photobleaching measurements. This routine will further improve the macroscopic model of singlet oxygen production for use in explicit dosimetry.

9694-14, Session 4

Combination strategy in photodynamic therapy of skin diseases

Xia Lei M.D., Jinjin Wu, Third Military Medical Univ. (China); Zheng Huang, Fujian Normal Univ. (China) and Univ. of Colorado Denver (United States)

In recent years, topical photodynamic therapy (PDT) has been widely used in dermatology and beyond oncological indications. Our clinical study suggests that the combination of PDT with other therapeutic modalities could further improve clinical outcomes in the treatment of several common skin disorders. This presentation will demonstrate our experience in: (i) treating skin cancers using surgery combined with ALA-PDT, (ii) treating flat wart, condyloma acuminata, and refractory planar wart using cryotherapy combined with ALA-PDT, (iii) treating severe acne in combination with clearing procedure and ALA-PDT, and (iv) treating the rosacea using fractional laser combined with ALA-PDT. The optimization of combination treatment protocols and outcomes will be discussed.

9694-15, Session 4

Fluorescence image-guided photothermal therapy of human oesophageal adenocarcinoma in vivo using multifunctional gold nanorods in vivo

Eli Nabavi, Mohan Singh, Yu Zhou, Maria Elena Gallina, Hailin Zhao, Daqing Ma, Anthony Cass, George Hanna, Daniel S. Elson, Imperial College London (United Kingdom)

One of the issues in current hyperthermal techniques used for cancer therapy is the low spatial selectivity which may harm the surrounding healthy tissues [1]. In this study gold nanorods (GNRs) functionalised with Cy5.5 and EGFR were used to locate the tumour and the strong surface plasmon resonance (SPR) properties provided the local heat necessary for thermal ablation of cancer cells in vivo. GNRs were fabricated using seed mediated methods with the CTAB layers being replaced by polyethylene glycol (PEG) via a PEGylation process. A UV-visible spectrometer revealed the presence of a deep absorption peak at ~800nm.

After establishing tumour xenografts in mice by subcutaneous inoculation of human oesophageal adenocarcinoma cells (FLO-1), functionalized GNRs were administered either intratumourally (IT) or intravenously (IV). Fluorescence spectroscopy and imaging were performed to localise and monitor the tumour area using the variation in intensity of the fluorescence signal. PTT was then performed using radiation from a 808nm continuous wave diode laser which irradiated the tumour for 3 minutes. This established a significant increase in temperature (17oC - 34oC depending on the optical density of the GNRs) in both IV and IT groups monitored with a thermal camera. Histological examination of tumour sites showed fully preserved tissue layers with no signs of proliferating tumour. Initial ICP-MS results revealed no evidence of toxic accumulation of gold in blood, kidneys, liver or spleen after 28 days.

References


9694-16, Session 4

Detection techniques for singlet oxygen production during photodynamic therapy

Buhong Li, Fujian Normal Univ. (China)

Singlet oxygen is widely considered to be the major cytotoxic reactive oxygen species (ROS) generated during photodynamic therapy (PDT). This talk summarizes recent advances and future perspectives in detection techniques for singlet oxygen production, and the advantages and limitations of each technique will be presented. In addition, our custom developed novel configuration of a near-infrared sensitive camera and adaptive optics for in vivo fast imaging of singlet oxygen luminescence around 1270 nm will be highlighted. For clinical PDT application, the challenges for direct measurement of singlet oxygen luminescence will be discussed.

9694-17, Session 4

Home-use cancer detecting plaster

Zeev Zalevsky, Arkady Rudnitsky, Victor Sheiman, Bar-Ilan Univ. (Israel); Andrey Tzoy, Aitmamat Toktosunov, Arkady Adashov, Kyrgyz State Medical Academy (Kyrgyzstan)

In this paper we present a novel concept in which special plaster is...
developed for early detection of cancer. The plaster contains an array of micro needles with small detection array connected to each needle which inspects the color of the surface of the skin versus time after being pinched with the needles. We were able to show in pre-clinical trials that the color varies differently if the skin is close to tumor tissue. Such a device can be affordable for the end user and can make our life significantly better as it may significantly reduce the probability of missing malignant tissues or discovering malignant tissue in its “too late” progress stage. In addition to the home use of the proposed plaster, it can also be used by medical surgeons in the stage they are preparing the patient for the medical operation and need to properly identify the exact borders of the tumor, which is to be removed.

9694-18, Session 4

Combination photodynamic therapy using 5-fluorouracil and aminolevulinate enhances tumor-selective production of protoporphyrin IX and improves treatment efficacy of squamous skin cancers (Invited Paper)

Edward V. Maytin M.D., Sanjay Anand, Cleveland Clinic Lerner Research Institute (United States)

In combination photodynamic therapy (cPDT), a small-molecule drug is used to modulate the physiological state of tumor cells prior to giving aminolevulinate (ALA; a precursor for protoporphyrin IX, PpIX). In our laboratory we have identified three agents (methotrexate, 5-fluorouracil, and vitamin D) that can enhance therapeutic effectiveness of ALA-based photodynamic therapy for cutaneous squamous cell carcinoma (SCC). However, only one (5-fluorouracil; 5-FU) is FDA-approved for skin cancer management. Here, we describe animal and human studies on 5-FU mechanisms of action, in terms of how 5-FU pretreatment leads to enhanced PpIX accumulation and improves selectivity of ALA-PDT treatment. In A431 subcutaneous tumors in mice, 5-FU changed expression of heme enzyme (strongly downregulating ferrochelatase), inhibited tumor cell proliferation (Ki67), enhanced differentiation (E-cadherin), and led to strong selective increases in apoptosis. Interestingly, enhancement of apoptosis by 5-FU did not involve changes in caspase activation, but instead depended upon selectively increased accumulation of p53 that persisted for 24 h post PDT. In a clinical study, human subjects with actinic keratoses (AK; preneoplastic precursors of SCC) were pretreated on one side of the face or scalp with 5-FU cream for 6 days; the control side was left untreated. On Day 7, one 5-FU pretreated lesion and one control lesion were biopsied. All remaining lesions were treated with ALA-PDT. Histology of lesions revealed 5-FU selective elevations in PpIX, E-cadherin, and p53. The overall clinical response to ALA-PDT was improved ~2-fold. In summary, 5-FU is a useful clinical adjuvant to ALA-PDT for squamous neoplasia of the skin.

9694-19, Session V

Vitamin D for combination photodynamic therapy of skin cancer in individuals with vitamin D deficiency: Insights from a preclinical study in a mouse model of squamous cell carcinoma

Sanjay Anand, Cleveland Clinic Lerner Research Institute (United States); Erik Thomas, The Cleveland Clinic (United States); Tayyaba Hasan, Harvard Medical School (United States); Edward V. Maytin M.D., Cleveland Clinic Lerner Research Institute (United States)

Combination photodynamic therapy (cPDT) in which vitamin D (VD) is given prior to aminolevulinate, a precursor (pro-drug) for protoporphyrin IX (PpIX), is an approach developed in our laboratory. We previously showed that 1,25-dihydroxyvitamin D3 (calcitriol), given prior to PDT, enhances accumulation of PpIX and improves cell death post-PDT in a mouse skin cancer model. However, since calcitriol poses a risk for hypercalcemia, we replaced systemic calcitriol with oral cholecalciferol (D3), administered as a high (tenfold, “10K”) diet over a ten-day period. Here, we ask whether VD deficiency might alter the response to cPDT. Nude mice were fed a VD-deficient diet for at least 4 weeks (“deficient”); controls were fed a normal 1,000 IU/kg diet (“1K”). Human A431 cells were implanted subcutaneous and mice were switched to the 10K diet or continued on their baseline diets (controls). In other experiments, mice received a human equivalent dose of 50,000 IU D3 by oral gavage, to simulate administration of a single, high-dose VD pill. At various times, tumors were harvested and serum was collected to measure levels of VD metabolic intermediates. A significant increase in PpIX levels and in the expression of differentiation and proliferation markers in tumor tissue was observed after VD supplementation of both the deficient and 1K mice. Further results describing mechanistic details of PpIX enhancement through alteration of heme- and VD-metabolic enzyme levels will be presented. Based on these results, a clinical study using oral vitamin D prior to PDT for human skin cancer should be performed.

9694-13, Session V

Photodynamic therapy for targeting extracellular biophysical regulators of tumor growth and invasive behavior in pancreatic cancer

Gwendolyn M. Cramer, Hamid El Hamidi, Dustin P. Jones, Ljubica Petrovic, Univ. of Massachusetts Boston (United States); Imran Rizvi, Brigham and Women’s Hospital (United States); Tayyaba Hasan, Massachusetts General Hospital (United States); Jonathan P. Celli, Univ. of Massachusetts Boston (United States)

A number of studies show that the mechanical properties (rheology) of the tumor microenvironment play a significant role in regulating growth, invasion and therapeutic response. This observation is particularly relevant to pancreatic tumors associated with an exceptional abundance of rigid, fibrotic stroma, which, in addition to obstructing drug delivery, plays multiple complex tumor-promoting roles. Here we specifically examine the consequences of biophysical signals from stromal mechanical composition on response to photodynamic therapy (PDT), and conversely, the potential of PDT to target these interactions. Using imaging and 3D tumor models with varying extracellular composition we are able to evaluate the impact of extracellular matrix (ECM) rheology on tumor growth, while in situ particle tracking microrheology provides concomitant longitudinal measurements of local mechanical changes associated with phenotypic alteration and therapeutic intervention. PTM allows us to resolve changes in matrix stiffness associated with fibrosis (increasing mechanical strength) as well as invasive behavior (local breakdown of ECM). We use this approach to show altered matrix remodeling in chemotherapy-resistant tumor sub-populations that exhibit elevated sensitivity to verteporfin PDT relative to the parent cell line. At the same time we examine the impact of PDT on the stromal compartment, in consideration of the potential role of PDT as a modality for stromal depletion. More broadly, the methods integrated here comprise a research platform for screening PDT and chemotherapy strategies targeting biophysical tumor-stroma interactions in pancreatic cancer and other solid tumors.
Modeling of the impact of initial oxygen concentration and blood flow variation on photodynamic therapy

Rozhin Penjweini, Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

Type II photodynamic therapy (PDT) is used for cancer treatment based on the combined action of a photosensitizer, a special wavelength of light and singlet oxygen (1O2) generation. Intra-patient and inter-patient variability of initial oxygen concentration ([3O2][0]), light fluence rate (?), 1O2 concentration and hemodynamic parameters such as blood flow and tissue oxygenation during PDT has been reported. Simulation of these variations is valuable, as would be a means for the rapid assessment of treatment effect. A mathematical model has been previously developed to incorporate the macroscopic kinetic equations for 1O2 generation, photosensitizers in ground and triplet states, 3O2, and tissue acceptors along with the diffusion equation for the light transport in tissue. In this study, several improvements of the model have been made to consider the effects of [3O2][0], and blood flow changes during PDT, on the magnitude of [1O2] and [3O2] in both tumor and normal tissues. For the modeling of [3O2] and [1O2], the forward calculation of the macroscopic kinetic equations is done in COMSOL Multiphysics. The finite-element calculation is implemented within COMSOL by varying the input parameters, such as [3O2]? and ? based on our mice studies. The blood flow variation is considered by making g to be ? dependent. The results show that [3O2]? has no effect on the long term 1O2 generation. In the well-oxygenated tissue, the exact [3O2] has little influence on treatment efficacy. When [3O2] becomes limiting, small changes in ? or [3O2]0 have large effects.

Adapting biomodulatory approaches to enhance photodynamic therapy outcomes in new contexts: pancreatic and oral cancers

Sriram R. Anbil, Harvard Medical School (United States) and Howard Hughes Medical Institute (United States) and The Univ. of Texas Health Science Ctr. at San Antonio (United States); Imran Rizvi, Brigham and Women’s Hospital (United States) and Harvard Medical School (United States); Amjad Rizvi, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Jonathan P. Khan, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Edward V. Maytin M.D., Cleveland Clinic Lerner Research Institute (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States) and Harvard Medical School (United States)

Biomodulation of cancer cell metabolism represents a promising approach to overcome tumor heterogeneity and poor selectivity, which contribute significantly to treatment resistance. To date, several studies have demonstrated that modulation of cell metabolism including the heme synthesis pathway serves as an elegant approach to improve the efficacy of aminolevulinic acid (ALA) based photodynamic therapy (PDT). However, the ability of biomodulation-enhanced PDT to improve outcomes in low resource settings and to address challenges in treating lethal tumors with exogenous photosensitizers remains underexplored. The ability of vitamin D or methotrexate to enhance PDT efficacy in a carcinogen-induced hamster cheek pouch model of oral squamous cell carcinoma and in 3D cell-based models for pancreatic ductal adenocarcinoma is evaluated. Challenges associated with adapting PDT regimens to low resource settings, understanding the effects of biomodulatory agents on the metabolism of cancer cells, and the differential effects of biomodulatory agents on tumor and stromal cells will be discussed.

Red and blue-excited wide-field fluorescence imaging to distinguish subsurface PpIX during photodynamic therapy of the skin

Ethan LaRochelle, Hayden H. Chun, Thayer School of Engineering at Dartmouth (United States); Tayyaba Hasan, Wellman Ctr. for Photomedicine (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States); Edward V. Maytin M.D., Cleveland Clinic Lerner Research Institute (United States); Michael S. Chapman M.D., Dartmouth Hitchcock Medical Ctr. (United States); Scott C. Davis, Thayer School of Engineering at Dartmouth (United States)

Actinic Kertoses (AK) are common pre-cancerous lesions associated with sun-damaged skin. While generally benign, the condition can progress to squamous cell carcinoma (SCC) and is a particular concern for immunosuppressed patients who are susceptible to uncontrolled AK and SCC. Among the FDA-approved treatment options for AK, ALA-based photodynamic therapy is unique in that it is non-scarring and can be repeated on the same area. However, response rates vary widely due to variations in drug and light delivery, PpIX production, and tissue oxygenation. Thus, developing modalities to predict response is critical to enable patient-specific treatment-enhancing interventions. To that end, we have developed a wide-field spectrally-resolved fluorescence imaging system capable of red and blue light excitation. While blue light excites PpIX efficiently, poor photon penetration limits the image content to superficial layers of skin. Red light excitation, on the other hand, can reveal fluorescence information originating from deeper in tissue, which may provide relevant information about PpIX distribution. Our instrument illuminates the skin via a fiber-based ring illuminator, into which is coupled sequentially a white light source, and blue and red laser diodes. Light emitted from the tissue passes through a high-speed filter wheel with filters selected to resolve the PpIX emission spectrum. This configuration enables the use of spectral fitting to decouple PpIX fluorescence from background signal, improving sensitivity to low concentrations of PpIX. Images of tissue-simulating phantoms and animal models confirm a linear response to PpIX, and the ability to image sub-surface PpIX inaccessible with blue light using red excitation.

Molecular targeted PDT with selective delivery of ICG Photo-Immunoconjugates

Sijia Wang, Gereon Hüttmann, Univ. zu Lübeck (Germany); Tayyaba Hasan, Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States) and Harvard Medical School (United States); Ramtin Ramhazadeh, Univ. zu Lübeck (Germany)

Light-induced inhibition of intracellular molecules holds great promise for a selective treatment of cancer and other diseases. Challenges for the targeting of intracellular proteins are the synthesis of effective photoimmuno-conjugates and their functional delivery inside living cells. In earlier studies we have shown, that photodynamic inactivation of the nuclear Ki-67 protein leads to an effective elimination of proliferating tumor cells. Here we show a selective treatment for EGFR and Ki-67 positive cancer cells after light-controlled delivery of indocyanine green (ICG) photo-immunoconjugates. The Ki-67 antibody TuBB-9, which recognizes...
an active state of the protein, was labeled with different ratios of ICG and encapsulated into immuno-liposomes that selectively deliver the conjugates to EGFR overexpressing cells. To overcome endosomal entrapment of the delivered agents, ovarian carcinoma cells were treated with the photosensitizer benzoporphyrin monoacid derivative (BPD) and irradiated first for endosomal escape of the TuBB-9-ICG constructs. 24 h after irradiation TuBB-9-ICG antibodies showed a relocation from spots in the cytoplasm to the cell nucleus. A second irradiation of the delivered TuBB-9-ICG led to a significant elimination of cells after Ki-67 inactivation.

9694-24, Session 6
Reducing background noise in near-infrared medical imaging: Routes to activated fluorescing
Mary K. Burdette, Yuriy P. Bandera, Stephen H. Foulger, Clemson Univ. (United States)

The majority of organic near-infrared emitters utilized in diagnostic and therapeutic imaging exhibit low quantum yields when optically excited. In an effort to reduce the background noise and to localize the emission, it is advantageous to “activate” the emission only in regions of interest. To this end, a colloidal nanoparticle platform was developed that can facilitate the transfer of quenched NIR fluorophores to the interior of cells and only within the cell is the emission permitted. This platform is demonstrated with sub-100 nm poly(propargyl acrylate) (PA) nanoparticles that have been surface modified with an azide modified bovine serum albumin (azBSA). The azBSA was covalently attached to the particles through a copper catalyzed azide alkyne Huisgen cycloaddition (i.e. “click” transformation). To the azBSA, alkyne modified silicon phthalocyanine (akSiPc) fluorophores were attached through a click reaction. As a free molecule in phosphate-buffered saline (PBS), the akSiPc exhibited a 780 nm emission with a 630 nm excitation, though once attached to the PA particles via the azBSA, the emission was quenched. The emission of the akSiPc could be recovered with the incubation of the PA/BSA/akSiPc particles with trypsin, a protease which cleaves peptide chains. The trypsin digested the azBSA and “freed” the quenched akSiPc. The PA/BSA/akSiPc particles were incubated with human non-small cell lung cancer cells (A549) and live cell scanning confocal microscopy at varying time points was employed to study the particles uptake into the cells and subsequent emission activation. It was speculated that particles localized in the lysosomes of the cells where the azBSA was digested, releasing the fluorophores.

9694-25, Session 6
Efficiency of photodynamic therapy using indocyanine green and infrared light on MCF-7 breast cancer cells in vitro
Mustafa K. Ruhi, Bogazici Univ. (Turkey); Ayse Ak, Erzincan Univ. (Turkey); Murat Gulsoy, Bogazici Univ. (Turkey)

Cancer is one of the main reasons of death in all around the world and breast cancer is the most frequent cancer type among women. The conventional treatment of cancer includes surgical intervention, radiation therapy and chemotherapy. Another therapeutic method, which has been studied and recently being used in clinical applications is Photodynamic Therapy (PDT). Most photosensitizers currently being investigated are sensitive to red light. However, it is known that infrared light has a better penetration into the skin or soft tissue i.e. breast tissue. Indocyanine Green (ICG), which is used in this study, is the unique photosensitizer that is sensitive to infrared light.

A wide literature review showed that there is no comprehensive PDT research on breast cancer based on using infrared light and ICG together. In our study, ICG and infrared light are used in order to investigate the PDT effect on breast cancer. Preliminary dosimeter experiments of varying ICG doses between 10 and 500 ?M were performed on MCF-7 breast cancer cells in vitro. In addition to ICG dose examination studies, cancer cells were illuminated by means of 809 nm diode laser with varying energy densities of 25, 50 and 100 J/cm2. MTT tests were performed 24, 48 and 72 hours following ICG dose determination and laser irradiation experiments to find out the cytotoxic and ineffective doses.

After determining the convenient drug and light doses, ICG concentrations from 25 to 100 ?M and laser irradiations of 25 J/cm2 and 50 J/cm2 were applied together on all cancer cells. Following the drug administrations and laser irradiations cell viability and cell death tests were performed at days mentioned previously. All experiments were repeated at least for three times. Results of preliminary studies show that ICG administration combined with 809 nm laser irradiation at certain dose and energy densities may have important cytotoxic effects on MCF-7 breast cancer cells in vitro.

9694-26, Session 7
Two-photon photodynamic properties of TBO-AuNR-in-shell nanoparticles
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Photodynamic therapy (PDT) is a light-activated chemotherapeutic treatment that utilizes singlet oxygen and reactive oxygen species induced oxidative reactions to react with surrounding biological substrates, which either kills or irreversibly damages malignant cells. We used multiphoton nonlinear optical microscopy to observe the photodynamic effects of TBO-AuNR-in-shell NPs. Excited by femtosecond Cr:forsterite laser operating at 1230nm, singlet oxygen were generated through a plasmon-enhanced two-photon nonlinear optical process. For cells took up NPs, this photodynamic effect can kill the cell. From nonlinear optical microscopy images, we found they shrunk after 3 minutes of illumination.

9694-27, Session 7
Dosimetry study of PHOTOFRIN-mediated photodynamic therapy in a mouse tumor model
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Although the clinical applications of photodynamic therapy (PDT) are expending, there are still unpredictable relationship and variability between PDT dose and therapeutic outcomes. An explicit dosimetry model using apparent reacted 1O2 concentration ([1O2]rx) has been developed as the PDT dosimetry tool. In this study, this explicit PDT dosimetry model was adopted to establish the correlation between calculated reacted [1O2]rx, total light fluence and tumor growth inhibition in Photofrin-mediated PDT in a mouse tumor model. Mice with radiation-induced fibrosarcoma (RIF) tumors were injected with Photofrin at a dose of 5 mg/kg, PDT was carried out 24h later with different fluence rates (50, 75 and 150 mW/cm2) and different fluence (50, 135 and 250 J/cm2) using a collimated light applicator coupled to a 630nm laser. The amounts of [1O2]rx was calculated using the PDT dosimetry model incorporating Photofrin concentration as
Site-specific antibody-liposome conjugation through copper-free click chemistry: a molecular biology approach for targeted photodynamic therapy

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Nanocarriers, such as liposomes, have the ability to potentiate photodynamic therapy (PDT) treatment regimens by the encapsulation of high payloads of photosensitizers and enhance their passive delivery to tumors through the enhanced permeability and retention effect. By conjugating targeting moieties to the surface of the liposomal nanoconstructs, cellular selectivity is imparted on them and PDT-based therapies can be performed with significantly higher dose tolerances, as off-target toxicity is simultaneously reduced. However, the maximal benefits of conventional targeted nanocarriers, including liposomes, are hindered by practical limitations including chemical instability, non-selective conjugation chemistry, poor control over ligand orientation, and loss of ligand functionality following conjugation, amongst others.

We have developed a robust, physically and chemically stable liposomal nanoplatform containing benzoporphyrin derivative photosensitizer molecules within the phospholipid bilayer and an optimized surface density of strained cyclooctyne moieties for ‘click’ conjugation to azido-functionalized antibodies. The clinical chimeric anti-EGFR antibody Cetuximab is site-specifically photocrosslinked to a recombinant bioengineered that recognizes the antibody’s Fc region, containing a terminal azide. The copper-free click conjugation of the bioengineered Cetuximab derivative to the optimized photosensitizing liposome provides exceptional control over the antibody’s optimal orientation for cellular antigen binding. Importantly, the reaction occurs rapidly under physiological conditions, bioorthogonally (selectively in the presence of other biomolecules) and without the need for toxic copper catalysts. Such state-of-the-art conjugation strategies push the boundaries of targeted photodynamic therapy beyond the limitations of traditional chemical coupling techniques to produce more robust and effective targeted therapeutics with applications beyond conventional treatments.

First prospective study assessing the combination of photodynamic therapy and proton radiation therapy: safety, outcomes, and potential synergy when treating malignant pleural mesothelioma

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Background: Photodynamic therapy (PDT) is an effective intraoperative therapy for mesothelioma, but external beam radiotherapy is limited by risk of fatal pneumonitis when treating large pleural volumes. Proton therapy (PT) reduces irradiation to normal tissues compared with photon therapy, which may reduce toxicities. PT may be more safely delivered to pleural surfaces and combined with surgery/PDT. Across malignancies, there are no clinical data combining PDT and PT. We assess toxicities and outcomes of our prospective cohort receiving both modalities.

Methods: All consecutive patients with mesothelioma treated with PDT from 2011-2015 and enrolled on a prospective IRB-approved PT protocol were included. PDT photosensitizer was porfimer sodium (2mg/kg;24hr drug-light interval) or 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH) (4mg/m2;48hr). Local control (no progression in the PT portal) and survival were measured from PT completion.

Results: Ten patients were included. Patients were predominantly male (80%). All patients were Caucasian with epithelioid histology and stage III-IV disease. PT was delivered to a median dose of 56.7CGE/1.8-2.0CGE adjutantly following lung-sparing radical pleurectomy/PDT (n=8) or as salvage therapy after surgery/PDT (n=2) at a median of 7.5 months after surgery/PDT and 9.9 months after mesothelioma diagnosis. Local control was 100% at 1-year and 2-years. Median survival from PT end was 19.0 months, and 1-year and 2-year survival rates were 58% and 29%. No patient experienced CTCAE4 grade ≥3 acute or late toxicity.

Conclusion: This is the first report combining PDT and PT and shows
the sequential combination is well tolerated. Our median survival of >30 months from diagnosis in stage III-IV patients compares favorably to our mesothelioma institutional survival for PT without PDT and for photon therapy with PDT, suggesting the possibility of spatial or systemic cooperativity.

9694-30, Session 8

Mechanistic exploration of a bi-directional photochemotherapeutic combination for pancreatic cancer

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It is increasingly evident that the most effective cancer treatments will involve interactive regimens that target multiple non-overlapping pathways, preferably such that each component enhances the others to improve outcomes while minimizing systemic toxicities. Toward this goal, we developed a combination of photodynamic therapy and irinotecan, which mechanistically cooperate with each other, beyond their individual tumor destruction pathways, to cause synergistic reduction in orthotopic pancreatic tumor burden. A three-way mechanistic basis of the observed synergism will be discussed: (i) PDT downregulates drug efflux transporters to increase intracellular irinotecan levels. (ii) Irinotecan reduces the expression of hypoxia-induced marker, which is upregulated by PDT. (iii) PDT downregulates irinotecan-induced survivin expression to amplify the apoptotic and anti-proliferative effects. The clinical translation potential of the combination will also be highlighted.

9694-31, Session 8

Cherenkov radiation fluence estimates in tissue for molecular imaging and therapy applications

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Cherenkov radiation has recently emerged as an interesting phenomenon for a number of applications in the biomedical sciences. Its unique properties, including broadband emission spectrum, spectral weight in the ultraviolet and blue wavebands, and local generation of light within a given tissue, have made it an attractive new source of light within tissue for molecular imaging and phototherapy applications. While several studies have investigated the total Cherenkov light yield from radionuclides in units of [photons/decay], further consideration of the light propagation in tissue is necessary to fully consider the utility of this signal in vivo. Therefore, to help further guide the development of this novel field, quantitative estimates of the light fluence rate of Cherenkov radiation from both radionuclides and radiotherapy beams in a biological tissue are presented for the first time. Using Monte Carlo simulations, these values were found to be on the order of 0.01 – 1 nW/cm² per MBq/g for radionuclides, and 1 – 100 uW/cm² per Gy/sec for external radiotherapy beams, dependent on the given waveband, optical properties, and radiation source. For phototherapy applications, the total light fluence was found to be on the order of nJ/cm² for radionuclides and mJ/cm² for radiotherapy beams. The results indicate that diagnostic potential is possible for Cherenkov radiation excitation of molecular probes, but phototherapy may remain elusive at such exceedingly low fluence values without additional photochemistry.

9694-32, Session 8

PDT dose dosimeter for pleural photodynamic therapy

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PDT dose is the product of the photosensitizer concentration and the light fluence in the target tissue. For improved dosimetry during plural photodynamic therapy (PDT), a PDT dose dosimeter was developed to measure both the light fluence and the photosensitizer concentration simultaneously in the same treatment location. Light fluence and spectral data were rigorously compared to other methods of measurement (e.g. photodiode, multi-fiber spectroscopy contact probe) to assess the accuracy of the measurements as well as their uncertainty. Photonsensitizer concentration was obtained by measuring the fluorescence of the sensitizer excited by the treatment light. Fluence rate based on the intensity of the laser spectrum was compared to the data obtained by direct measurement of fluence rate by a fiber-coupled photodiode. Phantom studies were done to obtain an optical property correction for the fluorescence signal. Measurements were performed in patients treated with HPPH and Photofrin for different locations in the pleural cavity. Multiple sites were measured to investigate the heterogeneity of the cavity and to provide cross-validation via relative dosimetry. Furthermore, tissue optical properties were determined using a spatially-resolved absorption spectroscopy measurement both before and after PDT at the same sites of the PDT dosimeter detectors. This novel method will allow for accurate real-time determination of delivered PDT dose and improved PDT dosimetry.

9694-33, Session 8

Analysis of superficial fluorescence patterns in nonmelanoma skin cancer during photodynamic therapy by a dosimetric tool

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Photodynamic Therapy (PDT) has become an increasingly used therapeutic technique against nonmelanoma skin cancer and its precursors. However the development of a customized dosimetry still requires optimizing the patient response to therapy. For this purpose, non-invasive treatment monitoring is one of the key elements to assess the photosensitizer activation during the photochemical process underlying PDT by its fluorescence. Hence dosimetric tools that consider all these aspects are crucial to prevent tumor recurrence or incomplete outcomes derived from an inadequate treatment administration.

In this work the superficial fluorescence patterns in three different types of nonmelanoma skin cancer tumors and their photodynamic treatment response are analysed by a fluorescence based dosimetric model for PDT with topical Methyl Aminolevulinate. Results show differences of even more than 50% in the fluorescence patterns as the treatment progresses depending on the malignant tissue type. They demonstrate the great relevance of the biological media as an additional dosimetric factor within the photodynamic context. These results contribute to the development of a future customized therapy with the assistance of dosimetric tools to interpret the fluorescence images obtained during the treatment monitoring as well as in differential photodiagnosis.
Optical spectroscopy of radiotherapy and photodynamic therapy responses in normal rat skin shows vascular breakdown products

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Photodynamic therapy (PDT) and radiotherapy are non-systemic cancer treatment options with different mechanisms of damage. So combining these techniques has been shown to have some synergy, and can mitigate their limitations such as low PDT light penetration or radiotherapy side effects (1,2). The present study monitored the induced tissue changes after PDT, radiotherapy, and a combination protocol in normal rat skin, using an optical spectroscopy system to track the observed biophysical changes. The Wistar rats were treated with one of the protocols: PDT followed by radiotherapy, PDT, radiotherapy and radiotherapy followed by PDT (3). Fluorescence and reflectance spectra and white light images were collected in order to observe the effects of these combined therapies, especially targeting vascular response. From the reflectance, information about oxygen saturation, met-hemoglobin and bilirubin concentration, blood volume fraction (BVF) and vessel radius were extracted from model fitting of the spectra. The rats were monitored for 6 days after treatment. Results showed that there was no significant variation in the vessel size or BVF after the treatments. However, the PDT caused a significant increase in the met-hemoglobin and bilirubin concentrations, indicating an important blood breakdown. These results may provide an important clue on how the damage establishment takes place, helping to understand the effect of the combination of those techniques in order to verify the existence of a known synergistic effect.

Fluence rate effects in intrathoracic PDT of murine tumors

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Photodynamic therapy (PDT) is being evaluated in the intraoperative setting for the treatment of cancers that grow on serosal surfaces, such as the pleura of lungs. PDT of large areas is associated with inhomogeneity in light distribution due to irregularity of the tissue surface and technical limitations in light delivery. This leads to heterogeneities in the fluence rate that is incident on the treated tissue, yet fluence rate can affect many biological aspects of PDT response. Using an orthotopic murine model, we studied fluence rate effects during PDT of the thoracic cavity. Specifically, the activation of survival signaling through the epidermal growth factor receptor was evaluated in nodules of PDT-treated thoracic disease. Studies were performed in disseminated non-small cell lung cancer, grown from H460 cells transfected with luciferase and red fluorescent protein. PDT with the photosensitizer HPPH and illumination to 50 J/cm² was delivered to the murine thoracic cavity using a cylindrical diffusing fiber at an incident fluence rate of 75 or 150 mW/cm². Nodules of disease with an incomplete PDT response were identified by bioluminescence imaging and excised for immunohistochemical analysis of EGFR activation. Proliferation (labeled by Ki67) was visualized in all treated and control nodules. Expression of total EGFR was also similar among the treated and control groups. However, illumination at 150 mW/cm² increased EGFR activation in proliferating cells, a trend that was not detected in mice treated at the lower fluence rate. Fluence rate-dependencies in activation of EGFR signaling could be significant to PDT response and will be further considered in our ongoing investigations that combine molecular targeting with PDT.

Synthesis and characterization of novel phthalocyanines and evaluation of photodynamic therapy properties

Aysun Korkmaz, Yusuf Yılmaz, Mehmet Kahraman, Gaziantep Univ. (Turkey)

Photodynamic therapy (PDT) is promising technique for an effective and minimally invasive treatment of several diseases, including some forms of cancers. However, there are several drawbacks of the approved photosensitizers (PS), such as insufficient light absorption at therapeutically relevant wavelengths and hampered the clinical effectiveness of PDT. However, phthalocyanines (Pc) are interesting PS-candidates due to the strong light absorption in the red spectral region and a high quantum yield of singlet oxygen for the cancer cell treatment. Thus, phthalocyanine compounds and their derivatives having high wavelength (near-IR) absorption, high triplet quantum yields, triplet state lifetime of singlet oxygen allow us to use PDT applications effectively. In this study, phthalocyanine compounds are synthesized and evaluated photophysical and photochemical properties for the possible application of PDT. Zinc is used as central atom for the Pc to obtain higher singlet oxygen production. The structures of the synthesized phthalocyanine are characterized by IR, UV-vis, 1H NMR, elemental analysis and MS. The results demonstrate that the synthesized Pc is a good candidate for the PDT applications for the cancers. The synthesized Pc will be also bound covalently to the nano surface via – SH functional group that can contribute to the production of singlet oxygen amount carrying phthalocyanines having diamagnetic metal.

Synthesis and characterization of novel phthalocyanines for optimization of photodynamic inactivation

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The dissemination of resistant bacterial strains is one of the most worrying threats to public health due to the excessive use of antibiotics. Photodynamic inactivation (PDI) is considered as a potential alternative for resistant infections which is a therapeutic modality that combines the use of light in the correct wavelength, molecular oxygen (O2) and photosensitive compounds to cause death to target cells. The mixture of curcuminoids (curcumin, demethoxycurcumin and bis-demethoxycurcumin) is a natural yellow phenolic compound, extracted from turmeric. Curcuma longa Linn, exhibiting several pharmacological actions and can be used as...
Acanthamoeba infections. This study evaluated the in vitro effectiveness of PDI in A. polyphaga using curcuminoids as photosensitizer (PS) besides observing morphological changes caused by this PS in this organism, in confocal microscopy. A. polyphaga trophozoites were grown at 37°C in PYG medium for 48 to 72 hours. After, the trophozoites were incubated with PS solution during one hour and the samples were irradiated using light-emitting diodes at 460 nm at light doses 30 and 50 J/cm². The results revealed reduction of 27.7%, 61.4% and 82.5% at 30 J/cm² and 75.2%, 85.0% and 95.9% at 50 J/cm², respectively, at curcuminoids concentrations of 500, 1000 and 1500 µg/mL. Throught fluorescence images it was possible to visualize the curcuminoids's uptake by the trophozoites. The PS showed toxicity to amoebae, in the dark, but the irradiation in PDI contributed to amoebae death effect. These data suggest that PDI may be an application of therapeutic intervention against Acanthamoeba infections, since it was effective in the inactivation of these amoebae.

9694-40, Session PMon

Use of combined iron chelator and fractionated ALA-PDT to enhance treatment of skin cancer in vivo

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The main advantage of ALA-PDT is the selective uptake of ALA and conversion into protoporphyrin IX (PpIX) in neoplastic cells. However, failures in treatment are still a common occurrence when ALA is used to treat large and deep tumors. Therefore, improvements to ALA-PDT efficacy are ideally needed. Several strategies have been studied to enhance the PDT effect from ALA-induced PpIX, such as the use of iron chelator, vitamin D for biological differentiation or fractionated PDT (PDT). The aim of this study was test a new combination (cPDT) approach using ALA-fPDT and DFO, since these two methods independently enhance the PpIX and should be synergistic. Optical measurements of PpIX fluorescence in tumor were acquired with a white light source, 405 nm (blue channel) and 639 nm (red channel) laser diodes prior to administration of ALA, before and after PDT. It was observed that in the group that received fPDT, there was a replenishment of PpIX during the dark interval of 2 h between the 1st PDT and 2nd PDT. Moreover, the production of PpIX was more pronounced in the group that received a pre-treatment with iron chelator for 3 consecutive days before the fPDT had been performed. It is expected that the cPDT approach would be a new method to increase the PpIX retention by arresting its conversion to heme. Furthermore, the re-oxygenation of tissue during the dark interval may increase the generation of singlet oxygen and therefore should make ALA-PDT more effective against skin cancer.
Comparison of two photosensitizers in photodynamic therapy using light pulses in femtosecond regime: an animal study

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Photodynamic therapy is a therapeutic modality for cancer treatment based on the interaction of light with a sensitizer agent and molecular oxygen into the target cells. The aim of this study is the evaluation of Photodynamic therapy using pulsed light source in the femtosecond regime through necrosis induced in healthy rat liver. The induced necrosis profile with CW laser and Pulsed Laser were evaluated in animal model which received Photogem (hematoporphyrin derivative) and Photodithazine (chlorine e6 derivative). The light sources used in those studies were a 630 and 660 nm CW diode laser and a Ti:Sapphire Regenerative Amplifier laser (1 kHz repetition rate and 100 fs pulse width) associated with an optical parametric amplifier (OPA) to convert to 630 and 660 nm. The induced necrosis with Photogem was greater with pulsed laser (2.0 ± 0.2 mm) in comparison with CW laser (1.0 ± 0.2 mm), while in Photodithazine the induced necrosis with was greater with CW laser (2.9 ± 0.2 mm) comparing the pulsed laser (2.0 ± 0.2 mm). These results indicate dependence of PDT mechanisms with photosensitizer and the light regime applied.

Intratumor photosensitizer injection for photodynamic therapy: Pre-clinical experience with methylene blue, Pc 4, and Photofrin

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For many photosensitizers, the drug is delivered intravenously. This necessitates a non-trivial drug-light interval in order for photosensitizer to accumulate in tumor tissue, which can result in significant systemic photosensitivity. Direct intratumor injection of photosensitizer could potentially eliminate these negative aspects of photodynamic therapy (PDT), while requiring a lower photosensitizer dose to achieve comparable drug concentration.

We have performed PDT using intratumor injection of 3 different photosensitizers, methylene blue (MB), Pc 4, and Photofrin, in a mouse tumor model. After a 0-15 minute drug-light interval, illumination was delivered by an appropriate diode laser. For animals receiving MB or Pc 4, surface illumination was delivered using a microlens-terminated fiber. For animals receiving Photofrin, interstitial illumination was delivered by a 1 cm diffuser.

In animals that received MB or Pc 4, tumor dimensions were measured every 2-3 days post-PDT, with a cure being defined as no palpable tumor 90 days post-treatment. For Photofrin, animals were sacrificed 24 hours post-PDT and their tumors were excised. These samples were H&E stained to assess PDT-induced necrosis. 55% of tumors were cured with MB-PDT, and a significant delay in tumor growth (p=0.01) was observed for Pc 4. For animals treated with Photofrin, the mean necrosis radius was 3.2±0.5 mm.

In conclusion, intratumoral injection of the photosensitizers methylene blue, Pc 4, and Photofrin is feasible, and results in an appreciable tumor response. Further investigation is necessary to optimize treatment protocols and assess the systemic photosensitivity induced by intratumor injection.
Regulation of cellular markers modulated upon irradiation of low power laser lights in burn injured mice

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Present study aims at understanding the importance of certain cellular markers in tissue regeneration regulated upon irradiation of low level laser light in burn injured mice. Swiss albino mice of either sex were selected for the study and subjected to thermal injury under anesthetic conditions. Following thermal injury, the animals were randomly divided into three groups: (i) the group treated with optimum laser dose (test), (ii) the group treated with 5% Povidone iodine (positive control) and (iii) the group not given any treatment (control). Burn tissue samples from each group were excised at day 6 post burn injury upon euthanization with an overdose of anesthesia and used for histological and immunohistochemical analysis. The excised tissue samples were then processed for paraffin block preparation and sectioning for both histological and immunohistochemical assessments. Haematoxylin & Eosin (H & E) staining was performed on the selected sections to assess proliferation and angiogenesis at various time points under study. For immunohistochemical analysis, sections were stained using specific antibodies like PCNA and Hsp70 against cellular markers regulated during enhanced tissue regeneration in burn injured mice treated with optimum laser dose. The evaluation of histological and immunohistochemical analysis were performed in a blinded fashion showing improved tissue restoration in animals with optimal laser treatments as compared to un-illuminated controls. The markers regulated in the process were observed to be quiet prominent in laser treatment groups as compared to the controls.

Biochemical changes on the repair of surgical bone defects grafted with biphasic synthetic micro-granular HA + bioglass induced by laser and LED phototherapies assessed by Raman spectroscopy

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This work aimed the assessment of the biochemical changes during bone mineralization induced by laser and LED irradiation in an animal model of bone repair using a spectral model based on Raman spectroscopy. Six groups were studied: Clot, Laser (7780 nm, 70 mW), LED (7850 nm ± 10 nm, 150 mW), Biomaterial (biphasic synthetic micro-granular hydroxyapatite (HA) + 7-tricalcium phosphate), Laser + Biomaterial and LED + Biomaterial. When indicated, defects were further irradiated at 48 h interval during 2 wks, 20 J/cm² per session. At 15th and 30th days, femurs were dissected and spectra of the defects were collected. Raman spectra were submitted to a model to estimate the relative amount of collagen, phosphate HA and carbonate HA, by using spectra of pure collagen, biomaterial and basal bone, respectively. At 15th days, the use of biomaterial associated to phototherapy reduced the collagen formation, whereas the amount of carbonate HA was not different in all groups. The phosphate HA was higher in the groups that received biomaterial grafts. At 30th days, it was observed an increase of collagen for the group Laser + Biomaterial, and a reduction in the carbonate HA for the LED + Biomaterial. The phosphate HA was higher for the groups LED + Biomaterial and Laser + Biomaterial, while decreased for the group Biomaterial. These results indicated that the use of Laser and LED phototherapies improved the repair of bone defects grafted with the biomaterial by increasing the collagen deposition and phosphate HA.

9695-6, Session 2

**Precision medicine: Molecular mechanisms will lead future optimizations with PBM therapy (Invited Paper)**

Praveen Arany D.D.S., Univ. at Buffalo (United States)

No Abstract Available

9695-7, Session 2

**Photobiomodulation of human dermal fibroblast pools in in vitro and ex-vivo culture: rational approach towards effective light parameters and nuances of cell physiology**

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Involvement of skin fibroblasts in skin ageing and regeneration makes them a target of phototherapy. However, the photobiomodulation literature is still lacking consensus on the molecular mechanisms and the rationality behind parameters choices.

This study aims at a systematic identification of parameters effectively modulating function of different phenotypes of human primary dermal fibroblasts in vitro and ex vivo and at unraveling mechanisms of light photoreception.

Post-surgery human facial skin was used to establish ex vivo wounded skin model and to isolate primary fibroblast of two phenotypes, which were cultured at 2% FBS. Treatment parameters spanning 447nm-850nm wavelength range, 0.1 - 100 mW/cm² 2 irradiance, 0.1 - 100 J/cm² 2 radiant exposure, were screened using a factorial design of experiment. Impact of light was assessed using AlamarBlue® proliferation assay, SircolTM collagen assay, and cell migration. Expression of photoreceptors was analysed using immunofluorescence and qRT-PCR.

Both mRNA and protein for CR1, 2 and opsin (1, 2, 3) photoreceptors were detected, suggesting their role in light reception. Shorter wavelengths (<500nm) were impacting cells more effectively than longer wavelengths. They stimulated reticular and inhibited papillary fibroblasts function in a dose-increasing manner. As expected, papillary fibroblasts showed a higher sensitivity to the irradiance and their collagen production was significantly increased by low dose of blue, cyan and high dose of infrared light. Translated parameters, applied to an ex-vivo and in vitro wounded skin models, accelerated wound closure.

We recommend careful selection of treatment parameters and culture conditions in parallel with identification of photoreceptors and engaged molecular pathways.

9695-8, Session 2

**Low level laser exposure influence on calcium channels and intracellular release in cultured astrocytes (Invited Paper)**

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Background, Traumatic brain injury (TBI) has become a focal point for a great deal of both molecular and clinical research. Both primary injury or mechanical damage (producing structural injuries such as cortical contusions, axonal shearing and microvascular damage) and less obvious secondary injuries are reported in the pathophysiology of TBI. Secondary injuries include obvious non-mechanical damage caused by metabolic events possibly related to neuronal or glial cell membrane stress. As a model system for the initial events of TBI we applied shear stress to cultured rat astrocytes in a microfluicid chamber using a fast pressure servo for stimulation and examined the intracellular Ca2+ levels in stressed cells and in cells which have been pretreated with low level laser energy (LLL). The introduction of drugs to alter intracellular Ca2+ were conducted to further assess the relationship between laser exposure and Ca2+.

Materials and Methods: Primary adult astrocytes were isolated from gelatin-sponge implants from adult Sprague Dawley rat brains. Low level laser stimulation (LLL) consisted of exposure to 660nm laser light at an average dose of 4 J/cm². Cells in microfluicid chambers were exposed to LLL in both normal Ca2+ and Ca2+ free environments. We tested whether LLL affected the response of cells to shear pulses of 23 dynes/cm² for 10ms. Cells were exposed to LLL and stimulated while the light was on and after the light was turned off. Ryanodine, sodium azide or thapsigargin were introduced to examine whether LLL affected normal Ca2+ and Ca2+ free environments. We tested whether LLL affected the response of cells to shear pulses of 23 dynes/cm² for 10ms. Cells were exposed to LLL and stimulated while the light was on and after the light was turned off. Ryanodine, sodium azide or thapsigargin were introduced to examine whether LLL affected normal Ca2+ and Ca2+ free environments.

Results: The laser energy had the following effects: 1) a delay in the response to shear stress 2) a reduction of the shear stress induced response and 3) an abrupt increase in the intracellular calcium following the exposure to 660nm light in the absence of shear stress. The results of experiments using agents to decrease fluxes in Ca2+ implied that illumination caused Ca2+ release from internal stores.

Conclusions: LLL stimulates Ca2+ release from the ER, possibly through ryanodine sensitive channels and elevates Ca2+ in the astrocytes. The results indicate that LLL produces significant effects on cultured astrocytes ant that these effects can have an impact on ATP and Ca2+ homeostasis. The experiments need to be extended to establish the action spectra of the absorber.

9695-9, Session 2

**In vitro measurements of oxygen consumption rates in hTERT-RPE cells exposed to low levels of red light**

Jeffrey C. Wigle, Cherry C. Castellanos, U.S. Air Force (United States)

Exposure to 2.88 J/cm² of red light induces an adaptive response against a lethal pulse of 2.0 ?m laser radiation in hTERT-RPE cells in vitro, but not...
in a knockdown mutant for vascular endothelial growth factor c (VEGF-C). Therefore, these mutants are expected to be useful in dissecting the molecular mechanisms of photobiomodulation. The generally accepted initiation sequence for photobiomodulation is that absorption of red light by cytochrome c oxidase (CCOX) of the electron transport chain increases the binding affinity of CCOX for O2 vs. nitric oxide (NO). This results in displacement of NO by O2 in the active site of CCOX, thereby increasing cellular respiration and intracellular ATP. We’ve previously reported that red-light exposure induces a small, but consistently reproducible, increase in NO levels in these cells. But the relative importance of NO and oxidative phosphorylation in photobiomodulation is unclear because little is known about the relative contributions of NO and ATP to the response. However, if NO dissociation from CCOX actually increases oxidative phosphorylation, one should see a corresponding increase in oxygen consumption. Using a Seahorse Extracellular Flux Analyzer, one can measure oxygen consumption rate (OCR) as a proxy for oxidative phosphorylation. Seahorse measurements using “mitochondria stress tests” to compare OCR in normal (WT) vs. VEGF-C(KD) cells show: (1) basal OCR in WT cells is significantly higher than in the VEGF-C(KD); and (2) the WT cells have a significant amount of “excess capacity,” whereas the VEGF-C(KD) have little or none. OCR measurements following red light exposure are ongoing.

9695-10, Session 3
Photobiomodulation dosimetry for nerve regeneration: a systematic review
James D. Carroll, THOR Photomedicine Ltd. (United Kingdom)

No Abstract Available

9695-11, Session 3
Efficacy of low level laser therapy on painful diabetic peripheral neuropathy (Invited Paper)
Arun Maiya, Manipal Univ. (India)
The prevalence of type 2 diabetes mellitus (T2DM) is rapidly increasing worldwide. It has been associated with many micro-vascular and macro-vascular complications.1 Among all the complications, peripheral neuropathy is considered to be the most common.2 It is estimated that the prevalence of peripheral neuropathy in T2DM patients is approximately 25-50% in developing countries.3 Diabetic peripheral neuropathy (DPN) accounts for more hospitalisation than all the other complications of T2DM. Painful DPN is associated with functional impairment & poor quality of life.4,5 Painful DPN is a result of injury to the Vasa nervorum, axons and atrophy of the axons leading to tissue damage.6 All nerve fibres may be injured, but small myelinated and unmyelinated fibres that transmit pain and temperature are most affected.6 In association with injury to the nerves, reduced microcirculation is responsible for the loss of protective sensation and atrophy of intrinsic foot muscles which later leads to development of foot complications like callus, ulcers, and infections of skin and bone in T2DM subjects with long duration of diabetes mellitus.7 In many subjects with diabetic neuropathy, pain develops as a symptom localised to the lower extremities, primarily the soles and toes.8 Current therapy for painful DPN is aiming to symptomatic relief through various drug administrations. These drugs are effective, but often associated with systemic side effects and do not retard the advancement of the underlying neuropathy.9 Other than pharmacological treatment, non-pharmacological management have also been used, including acupuncture10, infrared therapy11, and various electrotherapies, including transcutaneous electrical nerve stimulation (TENS) 12, and spinal cord electro stimulation.13 The efficacy of most conservative treatment options for painful DPN is still needs to be investigated. Among the electrotherapy modalities, low-level laser therapy has been used to manage nerve injuries and other pathologies of the nerve because it hold the potential to induce a biostimulational effect on the nervous system.14,15,16 In addition, low-level laser therapy has also been used in the management of diabetic complications such as foot ulcers.17 Even though low-level laser therapy is found to be very effective in nerve regeneration, there is a dearth of literature on effect of low-level laser therapy on painful DPN in T2DM population. Therefore the objective of the present study is to evaluate the effect of low-level laser therapy on Type 2 DM subjects with painful DPN.

9695-12, Session 3
Effect of interstitial low level laser therapy on tibial defect
Sangyeob Lee, Seulgi Jung, Donghyun Hwang, Jihoon Park, Myungjin Ha, Sungkon Yu, Edalat Radfar, Hansung Kim, Byungho Jung, Yongseu Univ. (Korea, Republic of)

Tibial defect is very common musculoskeletal disorder which make patient painful and uncomfortable. If tibial defect do not recover in early phase, nonunion may be occurred which needs costly treatment. So, many studies about bone regeneration are going to figure out fast bone healing in early phase. Low level laser therapy (LLLT) is already known that it is very convenient and good for beginning. However, light scattering and absorption obstruct musculoskeletal therapy which need accurate light delivery. In this study, we use laser needle system (LNS) which can overcome limit of light penetration depth. Experimental animals (mouse, C57BL/6) were divided into three groups: animals that applied LNS(=660nm; power 10mW; total energy 5J), animals that applied LNS(=660nm; power 20mW; total energy 10J) and animals not treated. All animals were performed surgical treatment to make tibial defect on right crest of tibia. Defect size was 1mm diameter circle. LNS groups(5J and 10J) were treated once per 48 hours with LNS. Bone volume and X-ray attenuation coefficient which measured on 0, 14th day by u-CT were used to evaluate effect of LLLT. Results show that bone volume of LNS groups (total energy 5J and 10J) were improved more than control. Also, u-CT images show that LNS groups (total energy 5J and 10J) have more structural improvement than control. X-ray attenuation coefficient of each groups have slightly different. These results suggest that LLLT through LNS may affect early phase of bone regeneration and can be used in various musculoskeletal disease.

9695-13, Session 3
Wearable light management system for light stimulated healing of large area chronic wounds
David Kallweit, Jan Mayer, Sören Fricke, Marc Schnieper, Rolando Ferrini, Ctr. Suisse d’Electronique et de Microtechnique SA (Switzerland)

Chronic wounds represent a significant burden to patients, health care professionals, and health care systems, affecting over 40 million patients and creating costs of approximately 40 billion € annually. We will present a medical device for photo-stimulated wound care based on a wearable large area flexible and disposable light management system consisting of a waveguide with incorporated micro- and nanometer scale optical structures for efficient light in-coupling, waveguiding and homogeneous illumination of large area wounds. The working principle of this innovative device is based on the therapeutic effects of visible light to facilitate the self-healing process of chronic wounds. On the one hand, light exposure in the red (656nm) induces growth of keratinocytes and fibroblasts in deeper layers of the skin. On the other hand, blue light (453nm) is known to have antibacterial effects predominately at the surface layers of the skin. In order to be compliant with medical requirements the system will consist of two elements: a disposable wound dressing with embedded flexible optical waveguides for the light management and illumination of the wound area, and a non-disposable compact module containing the light sources, a controller, a rechargeable
battery, and a data transmission unit. In particular, we will report on the developed light management system. Finally, as a proof-of-concept, a demonstrator will be presented and its performances will be reported to demonstrate the potential of this innovative device.

9695-15, Session 4
Effects of photothermal application in laser immunotherapy (LIT) for the treatment of metastatic cancer
Tomas Hode, Siu Kit Lam, Immunophotonics, Inc. (United States); Feifan Zhou, Wei Richard Chen, Univ. of Central Oklahoma (United States)
No Abstract Available

9695-16, Session 4
NIR exerts a hormetic biomodulation effect on neuronal function
Fatma Vatansever M.D., Ying-Ying Huang M.D., Michael R. Hamblin, Wellman Ctr. for Photomedicine (United States)
Near Infrared (NIR) light can act as a photomodulator of neuronal function. We have studied the photobiomodulation effect both in vitro and in vivo. Our studies showed that the hormetic nature of the photobiomodulation effect can be seen at the sub-cellular, cellular, ex vivo marker expression, and in vivo behavioral levels. The improvement in neurological severity score in brain-injured mice is better with only one application of 810nm light at 36J/cm² fluence and 50mW/cm² irradiance compared to 18J/cm² and irradiance of 25mW/cm². However when the exposure parameters are fixed and the application regimen is varied the hormetic response is found. We used 18J/cm² at 25mW/cm² of 810nm light given as 1-, 3-, or 14-daily applications and found that 3X light application dosage delivered the best results. We evaluated key indicators of neuronal function such as BDNF expression, neurogenesis, synaptogenesis, and cognitive performance modulation in response to 810nm NIR exposure with 18J/cm² fluence and 25mW/cm² irradiance. Our results indicated that marker expression and performance enhancement is at its peak with 3XNIR exposure, whereas 14XNIR reduced the expression/ improvement levels. Clearly NIR exposure exerts a hormetic response effect on neuronal and brain functioning.

9695-18, Session 4
Low-power laser irradiation did not stimulate breast cancer cells following ionizing radiation
Camila R. Silva, Claudinei Francisco M. Camargo, Martha S. Ribeiro, Instituto de Pesquisas Energéticas e Nucleares (Brazil)
Cancer has become a public health problem worldwide. Radiotherapy may be a treatment to a number of types of cancer, frequently using gamma-radiation with sources such as 137Cs and 60Co, with varying doses, dose rates, and exposure times to obtain a better outcome. Treatment with ionizing radiation can lead to serious complications such as burns and tissue radionecrosis. Low-power laser (LPL) has been reported in the literature as a stimulant for cell proliferation and tissue healing process. However, its effects on cancer cells are not yet well elucidated. The purpose of this work was to evaluate the effects of the LPL on breast cancer cultures following ionizing radiation. The breast cancer-MDA-MB-231 cells were gamma irradiated by a 60Co source, with doses of 2.5 Gy and 10 Gy. After 24h, cells were submitted to LPL irradiation using a red laser emitting at 660 nm, with output power of 40 mW and exposure time of 30 s and 60 s. The plates were uniformly irradiated, with energy of 1.2 J and 2.4 J, respectively. Cell viability was analyzed using the exclusion method with trypan blue. Our results show that breast cancer cells submitted to LPL after ionizing radiation remained 95 % viable. No statistically significant differences were observed between laser and control cells, which did not received LPL irradiation (P > 0.05). These findings suggest that LPL did not stimulate cancer cells and could be used in patients with complications post-radiotherapy.

9695-27, Session 4
Nitromedicine: a new concept
Salaheldin Halasa M.D., Nitromedicine (United States); Praveen Arany D.D.S., Univ. at Buffalo (United States); Michael R. Hamblin, Wellman Ctr. for Photomedicine (United States)
Nitromedicine is a new medical treatment paradigm, focused on increasing nitric oxide (NO) bioavailability and modulating redox-signaling pathways combined with phototherapy, electrotherapy and stem cell therapy. It has been known since the discovery of the biological role of NO in the 1980s, that supplying NO donors such can have many beneficial effects in different conditions by stimulating stem cells and modulating the immune response, but there also exists a substantial risk of side-effects with long-term use. Excess NO can inhibit mitochondrial metabolism by binding to cytochrome c oxidase (CCO) and can also produce reactive nitrogen species (Peroxynitrite) by interacting with reactive oxygen species (ROS). To avoid these potential damaging side-effects we propose to combine the use of NO donors with three additional components. Firstly we believe that addition of antioxidants such as hydrogen sulfide donors, polyphenols and vitamins can neutralize ROS and RNS. Secondly we believe that application of appropriate wavelengths and dosages of light (blue, red or near infrared depending on the exact condition being treated) will dissociate NO from CCO (and other storage sites) thus restoring mitochondrial ATP production and stimulating healing in many situations. Thirdly delivering electrons to the body might help to saturate the free radicals with electrons, eliminate underlying oxidative stress, stabilize mitochondria, prevent further formation of pathological free radicals and increase the nitric oxide bioavailability. This combination therapy may be applied to treat a large variety of oxidative stress-related diseases such as degenerative diseases, immunological diseases, chronic infectious diseases, cancers and a broad range of unmet medical needs involving chronic inflammation with an emphasis on pain management.

9695-28, Session 4
Biochemical responses of isolated lung CSCS after application of low intensity laser irradiation dose
Heidi Abrahamse, Univ. of Johannesburg (South Africa)
Studies have shown that using high fluences of Low Intensity Laser Irradiation (HF-LILI) produce apoptotic effects on normal and neoplastic cells. This study aimed to determine whether HF-LILI induce cell death in lung CSCS. Lung CSCS were isolated using the stem cell marker CD 133, characterized using flow cytometry and fluorescence microscopy, and applied in experiments which included treatment with LILI at wavelengths of 636, 825 and 1060 nm with fluences ranging from 5 J/cm² to 40 J/cm². Viability and proliferation studies, using Alamar blue assay and adenosine triphosphate luminescence (ATP), indicated an increase when treating lung CSCS with low fluences of 5 - 20 J/cm² and a decrease in viability and proliferation as well as an increase in apoptosis when applying a fluence of 40 J/cm² indicated by flow cytometry using Annexin V and propidium iodide (PI) dyes. Results indicate that LILI, when treating lung CSCS, can induce either a bio-stimulatory or bio-inhibitory effect depending on the
wavelength and fluence used. This study indicated successful apoptotic induction of lung CSCs. Future experiments should be able to conclude the exact mechanism behind HF-LILI, which can be used in the targeted treatments of CSC elimination, implementing HF-LILI in the same manner as PDT in the absence of a photosensitizer.

9695-31, Session 4
Photobiomodulation in retinal injury and disease
Sandeep Gopalakrishnan, Janis T. Eells, Univ. of Wisconsin-Milwaukee (United States)

Exposure of tissue to low-energy photon irradiation in the far-red (FR) to near-infrared (NIR) range of the spectrum, collectively termed “photobiomodulation” (PBM) has been demonstrated to restore the function of damaged mitochondria, up-regulate the production of cytoprotective factors and prevent cell death. FR/NIR photons penetrate diseased tissues including the retina and brain. Recent studies have demonstrated that the therapeutic actions of FR/NIR photobiomodulation are mediated by a key photoreceptor molecule, cytochrome c oxidase, the terminal electron acceptor of the electron transport chain, culminating in improved mitochondrial bioenergetics, increased synthesis of anti-oxidant and anti-inflammatory factors and improved cell survival. Investigations in rodent models of Light-Damage, Retinitis Pigmentosa, Diabetic Retinopathy and Age-related Macular Degeneration have demonstrated that PBM attenuates photoreceptor cell death, protects retinal function and exerts anti-inflammatory actions. Recent studies investigating the therapeutic efficacy of PBM in rodent and cultured cell models of Diabetic Retinopathy showed PBM ameliorated retinal lesions in vivo and reduced oxidative stress and cell death in vitro. Clinical investigations using PBM in non-center-involved Diabetic Macular Edema (DME) revealed a significant reduction in retinal edema in all subjects. Our research group is currently conducting a randomized clinical trial investigating the therapeutic efficacy of PBM in DME. This translational research project will bridge recent, promising basic research on the effects of PBM on cellular function and retinal disease in rodent models with a clinical treatment for a significant medical problem, diabetic macular edema.

9695-17, Session PSun
Assessment of the influence of Laser phototherapy on the bone repair process of complete fractures in tibiae of rabbits stabilized with Semi-Rigid Internal fixation treated with or without MTA implant: a histological study.
Luiz Guilherme P. Soares, Ctr. of Biophotonics, Univ. Federal da Bahia (Brazil); Aline C. P. Silva, Institute of Health Sciences, Univ. Federal da Bahia (Brazil); Anna Paula L. T. Silva, Bruno Luiz R. C. Neves, Nicole R. S. Santos, Jean N. dos Santos, Antonio L. Pinheiro, Ctr. of Biophotonics, Univ. Federal da Bahia (Brazil)

Beside biomaterials, Laser phototherapy has shown positive effects as auxiliary therapy in bone repair process, especially when involving large bone losses. The aim of this histological study was to evaluate, by light microscopy, the influence of laser phototherapy on the repair of complete tibial fractures in rabbits treated or not with semi-rigid internal fixation and Mineral trioxide aggregate - MTA. Twelve Rabbits were randomly divided into four groups with three animals each. After general anesthesia, complete fractures were created in one tibiae with a carboburundum disk. All animals (groups I-IV) had the fracture stabilized with semi-rigid fixation (wire osteosynthesis - WO). Group I was routinely fixed with WO; groups II and IV fracture was filled by blood clot and MTA implant. In Groups III and IV fracture was filled by blood clot and further irradiated with laser (780 nm, 70 mW, CW, $\varphi = 0.04$ cm², 20.4 J/cm², per session, t = 300s, 142.8 J/cm² per treatment). The phototheraphy protocol was applied immediately after the surgery and repeated each 48 hours during 15 days. Animal death occurred on the 30th postoperative day. After removal of the specimens, the samples were routinely processed, stained with H&E and evaluated by light microscopy. Histologically, the group treated with MTA implant and irradiated with laser showed the fracture filled by a more organized and mature trabecular bone, when compared with all other groups. It was concluded that the use of laser phototherapy + MTA was effective in improving bone repair of complete tibial fractures.

9695-19, Session PSun
Safety and efficacy of photo modulation therapy for weight loss
Ambereen Ahmed M.D., A&M Assorted Therapy, LLC (United States)

Photomodulation therapy uses light from a laser and is, therefore, a non-invasive, non-thermal treatment that is effective in reducing chronic pain and inflammation, and stimulating wound healing and tissue regeneration. Due to its accessibility and ease of use, it is also being explored as an alternative to lipoplasty for fat removal. This review covers literature focusing on the applications of photo modulation therapy, with a particular emphasis on the safety and efficacy of this therapy for fat loss. Based on the studies reported, photo modulation therapy is a safe technique that showed promising results in reducing the circumference of treated body parts. Also, photo modulation therapy is particularly effective when used in combination with exercise and physical therapy. The mechanism of action of how photo modulation therapy removes fat from the mammalian cells is controversial and requires further investigation. It was speculated previously that after the therapy, the cell will release the fat in vacuoles. However, most studies reported no such observation of fatty vacuoles when the cells were viewed under a microscope. It was likely that the laser treatment triggers a cellular response that converted triglycerides into fatty acids and glycerol, which can both pass through pores formed in the cell membrane and thus caused a shrinkage in adipocytes. However, no data supports this explanation, and further studies are required to understand completely this mechanism. Moreover, additional studies demonstrating the efficacy of photo modulation therapy in larger groups would be helpful in establishing this technique for regular clinical use.

9695-20, Session PSun
Wellbeing effect of LED photobiomodulation
Francois Michel M.D., Consultant (France); Daniel Barolet M.D., McGill Univ. (Canada)

LED photobiomodulation is known for its restorative effects on the skin and the rest of the body. While practicing aesthetic treatments on the skin of the face, a concomitant wellbeing effect has been observed obvious both in photographs of the treated areas and in patient behaviour. The latter has been corroborated by studies of the brain, with rigorously performed traumatic brain injury studies providing great insight. Taken alone the wellbeing effect has been described in only one pilot study.

In our study, an analogical questionnaire was created with the purpose of having a convenient tool for the assessment of quality of life following photobiomodulation treatments on the face as well as to gauge patients’ feelings regarding overall aesthetic improvement.
Photodynamic antimicrobial chemotherapy (PACT) against oral microorganisms with the use of blue LED associated to curcumin

Gustavo M. Pires Santos, Fernando J. P. Sampaio, Susana Carla P. Sampaio de Oliveira, School of Dentistry, Univ. Federal da Bahia (Brazil) and Institute of Health Sciences, Univ. Federal da Bahia (Brazil); Juliana S. C. Monteiro, School of Dentistry, Univ. Federal da Bahia (Brazil); Vanderlei S. Bagnato, Instituto de Física de São Carlos (Brazil); Antonio Luiz B. Pinheiro, School of Dentistry, Univ. Federal da Bahia (Brazil) and Institute of Health Sciences, Univ. Federal da Bahia (Brazil) and Univ. Camilo Castelo Branco (Brazil)

The use of curcumin as antimicrobial agent has been suggested. It is known that some compounds may be potentized by light at an appropriate wavelength. The aim of this study was to evaluate the effect of Photodynamic Antimicrobial Chemotherapy (PACT) using blue LED (?)450nm (220mW) associated to Curcumin at different concentrations on microorganisms of the oral cavity. Samples were collected from five individuals and inoculated into test tubes containing 8ml of TSB medium. Three culture plates of 24 wells were used and each well contained a suspension of the microorganisms and TSB medium totaling 1000L. 75, 37.5, 18.7, 9.4 and 4.77g/mL were added to the plates and a pre-irradiation time of 5 min was observed. A prototype blue LED device (São Carlos, SP, ?450 ± 5nm, 220mW, CW, Spot 0.785mm2; 2175, 50/J/cm2) was used for irradiation. After stirring, 200?L aliquots were taken from each well and ELISA test carried out immediately (triplicate) and turbidity assessed. After one hour of incubation in a bacteriological oven, 200?L aliquots were taken from the remaining wells for a second reading. It was observed that the concentrations of 75, 37.5, 18.7, 9.4 and 4.77g/ml when associated with LED showed inhibitions percentiles of 81%, 63.8%, 71%, 48.8% and -4.2% respectively in comparison to controls. Significant statistical difference (p=0.0181) was found between control x 75 ?g/ml. It is concluded that PACT with blue LED associated to Curcumin could be a potential mechanism for controlling the infection of the oral cavity.

Interaction of low level laser therapy in candida albicans fungal proliferation

Vanda M. Carneiro, Natália C. Araújo, Rebeca F. Menezes, Lara M. Moreno, Alexandrino P. Santos-Neto, Marleny E. M. Gerbi, Univ. Federal de Pernambuco (Brazil)

Candida albicans have important role in triggering infections in HIV + patients. The indiscriminate use of antifungal has entailed a resistance of Candida albicans, which requires new treatment alternatives for oral candidiasis. The use of low-intensity laser therapy promotes considerable improvement in curing illnesses caused by microorganisms as well as in healing wounds. This study aims to evaluate the effect of laser irradiation on the fungal proliferation of Candida albicans in immunosuppressed patients. Six Candida albicans strains were isolated from immunosuppressed patients and submitted to laser therapy. The experimental were divided in eight groups, according to two wavelength of lasers: InGaAlP 7685nm (P = 30mW, CW, 7-6 mm) and GaAlAs, 7830nm (P = 40mW, CW, 7-6 mm) that were applied in the following dosimetries: 6/J/cm2, 8/J/cm2, 10/J/cm2 and 12/J/cm2; it was also maintained control group not submitted to laser therapy. The results were not statistically significant (Kruskal’s-Wallis, p > 0.05), although there was a considerable reduction in proliferation of Candida albicans in the experimental groups of GaAlAs 7830nm with dosimetry of 6/J/cm2, which presented average scores lower than the other groups for the growth of Candida. More studies are needed, so that the laser therapy is a viable alternative in the treatment of fungal infections.

Low-level laser therapy (LLLT) in Russia: history of study of biomedomodulation action (BMA) mechanisms of low-intensity laser irradiation (LILI) and its therapeutic application practice

Sergey Moskvin, State Scientific Ctr. of Laser Medicine (Russian Federation)

In Russia (formerly USSR) study of BMA mechanisms of LILI began in 1964, immediately after the development of lasers. During the period from 1965 to 1972 several dozens of scientific conferences were held, hundreds of studies were published. Generally, secondary mechanisms and results of LILI effect on patients with various diseases were studied. This data was immediately implemented into practical medicine in the fields of oncology, surgery, dermatology and dentistry, and since 1974 LLLT is included in the standard of state medical care. For 50 years no less than 1000 books were published (monographs, collections, methodical and clinical materials), thousands of researches were carried out.

Primary mechanism and patterns of interaction of LILI with acceptors within cells can be represented in the following order: absorption of photon’s energy – emergence of a local temperature gradient – release of Ca+2+ from intracellular stores – stimulates Ca+2+-dependent processes. Understanding of this process allowed the explanation of all known secondary effects, optimize methods and extremely increase effectiveness of LLLT [Moskvin S.V., 2003-2015].

Owing to the knowledge of biomedomodulation action mechanisms of LILI numerous associated and combined LLLT techniques were developed and are widely used nowadays: locally, on projection of internal organs, laser acupuncture, reflexology, intracavitary, transdermal and intravenous laser blood illumination, magnetic-laser therapy, laser-phoresis, laser-vacuum massage, biomedomodulation, etc.

About 400,000 laser therapeutic devices are used in Russian practical healthcare. Unique, having no analogues in the world devices are produced – red pulsed laser diodes (wavelength 635 nm, power 5-40 W, pulse duration 100 ns, frequency 10,000 Hz) are designed specially for effective laser therapy.
Return to Contents

9695-25, Session PSun

PDT in periodontal disease of haart resistance patients

Elcio M. Giovani D.D.S., Gilberto A. Noro-Filho M.D., Bruno V. Caputo M.D., Renato Casarin M.D., Claudio Costa, Daniela Andrade, Camila C. Santos M.D., Univ. Paulista (Brazil)

HIV/AIDS patients present a change of microbiota associated with host immunodeficiency. Photodynamic therapy (PDT) showed as a promising and viable alternative in reducing microbiota. Present study evaluate effectiveness of photodynamic therapy in periodontal disease of AIDS patients with highly activity antiretroviral therapy (HAART) failure, measuring the clinical periodontal parameters and periodontal microbiota. Twelve patients with HAART resistance (R group) divided into two groups (control and PDT) and 12 patients with no HAART resistance (NR group) divided into two groups (control and PDT). The results show the difference in baseline of CD4 cells count, NR group 640.0 ± 176.2 cells/mm³ R group and 333.3 ± 205.8 cells /mm³ (p<0.05), and in 8.3% detectable viral load in NR group and 75% detectable (p <0.001) in R group. As clinical periodontal parameters (PD and CAL), PDT was more effective than the control group only in the NR group (p <0.05%), moreover, there was no difference in the evaluation of clinical periodontal parameters between the both R groups (p>0.05%). Microbiological evaluation in R group presents a general reduction in the Aa at 3 and 6 months. Furthermore, demonstrated a reduction of Pg in all groups at 6 months and in R group at 3 months. The impact assessment of photodynamic therapy in patients with different levels of immunosuppression determined that the combination of mechanical periodontal treatment with photodynamic therapy in patients with HAART failure did not cause additional benefits. Therefore, PDT in this study could not been indicated in HAART resistance patients.

9695-26, Session PSun

Assessment of the effects of laser photobiomodulation on peri-implant bone repair through energy dispersive x-ray fluorescence: A study of dogs

Rebeca F. Menezes, Natália C. Araújo, Vanda M. Carneiro, Lara Marques, Univ. Federal de Pernambuco (Brazil); Luiz Antonio Portela Guerra, University of Pernambuco (Brazil); Alexandrino P. Santos Neto, Marleny E. M. Gerbi, Univ. Federal de Pernambuco (Brazil)

The aim of this study was to evaluate the effects of the (AsGaA1 830nm ~ 40mW, CW, Ø ~ 0.3mm, 140J/cm²) laser radiation in the peri-implant bone using energy-dispersive x-ray fluorescence analysis of the concentration of calcium on titanium dental implants placed in shin bones of mongrel dogs. Two groups were settled: GI (control, n=20; two dental implants were made in the tibia of each animal = 10 animals), G2 (experimental laser, n=20; one dental implant was made in each animal + lasertherapy=20 animals). G2 was irradiated each 48 hours, during two weeks, in a total of seven 20J/cm² sessions, each one distributed in five 4J/cm² spots. The first irradiation took place on the trans-surgical, being one spot on the center of the surgical alveolus, before the placement of the implant and other four spots around it. In the following six sessions the radiation was applied to five spots: one of them the towards the bottom of the implant and the other four equally distributed around it. The specimens were removed 15 and 30 days post operation in order to measure of the perimplantar calcium concentration by deployment of the energy-dispersive x-ray fluorescence analysis. The Mann-Whitney statistical tests were applied to evaluate the findings (p < 0.05).

9695-29, Session PSun

Photodynamic ability of gold and silver nanoparticles in mediating cell death in breast and lung cancer cell lines

Heidi Abrahamse, Univ. of Johannesburg (South Africa)

Photodynamic therapy (PDT) showed as a promising option for treatment of periodontal disease in renal-transplanted patients and its effectiveness is similar to conventional therapy. The calcium concentration of the specimens submitted to the radiation had significant improvements. Laser radiation has been shown to accelerate bone repair at the upper and lower peri-implant region.

9695-30, Session PSun

How to market your laser phototherapy practice evidence based best practices

Terrance L. Baker, Sollay Cosmetic Medical & Laser Ctr. (United States)

How to market your expert witness practice is an intensive introductory workshop that is designed to show prospective, novice as well as established clinicians exactly how to start and build a successful medical therapeutic laser practice. This course is specifically designed for prospective and novice medical practitioners and requires no advance knowledge or training. Attendees will learn from experienced faculty in a step by step fashion how to start and build a successful medical therapeutic laser practice.
9696-1, Session 1

**Sub-diffuse structured light imaging provides macroscopic maps of microscopic tissue structure (Invited Paper)**

Stephen C. Kanick, Thayer School of Engineering at Dartmouth (United States)

The onset and progression of cancer introduces changes to the intra-cellular ultrastructural components and to the morphology of the extracellular matrix. While previous work has shown that localized scatter imaging is sensitive to pathology-induced differences in these aspects of tissue microstructure, wide adaptation this knowledge for surgical guidance is limited by two factors. First, the time required to image with confocal-level localization of the remission signal can be substantial. Second, localized (i.e. sub-diffuse) scatter remission intensity is influenced interchangeably by parameters that define scattering frequency and anisotropy. This similarity relationship must be carefully considered in order to obtain unique estimates of biomarkers that define either the scatter density or features that describe the distribution (e.g. shape, size, and orientation) of scatterers. This study presents a novel approach that uses structured light imaging to address both of these limitations.

Monte Carlo data were used to model the reflectance intensity over a wide range of spatial frequencies, reduced scattering coefficients, absorption coefficients, and a metric of the scattering phase function that directly maps to the fractal dimension of scatter sizes. The approach is validated in tissue-simulating phantoms constructed with user-tuned scattering phase functions. The validation analysis shows that the phase function can be described in the presence of different scatter densities or background absorptions. Preliminary data from clinical tissue specimens show quantitative images of both the scatter density and the tissue fractal dimension for various tissue types and pathologies. These data represent a novel wide-field quantitative approach to mapping microscopic structural biomarkers that cannot be obtained with standard diffuse imaging.

Implications for the use of this approach to assess surgical margins will be discussed.

9696-2, Session 1

**Imaging and modeling of collagen architecture in living tissue with polarized light transfer (Invited Paper)**

Jessica C. Ramella-Roman, Susan Stoff, Joseph Chue-Sang, Yuqiang Bai, Florida International Univ. (United States)

The extra-cellular space in connective tissue of animals and humans alike is comprised in large part of collagen. Monitoring of collagen arrangement and cross-linking has been utilized to diagnose a variety of medical conditions and guide surgical intervention. For example, collagen monitoring is useful in the assessment and treatment of cervical cancer, skin cancer, myocardial infarction, and non-arteritic anterior ischemic optic neuropathy. We have developed a suite of tools and models based on polarized light transfer for the assessment of collagen presence, cross-linking, and orientation in living tissue. Here we will present some example of such approach applied to the human cervix. We will illustrate a novel Mueller Matrix (MM) imaging system for the study of cervical tissue; furthermore we will show how our model of polarized light transfer through cervical tissue compares to the experimental findings. Finally we will show validation of the methodology through histological results and Second Harmonic imaging microscopy.

9696-3, Session 1

**Multi-modality spatial frequency domain imaging of tissue (Invited Paper)**

Bruce J. Tromberg, Univ. of California, Irvine (United States)

Spatial Frequency Domain Imaging (SFDI) utilizes structured light patterns to control optical path length and form images of tissue optical properties. Multiple contrast elements can be obtained using spatial frequency control, including tissue absorption, scattering, speckle, and fluorescence. Modulating spatial and spectral frequencies and orientation patterns provides additional contrast from tissue blood flow and scatter orientation while facilitating tomographic localization of buried structures. This talk describes the integration of these modalities into SFDI imaging devices and addresses the practical value of each contrast element in medical imaging and imaging guided therapy.

9696-4, Session 1

**Real-time endoscopic oxygenation imaging using single snapshot of optical properties (SSOP) imaging**

Joseph P. Angelo, Beth Israel Deaconess Medical Ctr. (United States); Martijn van de Giessen, Leiden Univ. Medical Ctr. (Netherlands); Sylvain Gioux, Beth Israel Deaconess Medical Ctr. (United States)

With 50% of all interventional procedures in the US being minimally invasive, there is a need for objective tools to help guide surgeons in this challenging environment. Tissue oxygenation is a useful biomarker of tissue viability and suitable for surgical guidance. Here we present our efforts to perform real-time quantitative optical imaging through a rigid endoscope using Single Snapshot of Optical Properties (SSOP) imaging. In particular, in this work we introduce for the first time 3 dimensionally-corrected dual wavelength optical properties imaging using SSOP through an endoscope, allowing accurate oxygenation maps to be obtained on tissue simulating phantoms and in vivo samples. We compared the results with state-of-the-art wide-field spatial frequency domain imaging (SFDI). Overall, results from the novel endoscopic imaging system agreed within 10% in absorption, reduced scattering, and oxygenation. Moreover, we introduce here real-time, video-rate quantitative optical imaging with 3D profile correction through an endoscope. These results demonstrate the potential of endoscopic SSOP as an objective surgical guidance tool for the clinic.

9696-5, Session 1

**Single-sensor real-time multispectral fluorescence and color imaging platform for image guided surgery**

Nikolas Dimitriadis, Martin Theuring, Bartlomiej Grychtol, Lars Maertins, Fraunhofer-Institut für Produktionstechnik und Automatisierung (Germany); Matthias Kolibabka, Eric Brandhorst, Hans-Peter Hammers, Ruprecht-Karls-Universität Heidelberg (Germany); Nikolaos C. Delioliadis, Fraunhofer-Institut für Produktionstechnik und Automatisierung (Germany)
Making fluorescence guided surgery the gold standard in clinical routine requires to develop novel imaging modalities taking full advantage of current state-of-the-art molecular targeting strategies. Here, we present an imaging platform combining conventional color imaging with multispectral fluorescence imaging to enrich the image with functional, morphological or disease related information obtained by molecular fluorescent contrast agents. The platform uses a single multi-channel sensor combined with a concept of temporal and spectral multiplexing and can easily be adapted to the fields of surgical microscopy, endoscopy or ophthalmoscopy. Preclinical experiments in the field of retinal angiography demonstrate the platform versatility.

9696-6, Session 2

Attenuation correction in molecular fluorescence imaging (Invited Paper)

Bin Yang, James W. Tunnell, The Univ. of Texas at Austin (United States)

Fluorescence-guided surgery has demonstrated more complete tumor resections in both preclinical models and clinical applications. However, intraoperative fluorescence-based imaging can be challenging due to attenuation of the fluorescence by intrinsic tissue scattering and absorption. Removing attenuation in fluorescence imaging is critical in many applications. We have developed both a model based approach and an experimental approach to retrieve attenuation corrected fluorescence based on spatial frequency domain imaging (SFDI).

In the model based approach, we extended an attenuation correction model initially developed for point measurement into wide-field imaging with SFDI. To achieve attenuation correction, tissue optical properties were evaluated at both excitation and emission wavelengths, which were later applied in the model. In an in-vitro phantom study, we achieved a relative flat intensity profile over entire absorption range compared to over 80% drop at the highest absorption level before correction. Similar performance was also observed in an ex-vivo tissue study. However, lengthy image acquisition and image processing make this method ideal for static imaging instead of video-rate imaging. To achieve video-rate correction, we developed an experimental approach to reduce absorption by limiting the imaging depth using a high spatial frequency pattern. The absorption reduced fluorescence image was obtained by performing a simple demodulation. The in-vitro phantom study suggested an approximate 20% intensity drop at the highest absorption level compared to over 70% intensity drop before correction. This approach enabled video-rate attenuation corrected imaging at 19 fps, making this technique viable for clinical image guided surgery.

9696-7, Session 2

The benefits of paired-agent imaging in molecular-guided surgery: an update on methods and applications (Invited Paper)

Kenneth M. Tichauer, Illinois Institute of Technology (United States)

One of the major complications with conventional imaging-agent-based molecular imaging, particularly for cancer imaging, is variability in agent delivery and nonspecific retention in biological tissue. Such factors can account to “swamp” the signal arising from specifically bound imaging agent, which is presumably indicative of the concentration of targeted biomolecule. In the 1950s, Pressman et al. proposed a method of accounting for these delivery and retention effects by normalizing targeted antibody retention to the retention of a co-administered “untargeted”/control imaging agent [1]. Our group resurrected the approach within the last 5 years, finding ways to utilize this so-called “paired-agent” imaging approach to directly quantify biomolecule concentration in tissue (in vitro, ex vivo, and in vivo) [2]. These novel paired-agent imaging approaches capable of quantifying biomolecule concentration provide enormous potential for being adapted to and optimizing molecular-guided surgery, which has a principle goal of identifying distinct biological tissues (tumor, nerves, etc...) based on their distinct molecular environment. This presentation will cover the principles and nuances of paired-agent imaging, as well as the current status of the field and future applications.


9696-8, Session 2

Real-time multispectral fluorescence lifetime values estimation and overlay onto tissue white-light video frames

Dimitris S. Gorpas, Dinglong M. Ma, Julien Bec, Diego R. Yankelevich, Laura Marcu, Univ. of California, Davis (United States)

Fluorescence lifetime imaging has been shown to be a robust technique for tissue biochemical and/or functional characterization. Fluorescence lifetime measurements present great potential for intraoperative tissue diagnosis and guidance of surgical procedures. We report a novel technique for real-time mapping of fluorescence parameters (i.e. lifetime values) onto the location from where the fluorescence measurements were taken. This is achieved by merging a 450 nm aiming beam generated by a diode laser with the excitation light in a single delivery/collection fiber and by continuously imaging the region of interest with a color CMOS camera. The interrogated locations are then extracted from the acquired frames via color-based segmentation of the aiming beam. Assuming a Gaussian profile of the imaged aiming beam, the segmentation results are fitted to ellipses that are dynamically scaled at the full width of three automatically estimated thresholds (50%, 75%, 90%) of the Gaussian distribution’s maximum value. This enables the dynamic augmentation of the white-light video frames with the corresponding fluorescence decay parameters. A fluorescence phantom and fresh tissue samples were used to evaluate this method with motorized and hand-held scanning measurements. At 640x512 pixels resolution the area of interest augmented with fluorescence decay parameters can be imaged at an average 34 frames per second. The developed method has the potential to become a valuable tool for real-time display of optical spectroscopy data during continuous scanning applications that subsequently can be used for tissue characterization and diagnosis.

9696-9, Session 2

A compact bio-inspired visible/NIR imager for image-guided surgery

Shengkui Gao, Misael Garcia, Chris Edmiston, Washington Univ. in St. Louis (United States); Timothy York, Southern Illinois Univ. Edwardsville (United States); Radoslav Marinov, Suman B. Mondal, Washington Univ. in St. Louis (United States); Nan Zhu, College of Optical Sciences, The Univ. of Arizona (United States); Gail P. Sudlow, Walter J. Akers, Julie A. Margenthaler, Washington Univ. School of Medicine in St. Louis (United States); Rongguang Liang, Marta Pepino, Washington Univ. in St. Louis (United States); Viktor Gruev, Washington Univ. in St. Louis (United States)

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A compact bio-inspired visible/NIR imager for image-guided surgery

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Inspired by the visual system of the morpho butterfly, we have designed, fabricated, tested and clinically translated an ultra-sensitive, light weight and compact imaging sensor capable of simultaneously capturing near infrared (NIR) and visible spectrum information. The visual system of the morpho butterfly combines photosensitive cells with spectral filters at the receptor level. The spectral filters are realized by alternating layers of high and low dielectric constant, such as air and cytoplasm. We have successfully mimicked this concept by integrating pixelated spectral filters, realized by alternating silicon dioxide and silicon nitrate layers, with an array of CCD detectors. There are four different types of pixelated spectral filters in the imaging plane: red, green, blue and NIR. The high optical density (OD) of all spectral filters (OD>4) allow for efficient rejections of photons from unwanted bands. The single imaging chip weighs 20 grams with form factor of 5mm by 5mm.

The imaging camera is integrated with a goggle display system. A tumor targeted agent, LS301, is used to identify all spontaneous tumors in a transgenic PyMT murine model of breast cancer. The imaging system achieved sensitivity of 98% and selectivity of 95%. We also used our imaging sensor to locate sentinel lymph nodes (SLNs) in patients with breast cancer using indocyanine green tracer. The surgeon was able to identify 100% of SLNs when using our bio-inspired imaging system, compared to 93% when using information from the lymphotropic dye and 96% when using information from the radioactive tracer.

9696-10, Session 3

Optical probes for molecular-guided surgery with photodestruction of residual microscopic tumors and controlled drug-release to suppress multiple molecular signaling pathways of treatment escape (Invited Paper)

Bryan Q. Spring, R. Bryan Sears, Lei Z. Zheng, Zhiming Mai, Massachusetts General Hospital (United States); Reika Watanabe, Elizabeth Villa, Univ. of California, San Diego (United States); Tayyaba Hasan, Massachusetts General Hospital (United States)

Residual tumor deposits missed by conventional treatments frequently seed local and distal recurrence utilizing a network of molecular signaling mechanisms. Beyond providing contrast for molecular-guided surgery, this talk will highlight new concepts in phototherapy to address residual cancer cells in danger zones of recurrence, including selective treatment of microscopic disease using molecular-targeted, activatable immunonjugates, and photo-initiated release of multikinase inhibitors that suppress multiple modes of tumor escape using optically active nanoparticles. These new approaches support an expanded role for the use of light in fluorescence-guided surgery—for phototherapy and for focused drug release to maximize tumor debulking with suppression of disease recurrence.

9696-11, Session 3

Engineered antibodies for optical imaging (Invited Paper)

Anna M. Wu, Univ. of California, Los Angeles (United States)

Antibodies have formed the basis of a spectrum of in vivo targeting approaches for detection and treatment of disease. Protein engineering has been employed to develop cancer-specific antibodies for clinical use as imaging agents, including the generation of engineered antibody fragments with optimized pharmacokinetics. Bioluminescence imaging has been enabled through fusion of engineered fragments to luciferases; alternatively, cysteine residues have been introduced to facilitate site-specific conjugation of fluorescent dyes. Preclinical examples of whole body imaging or intraoperative visualization confirm the potential utility, and translatability, of antibody-based optical probes.

9696-12, Session 3

In-vivo fluorescence lifetime imaging for monitoring the efficacy of HER2 expressing tumors (Invited Paper)

Yasaman Ardestipour, Viktor V. Chernomordik, Moinuddin Hassan, Rafal Zielinski, Jacek Capala, Amir Gandjbakhche, National Institutes of Health (United States)

No Abstract Available

9696-13, Session 3

Fluorescent probes for pancreatic cancer margin assessment in the operating room

Dianmu Zhang, Emily Schultz, Summer L. Gibbs, Oregon Health & Science Univ. (United States)

With a 5-year survival rate of only 6%, pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal cancers. The only curative treatment for PDAC is surgery where cure is directly related to margin status, and incomplete resections are considered palliative at best. Unfortunately, complete resection with negative margins remains difficult as 15-85% of patients are left with residual disease following surgery, due to limited margin assessment methods, which include direct visual and palpation as well as fast frozen sectioning in pathology. Intraoperative fluorescent imaging has the potential to significantly increase surgical success rate by improving margin assessment, but there are no available contrast agents to specifically identify PDAC cells. The goal of the current work is to develop fluorescent probes specific for cancer cells with altered expression levels of epidermal growth factor receptor (EGFR), which can facilitate precise assessment of resected pancreas specimens in the operating room. We have synthesized fluorescent derivatives of small molecule EGFR inhibitors including Gefitinib, Erlotinib and PD168393 for application to the resected tissue in the operating room. To differentiate between specific and nonspecific ex vivo tissue uptake, spectrally distinct non-specific probes were also synthesized for ratiometric imaging to quantify EGFR uptake. Specificity was tested on human pancreatic cancer cell lines with various EGFR expression levels. Competitive titrations were performed to determine the binding affinity of these fluorophore-conjugated molecules in comparison to their parent compounds. Cumulating studies were performed on resected human pancreas tissues from Whipple procedures to demonstrate differentiation of PDAC from benign pancreas.

9696-14, Session 3

Porphyrin lipid nanoparticles for enhanced photothermal therapy (PTT) in a patient-derived orthotopic pancreas xenograft cancer model

Christina MacLaughlin, Lili Ding, Cheng Jin, Pinjiang Cao, Juan Chen, Brian C. Wilson, Gang Zheng, David W. Hedley, Univ. Health Network (Canada)

The delivery of light-based therapies is advantageous for treatment of pancreatic cancer because surgery is highly invasive and radiotherapy cannot be delivered in curative doses. Despite the promise of PTT for...
ablation of pancreatic tumors, current studies lack clinical relevance for absence of a realistic model. Using photothermal sensitizers in combination with laser light for PTT can increase efficiency of conversion of light energy to heat, and confinement of thermal destruction to the tumor. Porphyrins have been previously employed as PDT and PTT photosensitizers, however their incorporation in to “porphysomes”, ordered lipid nanoparticles each containing ~80,000 porphyrins through conjugation of pyropheophorbide to phospholipids, carries two distinct advantages: 1) formation of an ordered macromolecular porphyrin structure imparts nanoparticles with enhanced photonic properties for imaging and therapy; 2) enhanced permeability and retention effect is exploited for optimal delivery of porphysomes to the tumor region thus high payload delivery. A patient-derived orthotopic pancreas xenograft tumor model was used to study feasibility of porphysome-enhanced PTT for pancreatic cancer treatment. Uptake of porphysomes by the orthotopic tumor was validated using ex vivo fluorescence imaging, and fluorescence measurement from homogenized tissues. PTT progress was monitored as changing tumor surface temperature using IR optical imaging. Histological analyses examined microstructure changes in tissue morphology, and tumor tissue viability following heat exposure. These studies provide insight into the biodistribution of porphysomes in a clinically relevant model of pancreatic cancer, and the relationship to PTT specificity and efficacy.

9696-15, Session 4
Optical surgical navigation for nodal staging: to see or not to see? (Invited Paper)
Eva M Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)
No Abstract Available

9696-16, Session 4
Lambertian characteristic of tissue phantoms used as near infrared imaging fluorescence calibrator
Maritoni Litorja, National Institute of Standards and Technology (United States); Simon G. Lorenzo, Louisiana State Univ. (United States); Banghe Zhu, Eva M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)
A stable solid tissue phantom consisting of quantum dots homogeneously dispersed with titanium dioxide in polyurethane matrix has been successfully used as a convenient calibrator to track near infrared fluorescence (NIRF) imaging instrument performance. It becomes more valuable as a quantitative tool when calibrated to provide the imager an optical response scale that is traceable to the International System of Units (SI). The use of SI is highly beneficial as it allows measurement comparisons across instruments, across time and locales. This facilitates assessment of the technology by the developers of both the optical systems and the molecular probe being measured remotely, by regulators and end users. The tissue phantom in this application is used effectively as a reference light source, thereby allowing the user to evaluate an imager’s capability as a light detector. Since the tissue phantom is being used as a reference material for measurement traceability to the SI, it needs to be examined for various characteristics that affect its utility as a standard. Here we examine the radiometric characteristics of the solid tissue phantom as a light source; specifically its property as a Lambertian source. This allows automatic corrections to be made as the imager-to-source geometric position during use in the clinic may vary from the calibrated position. The results are compared to that of a reference lamp typically used to calibrate photodetectors and imagers.

9696-17, Session 4
Goggle augmented imaging and navigation system (GAINS) for real-time fluorescence image-guided oncologic surgery
Suman B. Mondal, Shengkui Gao, Washington Univ. in St. Louis (United States); Nan Zhu, College of Optical Sciences, The Univ. of Arizona (United States); Gail P. Sudlow, Walter J. Akers, Washington Univ. School of Medicine in St. Louis (United States); Ryan C. Fields, Washington Univ. in St. Louis (United States); Julie A. Margenthaler, Washington Univ. School of Medicine in St. Louis (United States); Viktor Gruve, Washington Univ. in St. Louis (United States); Samuel Achilefu, Washington Univ. School of Medicine in St. Louis (United States)
Surgeons rely on visual and tactile evaluation to distinguish cancerous tissue from healthy tissue during oncologic surgery, which are insufficient for interrogating tumor boundaries and detecting very small tumor nodules. As a result oncologic surgery often suffers from incomplete tumor removal that increases the chances of cancer recurrence and requires repeat surgery. To overcome these challenges we, we have developed a wearable goggle augmented imaging and navigation system (GAINS) that can provide real-time intraoperative image guidance for oncologic surgery without disrupting the normal surgical workflow. GAINS detects near-infrared fluorescence from contrast agents accumulated in the tumors or sentinel lymph nodes as well as the color reflectance image from the tissue. GAINS projects accurately aligned color-fluorescence images to a head-mounted display worn by the surgeons without latency, allowing intraoperative visualization of tumors, and sentinel lymph nodes, including tumor boundaries and small nodules not otherwise apparent. Aided by tumor-targeted contrast agent, GAINS provided real-time image guidance for complete tumor resection in subcutaneous and metastatic mouse models of cancer with high sensitivity (100%) and specificity (98% ±7% standard deviation). Using indocyanine green contrast in human breast cancer and melanoma patients, GAINS detected sentinel lymph nodes with high sensitivity (100%). GAINS-guided tumor resection and sentinel lymph node mapping has the potential for improving surgical outcomes in oncologic surgery, decreasing the rate of repeat surgery and improve the accuracy of cancer staging.

9696-18, Session 4
A projective surgical navigation system for cancer resection
Qi Gan, Dong Wang, Jian Ye, Ze Shu Zhang, Xin R. Wang, Peng Fei Shao, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)
Invisible near infrared (NIR) fluorescence imaging technology can guide surgeons with precise and real-time location information of a tumor during cancer resection surgery. However, many intraoperative fluorescence imaging systems are based on wearable devices or stand-alone displays, leading to distraction of the surgeons and suboptimal outcome. To overcome these limitations, we design a projective fluorescence imaging system for surgical navigation. The system consists of a LED excitation light source, a monochromatic CCD camera, a host computer, a mini projector and a CMOS camera. A software program is written by C++ to call OpenCV functions for calibrating and correcting fluorescence images captured by the CCD camera upon excitation illumination of the LED source. The images are projected back to the surgical field by the mini projector. Imaging performance of this projective navigation system is characterized in a tumor surgery
The use of fluorescence imaging two aiding oncologic surgery is a fast growing field and biomedical imaging, revolutionizing open and minimally invasive surgery practices. We have designed, constructed, and tested a system for fluorescence image acquisition and direct display on the surgical field for fluorescence guided surgery. The system uses a near-infrared sensitive CMOS camera for image acquisition, a near-infra LED light source for excitation, and DLP digital projector for projection of fluorescence image data onto the operating field in real time. Instrument control was implemented in Matlab for image capture, processing of acquired data and alignment of image parameters with the projected pattern. Accuracy of alignment was evaluated statistically to demonstrate sensitivity to small objects and alignment throughout the imaging field. After verification of accurate alignment, feasibility for clinical application was demonstrated in large animal models of sentinel lymph node biopsy. Indocyanine green was injected subcutaneously in Yorkshire pigs at various locations to model sentinel lymph node biopsy in gynecologic cancers, head and neck cancer, and melanoma. Fluorescence was detected by the camera system during operations and projected onto the imaging field, accurately identifying tissues containing the fluorescent tracer at up to 15 frames per second. Fluorescence information was projected as binary green regions after thresholding and denoising raw intensity data. Promising results with this initial clinical scale prototype provided encouraging results for the feasibility of optical projection of acquired luminescence during open oncologic surgeries.

9696-20, Session 4
Intraoperative vascular imaging with augmented microscopy
Jeffrey R. Watson, The Univ. of Arizona (United States); Nikolay Martirosyan, Banner Univ. Medical Ctr. (United States); Summer Garland, The Univ. of Arizona (United States); Michael Lemole Jr., Banner Univ. Medical Ctr. (United States); Marek Romanowski, The Univ. of Arizona (United States)

A common difficulty in intracranial vascular angiography is the inability to simultaneously visualize and manipulate vascular anatomy upon near-infrared contrast injection. We previously introduced the augmented microscope with near-infrared fluorescence detection. Here we report on application of augmented microscopy in guiding vascular procedures in neurosurgery. We used normal Wistar rats to represent normal intracranial vasculature and orthotopically implanted C6 cells to represent vascular anomalies. Two near-infrared agents were compared, FDA approved indocyanine green (ICG) and gold-coated, plasmon-resonant liposomes incorporating lipophilic near-infrared dye (Au-IR-liposomes), developed earlier in our laboratory. While ICG is standard in clinical angiography, the Au-IR-liposomes present a new class of light-activated functional nanoparticles that enable spatially and temporally controlled delivery of pharmaceuticals or activating photothermal ablation. We demonstrated performance of augmented microscopy in several surgical procedures including craniotomy, intracranial angiography, and tumor resection. Augmented images of near-infrared contrast agent distribution in vasculature, as seen by the operating surgeon, were acquired through the microscope optical path. Vascular imaging under augmented microscopy guidance was compared to current surgical image guidance technique, i.e., standard bright-field microscopy. The augmented fluorescence channel improved accuracy of locating vessels of interest and reduced surgical time through a more efficient image guidance system. Though the vascular system remains the main route for delivering contrasts and therapies, we envision augmented microscopy being applied to agents that extravasate or localize to various solid tissues. In combination with new types of functional contrast agents, augmented microscopy will lead to improved surgical outcomes and new image guided surgery opportunities.

9696-21, Session 4
A portable fluorescence microscopic imaging system for cholecystectomy
Jian Ye, Chao Y. Yang, Qi Gan, Univ. of Science and Technology of China (China); Rong Ma, Chongqing Medical Univ. (China); Ze Shu Zhang, Peng Fei Shao, Shiwu Zhang, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

In this paper we proposed a portable fluorescence microscopic imaging system to prevent iatrogenic biliary injuries from occurring during cholecystectomy due to misidentification of the cystic structures. The system consisted of a light source module, a CMOS camera, a credit-card sized computer (Raspberry Pi) and 5 inch HDMI LCD. Specifically, the light source module was composed of 690nm and 850nm LEDs, allowing the CMOS camera to simultaneously acquire both fluorescence and background images. The system was controlled by the Raspberry Pi using Python programming with the OpenCV library under Linux. We chose Indocyanine green (ICG) as a fluorescent contrast agent and then tested fluorescence intensities of the ICG aqueous solution at different concentration levels by our fluorescence microscopic system compared with the commercial Xenogen IVIS system. The spatial resolution of the proposed fluorescence microscopic imaging system was measured by a 1951 USAF resolution target and the dynamic response was evaluated quantitatively with an automatic displacement platform. Finally, we verified the technical feasibility of the proposed system in mouse models of bile duct, performing both correct and incorrect gallbladder resection. Our experiments showed that the proposed system can provide clear visualization of the confluence between the cystic duct and common bile duct or common hepatic duct, suggesting that this is a potential method for guiding cholecystectomy. The proposed portable system only cost a total of $300, potentially promoting its use in resource-limited settings.

9696-500, Session HT
Targeted fluorescence image-guided surgery
Heather Franklin, Blaze Bioscience, Inc. (United States)
No Abstract Available
Fluorophore-conjugated antibodies for imaging and resection of GI tumors (Invited Paper)

Michael Bouvet, Univ. of California, San Diego (United States); Robert M. Hoffman, Univ. of California, San Diego (United States) and AntiCancer, Inc. (United States)

Introduction: Negative surgical margins are critical to prevent recurrence in cancer surgery. This is because with current technology in many cases negative margins are impossible due the inability of the surgeon to detect the margin. Our laboratory has developed fluorophore-labeled monoclonal antibodies to aid in cancer visualization in orthotopic nude mouse models of human gastrointestinal (GI) cancer in order to achieve negative margins in fluorescence-guided surgery (FGS). Methods: Tumor specific antibodies were conjugated with fluorophores of visible and near-infrared wavelengths. Orthotopic primary and metastatic human GI tumors in nude mouse models were readily visualized with fluorescence imaging after administration of fluorophore conjugated tumor specific antibodies. Results: The fluorescence signal was detectable 30 minutes after systemic antibody delivery and remained present for two weeks, with minimal in vivo photobleaching after exposure to standard operating room lighting. There was greatly improved ability to resect labeled tumor tissue using FGS. Comparison of different fluorophores revealed differences in sensitivity and photobleaching in vivo. Conclusions: These results indicate that fluorophore-labeled tumor specific antibodies enable enhanced visualization of tumors for FGS of GI cancers when tumor specific antibody expression is present, and that the choice of fluorophore significantly affects the signal intensity in the labeled tumor. The technologies described herein have the potential to change the paradigm of surgical oncology to engender significantly improved outcomes.

Fluorescence-based enhanced reality (FLER) for real-time estimation of bowel perfusion in minimally-invasive surgery (Invited Paper)

Michele Diana, IHU Strasbourg (France)

No Abstract Available

Affibody in fluorescence-guided surgery of glioma to mark the extent based upon tumor receptors

Ana Luiza Ribeiro de Souza, Thayer School of Engineering at Dartmouth (United States) and CAPES Foundation (Brazil); Kayla Marra, Jason R. Gunn, Jonathan T. Elliott, Thayer School of Engineering at Dartmouth (United States); Kimberley S. Samkoe, Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States) and Geisel School of Medicine (United States); Daniel R. Draney, LI-COR Biosciences (United States); Joachim Feldwisch, Affibody AB (Sweden)

The key to fluorescence guided surgical oncology is the ability to create specific contrast between normal and glioma tissue. The blood brain barrier that limits the delivery of substances to the normal brain is broken in tumors, allowing accumulation of agents in the tumor interior. However, for a clinical success, imaging agents should be in the infiltrative edges to minimize the resection of normal brain while enable the removal of tumor. The aberrant overexpression and/or activation of EGFR is associated with many types of cancers, including glioblastoma and the injection of a fluorescent molecule targeted to these receptors would improve tumor contrast during fluorescence guided surgery. Affibody molecules have intentional medium affinity and high potential specificity, which are the desirable features of a good surgical imaging agent. The aim of this study was evaluate the brain/glioma uptake of ABY029 labeled with near-infrared dye IRDye800CW after intravenous injection. Rats were either inoculated with orthotopic implantations of U251 human glioma cell line or PBS (shams control) in the brain. The tumors were allowed to grow for 2-3 weeks before carrying out fluorescent tracer experiments. Fluorescent imaging of ex vivo brain slices from rats was acquired at different time points after injection of fluorescently labeled EGFR-specific affibody to verify which time provided maximal contrast tumor to normal brain. Although the tumor was most clearly visualized after 1h of IRDye800CW-labeled ABY029 injection, the tumor location could be identified from the background after 48h. These results suggest that the NIR-labeled affibody examined shows excellent potential to increase surgical visualization for confirmed EGFR positive tumors.

Direct administration of nerve-specific fluorophores to guide nerve-sparing radical prostatectomy

Connor Barth, Summer L. Gibbs, Oregon Health & Science Univ. (United States)

Prostate cancer cure is the primary goal of radical prostatectomy, however preserving the nerve structures responsible for continence and potency are vital for maintained quality of life. Although the nerve-sparing surgical technique was developed over 30 years ago, nerve damage following radical prostatectomy continues to plague surgical treatment and is reported in some form in up to 60% of patients one to two years post surgery. Surprisingly, no method exists to enhance direct nerve visualization in the surgical suite, and nerve detection is completed through a combination of palpation and visualization when possible. Importantly, a few classes of fluorescent small molecules have recently been demonstrated to have nerve specificity following systemic administration including the distyrylbenzenes (DSB), select oxazines (oxazine 4 perchlorate), and certain cyanines (3,3’-diethylthiatricarbocyanine iodine). However, the prostate is a highly innervated organ, where direct labeling of the cavernous nerve and neurovascular bundle (NVB) would provide significantly improved imaging contrast by comparison to systemically labeling all nerve structures in the gland. In the current work, a direct administration methodology was optimized for application of an effective nerve-specific fluorophore, oxazine 4 perchlorate, during nerve-sparing radical prostatectomy. Fluorophore concentration, formulation, incubation time, and tissue washing procedures were optimized to enhance nerve signal to background ratio in rodent models. Optimization of the direct administration procedure yielded nerve signal-to-background ratios equal to or greater than systemic administration. These promising results will be scaled to more representative rodent and swine nerve models enabling imaging of the cavernous nerve and NVB using the optimized direct administration procedure.

Detection of breast positive surgical margins with fluorescence-guided microscopy imaging

Nicușor V. Iftimia, Dorin Preda, Jesung Park, Mitchell Antalek, Physical Sciences Inc. (United States)

We present a novel technology based on a high sensitivity/specificity cancer targeting agent and of a novel fluorescence-guided microscopy (FGM)
scheme for intraoperative assessment of surgical margins in breast cancer patients. Cancer cells are targeted using an optically silent peptide substrate coupled to a near infrared (NIR) fluorochrome that is cleaved by highly mediated breast cancer enzymes, like urokinase-type plasminogen activator (uPA), to become highly fluorescent when excited by a NIR laser beam. A FGM instrument is used to localize cancer-suspect areas on the lumpectomy specimen and visualize tissue morphology at the sub-cellular scale, such that a trained pathologist can read these images in real-time and confirm or rule-out cancer presence. The proposed technology will enable efficient assessment of surgical specimens during surgery, when it is mostly needed, and therefore help the clinician to determine if additional tissue excision is needed or not. The preliminary testing of this technology on breast surgical specimens will be discussed.

9696-27, Session 5

Quantitative in vivo immunohistochemistry for tumor margin assessment in head and neck surgical resection
Kimberley S. Samkoe, Geisel School of Medicine (United States); Kenneth M. Tichauer, Illinois Institute of Technology (United States); Eunice Chen, Geisel School of Medicine (United States); Jason R. Gunn, Thayer School of Engineering at Dartmouth (United States); P. Jack Hoopes, Wendy A. Wells, Geisel School of Medicine (United States); Tayyaba Hasan, Wellman Ctr. for Photomedicine (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

Ninety percent of patients with head and neck squamous cell carcinomas (HNSCC) have overexpression of epidermal growth factor receptor (EGFR), which is correlated with poor prognosis. Complete surgical resection of HNSCC tumors has a large impact on patient survival, where detection of tumor at or close to surgical margins increases the risk of death at 5-years by 90%. In addition, large surgical margins can greatly increase the morbidity experienced by the patient due to functional and cosmetic damage of oral and facial structures. Single fluorescence targeting agents are often used for tumor detection in in vivo pre-clinical imaging; however, the arising signal is qualitative at best because it is a complex mixture of vascular perfusion, vascular leakage, inhibited lymphatic clearance, and receptor binding. In vivo ratiometric receptor concentration imaging (RCI) allows quantification of receptor expression (hence identification of cancerous tissue) by utilizing co-administered paired-agents consisting of a targeted agent and non-targeted perfusion agent to reference the plasma delivery and leakage. A panel of HNSCC tumors with varying levels of EGFR expression (SCC-15 > SCC-25 > SCC-09) have been imaged using ABY-029, a clinically relevant anti-EGFR affibody labeled with IRDye 800CW, and affibody control imaging agent labeled with IRDye 680RD. RCI maps of in vivo tissue have been created and are spatially correlated with EGFR and CD31 immunohistochemistry and basic H&E staining. The RCI threshold parameters for distinguishing tumor from normal tissues (skin and muscle) and the accuracy of margin detection in these tumors will be presented. RCI surgical resection will be further developed using a novel multi-channel, gated fluorescence-guided surgery (FGS) imaging system that is capable of performing RCI in normal room light.

9696-28, Session 6

Optical contrast agents to guide surgical ablation and pathological evaluation of resected tissues (Invited Paper)
Eben L. Rosenthal, Stanford Univ. (United States); Jason M. Warram, The Univ. of Alabama School of Medicine (United States)

Surgical contrast agents are being developed for surgical navigation, but the opportunity for multimodality imaging of tissues throughout the process of resection and pathological imaging has not been defined. Using fluorescently labeled anti-EGFR antibodies we have used multimodality imaging strategies to guide surgery and reduce sampling error in pathology.

9696-29, Session 6

BLZ-100 tumor fluorescent reporter (Invited Paper)
Heather Franklin, Blaze Bioscience, Inc. (United States)
No Abstract Available

9696-30, Session 6

Comparison of lymphatic uptake and active pumping patterns for small and large sized fluorescent molecules imaged in vivo
Alisha V. DSouza, Jason R. Gunn, Kayla Marra, Jonathan T. Elliott, Thayer School of Engineering at Dartmouth (United States); Kimberley S. Samkoe, Geisel School of Medicine (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

With increasing shift towards targeted therapy and intra-lymphatic drug delivery, there is growing need to study the delivery and characteristics of tracer/drug uptake within such systems. The lymphatic system and vasculature is often looked at as the under-studied counterpart of the blood circulation system and models to study it are still rudimentary owing to limited real-time in vivo imaging capability. NIR fluorescence imaging has recently emerged as a promising imaging modality with high sensitivity to small dye volumes, and can produce high spatial and temporal resolution images of shallow lymphatic vessels and nodes in preclinical and clinical settings. These advantages combined with the ability to deliver dyes intra-lymphatically allows almost infinite contrast-to-background ratios with micro doses of tracers, and eliminates the need for systemic administration, thus preventing harmful toxicity effects.

Our goal was to quantitatively study and compare various parameters of uptake of differently sized fluorescent tracers through the collector lymphatics from injection site to the downstream lymph nodes. Far-red and near-infrared fluorescent dyes were injected intradermally into forepaws of mice to visualize sections of the lymphatic vasculature especially the collecting lymphatics and lymph nodes using a broad-beam microscopic dual channel planar commercial fluorescence imager. We demonstrate functional lymphatic imaging in the forelimb and axilla of mice and compare transport kinetics and pumping by intrinsic lymphatic contractility during the uptake of small and large untargeted fluorescent tracers, namely Methylene Blue:BSA and Mouse IgG Isotype Control-IRDye680 RD, which have a size ratio 1:3 respectively. We found that when injection conditions were consistent large variability existed in overall fluorescent dye uptake, which is significantly slower for large antibody tracers (factor of 10, p=0.02) than smaller protein tracers that are used more commonly in the clinic today. However, collecting lymph vessel pulsatile contraction appears to be unaffected by molecule size and is 3.67 pumps/min and 3.13 pumps/ min respectively and the difference between the groups is not statistically significant (p =0.18).
A standardized model for predicting flap failure using indocyanine green dye

Lindsay Moore, The Univ. of Alabama School of Medicine (United States); Terence M. Zimmermann, Mayo Clinic (United States); Jason M. Warram, The Univ. of Alabama School of Medicine (United States); Benjamin Greene, The Univ. of Alabama at Birmingham School of Medicine (United States); Melissa L. Korb, The Univ. of Alabama School of Medicine (United States); Eben L. Rosenthal, Stanford Univ. (United States)

Objective Techniques that provide a non-invasive method to evaluate intraoperative skin flap perfusion are currently available but underutilized. We hypothesize that intraoperative vascular imaging can be used to reliably assess flap perfusion and elucidate areas of future necrosis by means of a standardized critical perfusion threshold.

Methods Five animal groups (negative controls, n=4; positive controls, n=5; chemotherapy, n=5; radiation, n=5; chemoradiation, n=5) underwent pre-flap treatments two weeks prior to undergoing random pattern dorsal fasciocutaneous flaps with a length to width ratio of 2:1 (3 x 1.5 cm). Flap perfusion was assessed via laser-assisted indocyanine green (ICG) dye angiography using an open-field, near infrared fluorescence imaging device and compared to standard clinical assessment for predictive accuracy of flap necrosis.

Results For estimating flap failure, clinical prediction achieved a sensitivity of 79.3% and specificity of 90.5%. When average flap perfusion was more than three standard deviations below the average flap perfusion for the negative control group at the time of the procedure (144.3±17.05 absolute perfusion units), laser-assisted ICG angiography achieved a sensitivity of 81.1% and specificity of 97.3%. When absolute perfusion units (APUs) were seven standard deviations below the average flap perfusion for the negative control group, the specificity of necrosis prediction was 100%.

Conclusions This study yielded a standardized, quantitative method of assessing flap perfusion using APUs and standard deviations from an estimated normal perfusion baseline. APUs can improve the specificity of intraoperative prediction of tissue viability, and a positive predictive threshold of flap failure can be standardized for clinical use.

Near-infrared (NIR) fluorescence imaging of head and neck squamous cell carcinoma for fluorescence-guided surgery

Lindsay Moore, Jason M. Warram, Esther de Boer, William R. Carroll, Anthony Morlanndt, Kirk P. Withrow, The Univ. of Alabama School of Medicine (United States); Eben L. Rosenthal, Stanford Univ. (United States)

During fluorescence-guided surgery, a cancer-specific optical probe is injected and visualized using a compatible device intraoperatively to provide visual contrast between diseased and normal tissues to maximize resection of cancer and minimize the resection of precious adjacent normal tissues. Six patients with squamous cell carcinomas of the head and neck region (oral cavity (n=4) or cutaneous (n=2)) were injected with an EGFR-targeting antibody (Cetuximab) conjugated to a near-infrared (NIR) fluorescent dye (IRDye800) 3, 4, or 7 days prior to surgical resection of the cancer. Each patient’s tumor was then imaged using a commercially available, open-field NIR fluorescence imaging device each day prior to surgery, intraoperatively, and post-operatively. The mean fluorescence intensity (MFI) of the tumor was calculated for each specimen at each imaging time point. Adjacent normal tissue served as an internal anatomic control for each patient to establish a patient-matched “background” fluorescence. Resected tissues were also imaged using a closed-field NIR imaging device. Tumor to background ratios (TBRs) were calculated for each patient using both devices. Fluorescence histology was correlated with traditional pathology assessment to verify the specificity of antibody-dye conjugate binding. Peak TBRs using the open-field device ranged from 2.2 to 11.3, with an average TBR of 4.9. Peak TBRs were achieved between days 1 and 4. This study demonstrated that a commercially available NIR imaging device suited for intraoperative and clinical use can successfully be used with a fluorescently-labeled dye to delineate between diseased and normal tissue in this single cohort human study, illuminated the potential for its use in fluorescence-guided surgery.
This Proof-of-Concept study comprises 10 patients. Currently, seven patients are included and have been analyzed. The peritoneal cancer Index using fluorescence imaging decreases in all patients. A total of 87 lesions were imaged and histologically analyzed. In 31 out of 58 fluorescent lesions (54%) cancerous cells were found during histological analysis. All 23 non-fluorescent lesions were cancer negative. Additionally, in two patients the method detected cancer tissue that was initially missed by inspection and palpation. In one patient this concerned a positive resection margin and in another case a para-aortal lymph node metastasis was found. The Ex vivo fluorescence analysis of paraffin slices of tumor and normal lesions showed a tumor to normal ratio of 6.27. False positive lesions ex vivo were containing foreign bodies.

Intraoperative near-infrared fluorescence imaging of peritoneal carcinomatosis during the HIPEC procedure is technically feasible and safe. The peritoneal cancer index decreases with a mean of 3 points which might indicate overtreatment in selected patients. The high sensitivity (100%) gives the surgeon potentially a real-time tool for intraoperative decision making. Additionally, the technique enables the evaluation of possible positive resection surfaces, which might be of use on a future study concerning locally advanced rectal cancer. We state that these preliminary results are promising to improve future surgical procedures.

9696-36, Session 7

Ongoing advances in quantitative PpIX fluorescence guided intracranial tumor resection

Jonathan D. Olson, Stephen C. Kanick, Jaime J. Bravo, Thayer School of Engineering at Dartmouth (United States); David W. Roberts, Dartmouth Hitchcock Medical Ctr. (United States); Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States)

Aminolevulinc-acid induced protoporphyrin IX (ALA-PpIX) is being investigated as a biomarker to guide neurosurgical resection of brain tumors. ALA-PpIX fluorescence can be observed visually in the surgical field; however, raw fluorescence emissions can be distorted by factors other than the fluorophore concentration. Specifically, fluorescence emissions are mixed with autofluorescence and attenuated by background absorption and scattering properties of the tissue. Recent work at Dartmouth has developed advanced fluorescence detection approaches that return quantitative assessments of PpIX concentration, which are independent of background optical properties. The quantitative fluorescence imaging (qFI) approach has increased sensitivity to residual disease within the resection cavity at the end of surgery that was not visible to the naked eye through the operating microscope.

This presentation outlines clinical observations made during an ongoing investigation of ALA-PpIX based guidance of tumor resection. PpIX fluorescence measurements made in a wide-field hyperspectral imaging approach are co-registered with point-assessment using a fiber optic probe. Data show variations in the measured PpIX accumulation among different clinical tumor grades (i.e. high grade glioma, low grade glioma), types (i.e. primary tumors, metastases) and normal structures of interest (e.g. normal cortex, hippocampus). These results highlight the contrast enhancement and underscore the potential clinical benefit offered from quantitative measurements of PpIX concentration during resection of intracranial tumors.
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9697-85, Session PSun

Degree of polarization (uniformity) and depolarization index: unambiguous depolarization contrast for optical coherence tomography

Norman Lippok, Martin Villiger, Brett E. Bouma, Wellman Ctr. for Photomedicine (United States)

The degree of polarization (uniformity) has attracted increased interest as a functional contrast in optical coherence tomography (OCT). However, its computation from a single polarization state suggests an ambiguity that is strongly dependent on a sample's orientation. We here propose an improved metric to present depolarization with respect to the optical system rather than the propagating field. Using numerical simulations and optical frequency domain imaging, we evaluate the conventional DOP(U) for different polarization states and compare its performance with the unambiguous depolarization index.

9697-86, Session PSun

Self-phase modulation induced spectral broadening in wavelength swept SOA fiber ring lasers

Norman Lippok, Brett E. Bouma, Wellman Ctr. for Photomedicine (United States)

To date, only extended-cavity semiconductor lasers that employ intracavity scanning filters have met the requirements of biomedical imaging applications with fast repetition rates, narrow instantaneous linewidths and broad tuning ranges. While spectral broadening is a well-known behavior of gain saturated SOAs, it has not been investigated in conjunction with a rapidly wavelength swept bandpass filter for OFDI applications. We here revisit the self-phase modulation induced by SOAs and discuss how the frequency shift and spectral broadening is directly linked to the wavelength scanning speed and highlight its contribution to cavity loss and thus laser performance.

9697-87, Session PSun

Extended bandwidth wavelength swept laser source for high resolution optical frequency domain imaging

Sahar Hosseinzadeh Kassani, Changsu Jun, Norman Lippok, Martin Villiger, Brett E. Bouma, Wellman Ctr. for Photomedicine (United States)

Improving the axial resolution by providing wider bandwidth wavelength swept lasers remains a critical issue for Optical Frequency Domain Imaging (OFDI). Here, we demonstrate a wide tuning range all-fiber wavelength swept laser by combining two ring cavities that share a single Fabry-Perot tunable filter for ultrahigh resolution OFDI. The two cavities contain semiconductor optical amplifiers with central wavelengths of 1280 nm and 1220 nm, respectively. By modulating the amplifiers, we obtained consecutive wavelength sweeps in the two spectral regions that were fused together in post-processing to achieve a total scanning range of 220 nm, corresponding to 3.8 μm axial resolution in air.

9697-88, Session PSun

Dual mode-locked swept sources for SS-OCT

Radu F. Stancu, Adrian G. H. Podoleanu, Univ. of Kent (United Kingdom)

A novel dual-mode-locking mechanism was developed in order to tune an akenetic swept source (AKSS) based on dispersive cavity at a repetition rate close to, but slightly different from the inverse of the cavity roundtrip. Several optical source configurations emitting in the 1060 nm or 1550 nm wavelength region were developed, characterized and tested in OCT applications. For the 1550 nm swept source employing a Faraday rotating mirror in a dispersive cavity, sweeping rates in the range of MHz were achieved, from 782 kHz to up to 5 times this value, with proportional decrease in the tuning bandwidth. Linewidths smaller than 60 pm and output powers exceeding a few mW were measured. The 1060 nm swept source implemented was used to generate OCT images of a pressure sensitive adhesive.

9697-89, Session PSun

Band multiplexing of wide-wavelength swept source based on multi-wavelength generation by filterless active mode-locking

Gyeong Hun Kim, Hwi Don Lee, Hyung-Seok Lee, Chang-Seok Kim, Pusan National Univ. (Korea, Republic of)

Recently, a novel wavelength swept laser using active mode locking (AML) technique is shown to generate a wide wavelength band to utilize the whole of gain region of SOA because AML technique is a programmable all-electrical wavelength selection mechanism without any mechanical filter. The high sweep rate over 1 MHz was also demonstrated with this AML swept laser by applying the radio-frequency (RF) modulation signal into the gain medium directly without a wavelength selection filter in the laser cavity. Assuming we can have an over-limit performance of swept laser with ultra-high speed and ultra wide wavelength band, the excessive speed or wavelength region of laser output can be considered to be a useless contribution for OCT imaging. Therefore, it is required to consider the novel multiplexing methods to divide the repetition speed or wavelength region of swept laser for the effective utilization for OCT imaging system.

Here, we propose a new light source for multi-wavelength generation based on filter-less AML laser and the possibility of multi-OCT imaging applications through the band multiplexing method. In the AML laser, the selection of lasing wavelength is determined by the RF frequency of modulation signal on the gain medium and the free spectral range (FSR) of each wavelength of the laser cavity. We demonstrate a multi-wavelength selection by using a modified configuration of multiple optical paths or multiple RF signals for the satisfaction of multiple AML conditions.
9697-90, Session PSun

**Computational point spread function engineering for optical coherence tomography**

Jeffrey A. Mulligan, Steven G. Adie, Cornell Univ. (United States)

In order to address traditional trade-offs in optical imaging with respect to resolution, depth-of-field and signal collection vs. distance from focus, we propose computational point spread function engineering. We show that hardware and computational reduction of NA produce similar away-from-focus OCT resolution. We also show, through experiment and simulation, that phase aberrations affect both the phase and magnitude of OCT signals in the spatial frequency domain. Our results suggest that hardware based and computational point spread function engineering can be used together to yield superior image formation for 3D microscopy than either method alone.

9697-91, Session PSun

**Wavelength dependence of penetration depth of OCT imaging for biological tissue in 0.8-1.9 um wavelength region**

Hiroyuki Kawagoe, Masahito Yamanaka, Norihiko Nishizawa, Nagoya Univ. (Japan)

We investigated the wavelength dependence of the penetration depth of optical coherence tomography (OCT) for water-rich and highly scattering samples in 0.8-1.9 um wavelength region. To compare the broadband wavelength dependence of OCT imaging, we generated supercontinuum (SC) sources in 0.8, 1.1, 1.3, 1.6, 1.7, and 1.9 um wavelength regions based on ultrashort pulse lasers and highly nonlinear fibers, and constructed time-domain OCT systems using the generated SC sources. For quantitative comparison, we used lipid dilutions with different lipid concentrations as biological tissue phantoms. The result shows that the attenuation coefficients in 0.8-1.3 um wavelength regions were significantly increased with increasing the lipid concentration, on the other hand, those in 1.6-1.9 um wavelength regions were almost constant regardless of the lipid concentration. Besides, the deepest penetration was achieved at 1.3 um wavelength for low scattering samples, and in 1.6-1.7 um wavelength regions for highly scattering samples.

9697-92, Session PSun

**Influence of aberrations on the image quality in optical coherence microscopy**

Hinnerk Schulz-Hildebrandt, Mario Pieper, Peter König, Gereon Hüttmann, Univ. zu Lübeck (Germany)

Endoscopic optical coherence microscopy (eOCT) is able to provide cross-sectional images with a few micrometer resolution. Our eOCT device uses 0.45 NA GRIN optics and a supercontinuum light with a custom-made spectrometer covering a bandwidth from 550nm to 950nm. Using GRIN optics at high NA and the ultrabroad bandwidth, the image quality is significantly reduced by chromatic and spherical aberrations. To investigated the influences of spherical and chromatic aberrations on the OCT imaging formation we present an extensive numerical simulations based on numerical beam propagation.

Point spread functions (PSF) were simulated to calculate resolution and depth of focus (DOF). Quality of enface OCT images was assessed by convolution with simulated object structures.

With increasing amount of the spherical aberrations the depth of focus increase while lateral resolution decreased slightly. Axial resolution not change significantly. Also with chromatically length aberration an increase DOF was observed. However the axial resolution declined dramatically caused by the focal shift induced depth dependent damping of parts of the spectrum.

Our rigorous modeling of OCT imaging formation allows systematically evaluation and improvement image quality in low scattering tissues. We found that the aberrations influenced the OCT imaging in a complex way, improving the depth field but also introducing speckle noise and false image structures.

9697-93, Session PSun

**Quantitative OCT model links optical properties to microscale sample organization**

Mitra Almasian, Academisch Medisch Centrum (Netherlands); Nienke Bosschaart, Univ. Twente (Netherlands); Ton G. van Leeuwen, Dirk J. Faber, Academisch Medisch Centrum (Netherlands)

Optical coherence tomography (OCT) derived attenuation and backscattering coefficient may be used as a tool to differentiate between healthy and cancerous tissue. In this work describe the OCT derived parameters for bi-disperse random media (spherical scatters of varying size and concentration). For our model combine Mie-theory with the pair correlation function in the Percus-Yevick approximation and the Extended Huygens Fresnel model to account for multiple scattering.

To validate the proposed model we have compared calculations with OCT experiments. The OCT-signal (Santec InnerVision OCT system, 1300 nm) from samples of bi-disperse silica beads with varying volume fraction was recorded. From the OCT-images the attenuation coefficient and backscattering coefficient were derived by a single exponential fit. Hereafter, the experimental and theoretical values are compared.

We have previously modelled the optical properties of single-sized, discrete random media of varying concentration with this model. We have shown that our model provides an adequate description of experimentally obtained values of the OCT attenuation and backscattering coefficient of mono-disperse samples, demonstrating sensitivity of OCT-derived optical properties to the microscale organization of the samples. Tissue scattering is more complex because of the different sizes of scattering structures involved. As a first step to describe these complex geometries, in this work study suspensions of spheres of two sizes of silica beads as a more appropriate model for tissue scattering. We believe that this is an essential step towards a qualitative expression to link OCT-derived optical properties to clinically relevant morphological properties of tissue.

9697-94, Session PSun

**Coarse-grained and fine-grained parallel optimization for real-time en-face OCT imaging**

Konstantin Kapinchev, Adrian Bradu, Frederick Barnes, Adrian G. H. Podoleanu, Univ. of Kent (United Kingdom)

This paper responds to the recent increased interest in the en-face (C-scan) OCT display. Compared with cross-sectional (B-scan) imaging, the production of en-face images is more computationally demanding, due to the increased size of the data handled by the digital signal processing (DSP) algorithms. A sequential implementation of the DSP leads to a limited number of real-time generated en-face images. There are OCT applications, where simultaneous production of large number of en-face images from multiple depths is required, such as real-time diagnostics and monitoring of surgery and ablation. In sequential computing, this requirement leads to a significant increase of the time to process the data
9697-97, Session PSun

Supercontinuum white light source used to increase sensitivity and resolution of parallel line-field spectral domain optical coherence tomography

Jessica Barrick, The Univ. of North Carolina at Chapel Hill (United States); Michael Gardner, The Univ. of Texas at Austin (United States); Amy L. Oldenburg, The Univ. of North Carolina at Chapel Hill (United States)

We describe a line-field Fourier domain optical coherence tomography (LF FD OCT) system employing a supercontinuum light source to provide greater power and bandwidth. LF FD OCT parallelsizes OCT in comparison to spot-scanning methods by illuminating the sample with a line; a 2D camera subsequently collects laterally-resolved spectral interferograms.

One of the major limitations to LF FD OCT is the spread of optical power across A-lines. To address this limitation, we use a supercontinuum white-light source (NKT Photonics, low-noise EXR-9), filtered to achieve a center wavelength and bandwidth of 800nm and 300nm, respectively, corresponding to a coherence length of 17m. The beam is delivered into a free-space Michelson interferometer and focused into a vertical line on the sample (with beam height 8.6mm and width 12 μm) using a cylindrical lens. Backscattered light from the sample is recombined with the reference beam and dispersed onto a 2D CMOS camera (Photron, Fastcam SA3) by a diffraction grating. LF FD OCT thus allows for depth information from transverse channels to be simultaneously recorded with a B-mode frame rate of 1kHz.

The supercontinuum source allows for up to 400mW to be delivered to the sample, corresponding to ~0.5mW of power per 12μm spot size. While the potential effects of heating in this geometry need to be investigated, this is an order of magnitude increase in power compared to previous publications. This system will enable new applications for ultrahigh-resolution, phase-resolved OCT with higher SNR and/or speed than competing FD OCT technologies.

9697-98, Session PSun

Broadband master/slave interferometry using a super-continuum source

Michael Maria, Manuel J. M. Marques, Christopher Costa, Adrian Bradu, Univ. of Kent (United Kingdom); Thomas Feuchter, Lasse Leick, NKT Photonics A/S (Denmark); Adrian G. H. Podoleanu, Univ. of Kent (United Kingdom)

We report on a demonstration of a broadband master-slave interferometry (MSI) system using a super-continuum (SC) light source. Principle of MSI, based on channeled spectrum comparison, is extended to optical source with bandwidth close to 100 nm around 830 nm. A-scans and B-scan of phantom multi-layer object and finger nail are presented and compared with conventional Fourier domain OCT (FD-OCT) image produced with and without calibration. MSI insensitivity to dispersion from the interferometer and not needs of camera calibration help to handle very broad spectrum with minimum software compensation of system non-linearity. MSI B-scans present constant axial resolution over the entire depth range while conventional (calibrated and un-calibrated) FD-OCT methods are limited by the effect of dispersion. Data calibration improves axial resolution but only over a small depth range for conventional FD-OCT.
9697-99, Session PSun

Theory of Fourier phase within the framework of Fourier-domain optical coherence tomography
Shikhar Uttam, Yang Liu, Univ. of Pittsburgh (United States)

Fourier phase in Fourier-domain optical coherence tomography (FD-OCT) has been shown to estimate the sub-resolution change in the optical depth location of a strong interface in the refractive index profile of a sample using spectral-domain phase microscopy (SDPM), a derivative of FD-OCT. Here we present theoretical results that show Fourier phase not only estimates this sub-resolution change but also the mean spatial frequency of the coherence-gated refractive index profile. We derive this result from first principles, and specifically show how the sub-resolution change and mean spatial frequency are jointly estimated using Fourier phase of FD-OCT. We further show how under certain specific conditions along with a strong interface, Fourier phase reduces to estimating only the sub-resolution change in the depth location of the strong interface. On the other hand, in the slowly-varying refractive index regime we show how Fourier phase can be used to isolate the effect of mean spatial frequency of the coherence-gated refractive index profile. This latter result is the basis for depth-resolved spatial-domain low-coherence quantitative phase microscopy (dr-SLQPM). We, therefore, show that both SDPM and dr-SLQPM are special cases of the general theory of Fourier phase, and in the process elucidate the meaning of Fourier phase in FD-OCT. We provide analytical expressions for Fourier phase in the settings of both a strong interface, and a slowly-varying refractive index. We further provide numerical simulations to support our results.

9697-101, Session PSun

Narrow linewidth broadband tunable semiconductor laser at 840 nm with dual acousto-optic tunable configuration for OCT applications
Alexander Chamorovskiy, Mikhail V. Shramenko, Andrei Lobintsov, Superlum (Ireland); Sergey Yakubovich, Moscow State Institute of Radiotechnics, Electronics and Automation (Russian Federation)

We demonstrate a tunable narrow linewidth semiconductor laser for the 840 nm spectral range. The laser has a linear cavity which is comprised of polarization maintaining (PM) fiber. A Broadband semiconductor optical amplifier (SOA) in in-line fiber-coupled configuration acts as a gain element. It is based on InGaAs quantum-well (QW) active layer. SOA allows for tuning bandwidth exceeding 25 nm around 840 nm. Small-signal fiber-to-fiber gain of SOA is around 30 dB. A pair of acousto-optic tunable filters (AOTF) with a quasi-collinear interaction of optical and acoustic waves are utilized as spectrally selective elements. AOTF technology benefits in continuous tuning, broadband operation, excellent reproducibility and stability of the signal, as well as a high accuracy of wavelength selectivity due to the absence of mechanically moving components. A single AOTF configuration has typical linewidth in 0.05-0.15 nm range due to a frequency shift obtained during each roundtrip. A sequential AOTF arrangement enables instantaneous linewidth generation of <0.01 nm by compensating for this shift. Linewidth as narrow as 0.0036 nm is observed at 846 nm wavelength using a scanning Fabry-Perot interferometer with 50 MHz spectral resolution. Output power is in the range of 1 mW. While the majority of commercial tunable sources operate in 1060-1550 nm spectral ranges, the 840 nm spectral range is beneficial for optical coherence tomography (OCT). The developed narrow linewidth laser can be relevant for OCT with extended imaging depth, as well as spectroscopy, non-destructive testing and other applications.

9697-102, Session PSun

Optical coherence tomography with gapped spectrum
Nanshuo Wang, Xinyu Liu, Xiaojun Yu, Si Chen, En Bo, Yuemei Luo, Dongyao Cui, Linbo Liu, Nanyang Technological Univ. (Singapore)

The axial point spread function (PSF) of optical coherence tomography (OCT) is determined by the detected light spectral shape and bandwidth. However, the extension or shaping of the light spectrum is limited by the laser source, water absorption and the detector response. In most cases, several non-overlapped windows in the spectrum are available for OCT. Conventionally, only one spectral window is selected to be used in imaging to avoid artifacts in axial PSF. In this report, we present to use a gapped data estimation method, the so-called gapped-APES to estimate the missing part between the spectral bands and produce images with reduced artifacts and high resolution, comparable to the full spectrum images. We demonstrated this method by using a light source consisted of two non-overlapped-spectrum superluminescent diodes of 755nm-805nm and 880nm-930nm. A glass-slides stack and polystyrene calibration particles in water were imaged to demonstrate the performance of this algorithm. We proved that this method could produce images with less sideloobe artifacts and higher resolution, comparing to the direct Fourier transform of the gapped spectrum. This technique is promising to bridge various bands used by different OCT systems and thereby obtaining a virtual broadband spectrum in favor of a high axial resolution. In addition, this technique may also help to build low-cost gapped spectrum OCT systems.

9697-103, Session PSun

 Depths-encoded angular compounding for speckle reduction in optical coherence tomography
Zhaoyuan Cao, Jie Qian, Xinjian Chen, Jianhua Mo, Soochow Univ. (China)

Optical coherence tomography (OCT) is one of the successful inventions in medical imaging as a clinic routine in the past decades. This imaging technique is based on low coherence interferometer and consequently suffers from speckle noise inherently, which can degrade image quality and obscure micro-structures. Therefore, effective speckle reduction techniques have been always desired and researched since optical coherence tomography was invented. In this study, we proposed an angular compounding method to reduce speckle noise of OCT image. Two different angular light paths are created on the sample arm using two beam splitters. The epi-detection scheme results in three combinations of the two angular light paths are created on the sample arm using two beam splitters. We demonstrated this method by using a light source consisted of two non-overlapped-spectrum superluminescent diodes of 755nm-805nm and 880nm-930nm. A glass-slides stack and polystyrene calibration particles in water were imaged to demonstrate the performance of this algorithm. We proved that this method could produce images with less sideloobe artifacts and higher resolution, comparing to the direct Fourier transform of the gapped spectrum. This technique is promising to bridge various bands used by different OCT systems and thereby obtaining a virtual broadband spectrum in favor of a high axial resolution. In addition, this technique may also help to build low-cost gapped spectrum OCT systems.

9697-104, Session PSun

Design of a scan-lens and tube-lens for OCT applications
Farid Atry, Ramin Pashaie, Univ. of Wisconsin-Milwaukee (United States)

Optical Coherence Tomography (OCT) is an emerging imaging technology capable of providing tomographic images with micron scale resolution covering up to a few millimeters deep inside a semi-transparent tissue. With
recent advances in the OCT field, this imaging technology is capable of obtaining fast 3D images of microvasculature and hemodynamics in living tissue. There is an increasing demand for combining OCT with other optical techniques such as fluorescence microscopy, or optical neurostimulation methods. To combine different light beams with the OCT beam path, it is necessary to add a scan-lens and tube-lens into an OCT scanning head to open sufficient space for the necessary auxiliary components, such as optical filters and beam splitters. Adding a scan/tube lens has impact on the lateral resolution of the OCT scanner by introducing optical aberrations to the imaging system. In this paper off-the-shelf lenses are used to design inexpensive configurations of intermediary lensing mechanisms and Zemax simulation software is used to estimate optical aberrations in each design. Then the resolution provided by each lens system is measured practically and compared with the simulation results. Our results show that a Plössl-type compound lens can strike a balance between different aberrations over a large field of view while keeping the complexity and price of the intermediary lens mechanism relatively low.

9697-105, Session PSun
**Extending the effective imaging depth in spectral domain optical coherence tomography by dual spatial frequency encoding**

Tong Wu, Qingqing Wang, Youwen Liu, Jiming Wang, Nanjing Univ. of Aeronautics and Astronautics (China)

We present a simple and low-cost method for extending the effective imaging depth range in spectral domain optical coherence tomography (SDOCT). This method uses two reference arms with independent optical delay to image two different depths within the sample. Two depth images can be reconstructed by the dual spatial frequency encoding technique which is implemented by two galvo scanners in the reference arms with different offsets relative to the pivot axes. Two set of the spectral interferogram corresponding to the positions can be generated. To differentiate the depth profiles, the reference light beam is offset with different distances away from the pivot point when it deflects from the GS mirror. When the GSs in the sample arm and reference arms are scanned simultaneously, due to the different offset distances the spatial spectrograms will be carried to two modulation frequencies. Two spatial spectrograms can be filtered out to obtain the two full-range SDOCT images corresponding to the depths in the sample, then realizing the extended range imaging. The extended range SDOCT system provides an approximately 1.9 fold increase in effective -6 dB imaging range compared with the conventional single-reference-arm SDOCT. The parameter choice criterion for ensuring a high signal to cross talk ratio is also presented and discussed. Phantom and ex vivo biological images demonstrate the expected increase in imaging depth range.

9697-106, Session PSun
**System calibration and optimization for swept-source optical coherence tomography**

Tan-Lin Liao, Cheng-Han Huang, Chun-Jung Huang, Chia-Wei Sun, National Chiao Tung Univ. (Taiwan)

We discuss the experimental problems in constructing an SS-OCT system, including image processing, noise control, and the relationship between signal and data acquisition. To ensure the image quality and the imaging depth, we made sure the signals of all the hardware were synchronized, including the scanning lens synchronization signal, scanning lens drive control signal and the trigger signal of the swept source laser. After that, additional signal processing is needed to improve image quality and signal to noise ratio (SNR). The data analysis process in our programing comprises spectral shaping with Hamming window correction, DC noise deduction and fast Fourier transforms (FFT).

9697-107, Session PSun
**Dual scan based SD-OCT for depth enhanced investigation on leaf samples using a single spectrometer**

Naresh Kumar Ravichandran, Kiboom Park, BIO PHOTRONICS (Korea, Republic of); Seung-Yeol Lee, Kyungpook National Univ. (Korea, Republic of); Jaeyul Lee, Jaewon Song, Light Wave Lab. (Korea, Republic of); Hee-Young Jung, Kyungpook National Univ. (Korea, Republic of); Mansik Jeon, Jeeyhun Kim, BIO PHOTRONICS (Korea, Republic of)

In conventional optical coherence tomography (OCT) systems, the intensity of backscattering signal deteriorates, due to multiple scattering of light in tissues. This limits the depth range of samples to few millimeters. In this study, we proposed a dual scanning approach to increase the depth range of OCT systems. We proposed a method to overcome this limitation by using a dual scanning of the sample, from top and bottom surface of the sample, by using a single spectrometer. The system was built with a broadband source of 860 nm center wavelength with full width half maximum of 165 nm and 4096 pixels line scan camera. Each sample arm is matched path length with its respective reference arm. The inverted image of sample arm 2 (scanning on bottom surface of sample) is merged in accordance to the image of sample arm 1. Thickness of sample is manually measured and the images of sample arm 1 and sample arm 2 are merged to match the thickness of sample. The obtained image gives more depth resolved details of sample as compared to images obtained using individual sample arm. Using dual scanning we increased the depth range to twice that of conventional OCT system. The results are supported using A-scan analysis of obtained images. Application of dual scan analysis may help in, non-destructive and non-invasive study of entire samples from top and bottom surface at the same time.

9697-108, Session PSun
**Use of high NA fibers in swept source OCT for improved image quality**

Bharadwaj Muralidharan, Tianyi Wang, Thomas E. Milner, The Univ. of Texas at Austin (United States)

Speckle noise is common to all coherent imaging techniques and different approaches have been employed to reduce the degrading effects. In fiber-based optical coherence tomography (OCT), speckle noise of the recorded image is determined in part by the mode field diameter of the fiber in the sample path of the interferometer. In this work, we analyzed the impact of speckle noise in images recorded from a customized OCT system (1510 nm) utilizing either a SMF28 fiber or a high numerical aperture fiber (UHNAP) in the sample path of the interferometer. The fibers used in the reference arm is matched to those in the sample arm to minimize dispersion. High scattering photons were used to test the signal to noise ratio, contrast to noise ratio and their variations through the image, formed by the two different settings (i.e., different fibers) of the OCT system. We hypothesize that the use of high numerical aperture fiber with a smaller core diameter will reduce the speckle noise compared to the SMF28 fiber.
9697-109, Session PSun

**Optical coherence tomography probe design for reduced artifact generation and manufacturability**

Daniel Staloff, Corning Tropel Corp. (United States); Lovell E. Comstock, William Miller, Horst Schreiber, Corning Incorporated (United States)

Many fiber based probes used in Optical Coherence Tomography (OCT) are comprised of a spacer, GRIN lens or fiber, and a microprism. This design form suffers from many material interfaces, which induce back reflections into the sample arm of the interferometer. With so many interfaces, these probes can produce artifacts in the system’s imaging window. We present a design which has just two interfaces to minimize image artifacts. The two components of this design are the fiber endface and a reflective optic. Under certain conditions, these two components can have back reflections below -90dB which will minimize image artifacts. This will result in high fidelity imaging for medical diagnostics.

9697-1, Session 1

**Novel real-time volumetric tool segmentation algorithm for intraoperative microscope integrated OCT**

Christian Viehland, Brenton Keller, Oscar Carrasco-Zevallos, Duke Univ. (United States); David Cunefare, Duke Univ. School of Medicine (United States); Liangbo Shen, Duke Univ. (United States); Cynthia Toth M.D., Duke Univ. School of Medicine (United States); Sina Farsiu, Joseph A. Izatt, Duke Univ. (United States)

Optical coherence tomography (OCT) allows for micron scale imaging of the human retina and cornea. Current generation research and commercial intrasurgical OCT prototypes are limited to live B-scan imaging. Our group has developed an intraoperative microscope integrated OCT system capable of live 4D imaging. With a heads up display (HUD) 4D imaging allows for dynamic intrasurgical visualization of tool tissue interaction and surgical maneuvers. Currently our system relies on operator based manual tracking to correct for patient motion and motion caused by the surgeon, to track the surgical tool, and to display the correct B-scan to display on the HUD. Even when tracking only bulk motion, the operator sometimes lags behind and the surgical region of interest can drift out of the OCT field of view. To facilitate imaging we report on the development of a fast volume based tool segmentation algorithm. The algorithm is based on a previously reported volume rendering algorithm and can identify both the tool and retinal surface. The algorithm requires 45 ms per volume for segmentation and can be used to actively place the B-scan across the tool tissue interface. Alternatively, real-time tool segmentation can be used to allow the surgeon to use the surgical tool as an interactive B-scan pointer.

9697-2, Session 1

**Megahertz FDML laser with up to 143nm sweep range for ultrahigh resolution OCT at 1050nm**

Jan Philip Kolb, Univ. zu Lübeck (Germany) and Ludwig-Maximilians-Univ. München (Germany); Thomas Klein, Optores GmbH (Germany) and Ludwig-Maximilians-Univ. München (Germany); Matthias Eibl, Tom Pfeiffer, Univ. zu Lübeck (Germany) and Ludwig-Maximilians-Univ. München (Germany); Wolfgang Wieser, Optores GmbH (Germany) and Ludwig-Maximilians-Univ. München (Germany); Robert A. Huber, Univ. zu Lübeck (Germany)

We present a new design of a Fourier Domain Mode Locked-Laser (FDML-Laser), which provides a new record in sweep range at ~1µm center wavelength: At the fundamental sweep rate of 383 kHz we reach 143nm bandwidth – with 4x buffering and 153MHz sweep rate 120nm are achieved. The laser is fully characterized at 1.55 MHz sweep rate: The FWHM of the point spread function (PSF) is 7.4µm (in air) and the 6dB roll off point lies at 1.4nm. Additionally, human in vivo retinal imaging is performed showing rich detail, especially visible near the retinal pigment epithelium.

9697-3, Session 1

**Phase stable photoreceptor imaging with line-field spectral domain OCT**

Daniel J. Fechtig, Laurin Ginner, Abhishek Kumar, Michael Pircher, Medizinische Univ. Wien (Austria); Tilman Schmoll, Carl Zeiss Meditec, Inc. (United States); Lara M. Wurster, Wolfgang Drexler, Rainer A. Leitgeb, Medizinische Univ. Wien (Austria)

We present real-time photoreceptor imaging with a line-field parallel spectral domain OCT modality, utilizing a commercially available 2D CMOS detector array operating at and imaging speed of 500 B-scans/s. Our results demonstrate use of the first time in vivo structural and functional retinal assessment with a line-field OCT setup providing sufficient sensitivity, lateral and axial resolution and 3D acquisition rates in order to resolve individual photoreceptor cells. The phase stability of the system is manifested by the high phase-correlation across the lateral FOV on the level of individual photoreceptors. The setup comprises a Michelson interferometer illuminated by a broadband light source, where a line-focus is formed via a cylindrical lens and the back-propagated light from sample and reference arm is detected by a 2D array after passing a diffraction grating. The spot size of the line-focus on the retina is 5µm, which corresponds to a PSF of 50µm and an oversampling factor of 3.6 at the detector plane, respectively. A full 3D stack was recorded in only 0.8 s. We show representative enface images, tomograms and phase-difference maps of cone photoreceptors with a lateral FOV close to 2°. The high-speed capability and the phase stability due to parallel illumination and detection may potentially lead to novel structural and functional diagnostic tools on a cellular and microvascular imaging level. Furthermore, the presented system enables competitive imaging results as compared to respective point scanning modalities and facilitates utilizing software based digital aberration correction algorithms for achieving 3D isotropic resolution across the full FOV.

9697-4, Session 1

**A novel 1050nm handheld optical frequency domain imaging system for pediatric retinoblastoma patients: translation from laboratory bench to clinical study**

Oleg Nadiarnykh, Vrije Univ. Amsterdam (Netherlands); Annette C. Moll, Vrije Univ. Medical Ctr. (Netherlands); Johannes F. de Boer, Vrije Univ. Amsterdam (Netherlands)

We demonstrate a novel optical coherence tomography system specifically developed and validated for clinical imaging of retinoblastoma tumors in pediatric patients. The existing treatment options for this malignant tumor of the retina aim at reduction of tumor (re)growth risks, and vision preservation. The choice of optimal treatment strongly depends on skilled and detailed clinical assessment. Due to the limitations of the existing real-time diagnostic tools the patients at risk are periodically monitored...
with retinal imaging to confirm the absence of new tumor seedings. Three-dimensional visualization of tissue layer and microvasculature at improved axial and lateral resolution of interference-based OCT imaging provides sensitivity for detection of vital tumor tissue concurrent with local treatment. Our METC-approved system accommodates for the range of optical parameters of infants’ eyes, and uses the 1050nm wavelength to access the deeper choroidal layers of retina. The prototype is designed for patients in supine position under general anesthesia, where ergonomic handheld module is connected to fiber-based optical setup via umbilical cord. The system conforms to clinical safety requirements, including fully isolated low-voltage electric circuit. Focusing is performed with a mechanically tunable lens, where resolution is 6 µm axially, and varies with focusing at 10-18µm laterally.

We will present optical design, performance limitations, and results of the ongoing clinical study, including the increased OCT diagnostic sensitivity in three dimensions in comparison with the established clinical imaging modalities. We will discuss images of early, active, and treated tumors, as well as follow-up on patients after local and systemic treatments.

9697-5, Session 1

In vivo tear film thickness measurement and tear film dynamics visualization using spectral domain OCT and an efficient delay estimator

Valentin Aranha dos Santos, Medizinische Univ. Wien (Austria) and Technische Univ. Wien (Austria); René M. Werkmeister, Leopold Schmetterer, Medizinische Univ. Wien (Austria); Martin Gröschl, Technische Univ. Wien (Austria); Gerhard Garhofer, Medizinische Univ. Wien (Austria)

Dry eye syndrome is a highly prevalent disease of the ocular surface characterized by an instability of the tear film. Traditional methods used for the evaluation of tear film stability are invasive or show limited repeatability. Here we propose a new noninvasive fully automated approach to measure tear film thickness using an efficient delay estimator and ultrahigh resolution spectral domain OCT. Silicon wafer phantoms with layers of known thickness and group index were used to validate the estimator-based thickness measurement. A theoretical analysis of the fundamental limit of the precision of the estimator is presented and the analytical expression of the Cramer Rao lower bound (CRLB), which is the minimum achievable variance that may be achieved by any unbiased estimator, is derived. The performance of the estimator against noise was investigated using simulations. We found that the proposed estimator reaches the CRLB. The technique was applied in vivo in healthy subjects and dry eye patients. Series of tear film thickness maps were generated, allowing for the visualization of tear film dynamics. Our results show that the central tear film thickness can be precisely measured in vivo with a coefficient of variation of about 0.65 % and that repeatable tear film dynamics can be observed. The presented method has the potential of being an alternative to breakup time measurements (BUT) and could be used in clinical setting to study patients with dry eye disease and monitor their treatments.

9697-7, Session 2

Angiographic imaging using an 18.9 MHz swept-wavelength laser that is phase-locked to the data acquisition clock and resonant scanners

Serhat Tozburun, Cedric Blatter, Wellman Ctr. for Photomedicine (United States); Meena Siddiqui, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Ahhyun S. Nam, Massachusetts Institute of Technology (United States); Benjamin J. Vakoc, Wellman Ctr. for Photomedicine (United States)

In this study, we present an angiographic system comprised from a novel 18.9 MHz swept wavelength source integrated with a MEMs-based 23.7 kHz fast-axis scanner. The system provides rapid acquisition of frames and volumes on which a range of Doppler and intensity-based angiographic analyses can be performed. Interestingly, the source and data acquisition computer can be directly phase-locked to provide an intrinsically phase-stable imaging system supporting Doppler measurements without the need for individual A-line triggers or post-processing phase calibration algorithms. The system is integrated with a 1.8 Gigasample (GS) per second acquisition card supporting continuous acquisition to computer RAM for 10 seconds. Using this system, we demonstrate phase-stable acquisitions across volumes acquired at 60 Hz frequency. We also highlight the ability to perform c-mode angiography providing volume perfusion measurements with 30 Hz temporal resolution. Ultimately, the speed and phase-stability of this laser and MEMs scanner platform can be leveraged to accelerate OCT-based angiography and both phase-sensitive and phase-insensitive extraction of blood flow velocity.

9697-8, Session 2

Integrated RFA/OCT catheter for real-time guidance of cardiac radio-frequency ablation therapy

Xiaoyong Fu, Colin Blumenthal, Deniz Dosluoglu, Yves T. Wang, Michael W. Jenkins, Rakesh Souza, Christopher Snyder, Mauricio Arruda, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Currently, cardiac radiofrequency ablation is guided by indirect signals. We demonstrate an integrated radiofrequency ablation (RFA) and optical coherence tomography (OCT) probe for directly monitoring of the RFA process.
CONCLUSIONS This first-in-human OCT-NIRAF study demonstrates that dual-modality microstructural and fluorescence intracoronary imaging can be safely and effectively conducted in human patients. Our findings show that NIRAF is associated with a high-risk morphologic plaque phenotype. The focal distribution of NIRAF in these lesions furthermore suggests that this endogenous imaging biomarker may provide complementary information to that obtained by structural imaging alone.

9697-10, Session 2

Automatic classification of atherosclerotic plaques imaged with intravascular OCT

Jose D. Rico-Jimenez, Texas A&M Univ. (United States); Daniel U. Campos-Delgado, Univ. Autónoma de San Luis Potosí (Mexico); Martin Villiger, Brett E. Bouma, Wellman Ctr. for Photomedicine (United States); Javier A. Jo, Texas A&M Univ. (United States)

A novel computational method for plaque tissue characterization based on Intravascular Optical Coherence Tomography (IV-OCT) is presented. IV-OCT is becoming a powerful tool for the clinical evaluation of atherosclerotic plaques; however, it requires a trained expert for visual assessment and interpretation of the imaged plaques. Moreover, due to the inherit effect of speckle and the scattering attenuation of the optical scheme the direct interpretation of OCT images is limited. To overcome these difficulties, we propose to automatically identify the A-line profiles of the most significant plaque types (normal, fibrotic, or lipid-rich) and their respective abundance by using a probabilistic framework and blind alternated least squares to achieve the optimal decomposition. In this context, we present preliminary results of this novel probabilistic classification tool for intravascular OCT that relies on two steps. First, the B-scan is pre-processed to remove catheter artifacts, segment the lumen, select the region of interest (ROI), flatten the tissue surface, and reduce the speckle effect by a spatial entropy filter. Next, the resulting image is decomposed and its A-lines are classified by an automated strategy based on alternating-least-squares optimization. Our early results are encouraging and suggest that the proposed methodology can identify normal tissue, fibrotic and lipid-rich plaques from IV-OCT images.
Pacing-induced congenital heart defects assessed by OCT

Stephanie M. Ford, Univ. Hospitals Rainbow Babies & Children’s Hospital (United States); Matthew T. McPheeters, Yves T. Wang, Shi Gu, Yong Qiu Doughman, Case Western Reserve Univ. (United States); James P. Strainic M.D., Univ. Hospitals Rainbow Babies & Children’s Hospital (United States); Andrew M. Rollins, Michiko Watanabe, Michael W. Jenkins, Case Western Reserve Univ. (United States)

The role of hemodynamics in early heart development is poorly understood. In order to successfully assess the impact of hemodynamics on development, we need to monitor and perturb blood flow, and quantify the resultant effects on morphology. Here, we have utilized cardiac optical pacing to create regurgitant flow in embryonic hearts and OCT to quantify regurgitation percentage and resultant morphology. Embryonic quail in a shell-less culture were optically paced at 3 Hz (well above the intrinsic rate or 1.33-1.67 Hz) on day 2 of development (3-4 weeks human) for 5 minutes. The pacing fatigued the heart and led to a prolonged period (>1 hour) of increased regurgitant flow. Embryos were kept alive until day 3 (cardiac looping – 4-5 weeks human) or day 8 (4 chambered heart – 8 weeks human) to quantify resultant morphologic changes with OCT. All paced embryos imaged at day 3 displayed cardiac defects. The extent of regurgitant flow immediately after pacing was correlated with cardiac cushion size 24-hours post pacing (p-value < 0.01) with higher regurgitation leading to smaller cushions. Almost all embryos (16/18) surviving to day 8 exhibited congenital heart defects (CHDs) including 11/18 with valve defects, 5/18 with ventricular septal defects and 5/18 with hypoplastic right ventricles. Our data suggests that regurgitant flow leads to smaller cushions, which develop into abnormal valves and septa. Our model produces similar phenotypes as found in our fetal alcohol syndrome and velo-cardio-facial/DiGeorge syndrome models suggesting that hemodynamics plays a role in these syndromes as well. Utilizing OCT and optical pacing to understand hemodynamics in development is an important step towards determining CHD mechanisms and ultimately developing earlier treatments.

Dynamic contrast optical coherence tomography: quantitative measurement of microvascular transit-time distributions in vivo

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

Transit time is a fundamental microcirculatory parameter that is critical in determining oxygen delivery from capillaries to surrounding tissue. Recently, it was demonstrated theoretically that capillary transit-time heterogeneity potentially leads to non-uniform oxygen extraction in micro-domains. However, in spite of its importance, capillary transit-time distribution has been challenging to quantify comprehensively and efficiently at the microscopic level. Here, we introduce a method, called Dynamic Contrast Optical Coherence Tomography (DyC-OCT), based on dynamic cross-sectional OCT imaging of the kinetics of an intravascular tracer during its passage through the field-of-view. DyC-OCT is used to quantitatively measure the transit-time distribution in microvascular networks in cross-section at the single-capillary level. Transit-time metrics are derived from analysis of the temporal characteristics of the dynamic scattering signal, related to tracer concentration, using indicator-dilution theory. Since DyC-OCT does not require calibration of the optical focus, quantitative accuracy is achieved even deep in highly scattering brain tissue where the focal spot degrades. After direct validation of DyC-OCT against the dilution curves measured using a fluorescent plasma label in the surface pial vessels of a mouse brain, imaged through a thinned-skull, glass coverslip-reinforced cranial window, the laminar transit-time distribution was investigated in microvasculature across the entire depth of the mouse somatosensory cortex.

Conference 9697: Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine XX

investigate retinal vascular dynamics at unprecedented temporal resolution. Motion of retinal tissue, that is induced by expansion of the vessels therein, is measured with an accuracy of about 10 nm. The pulse shape of arterial and venous pulsation, their temporal delay as well as the frequency dependent pulse propagation through the capillary bed are determined. For the first time, imaging speed and motion sensitivity are sufficient for a direct measurement of pulse waves propagating with more than 600 mm/s in retinal vessels of a healthy young subject.

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cortex. Laminar trends were identified, with the earliest transit times in
the middle cortical layers, and the lowest heterogeneity in cortical layer 4.
The new DyC-OCT technique affords a novel perspective of microvascular
networks, with the unique capability of performing simultaneous
measurements of transit-time distributions across cortical laminae.

9697-16, Session 3
Visible light optical coherence tomography for microvascular oximetry in ocular circulation
Siyu Chen, Ji Yi, Hao F. Zhang, Northwestern Univ. (United States)

Visible light optical coherence tomography (vis-OCT) is intrinsically capable
of optical determination of blood oxygen saturation (sO2). Thanks to its 3D
sectioning ability, confounding factors that plague multi-wavelength
fundus photography can be avoided. We further supplemented it with
motion-enhanced angiography (vis-OCTA), which allowed us to resolve
retinal micro vessels without losing spectral information. As a result,
spectroscopic vis-OCTA can extract microvascular sO2 which are generally
inaccessible. Here we extend the theoretical formulation of vis-OCTA
oximetry to include optical attenuation, scattering and motion contrast.
The model allows robust estimation of sO2, while also promising reduction
of illuminating power to 1/3 of current value of ~1 mW. To demonstrate the
capability of our approach, we performed oxygen challenge while taking
vis-OCTA measurements on rat ocular circulation in vivo. We supplied the
experiment animal with the following gas mixture: normal air, 5% CO2 air,
pure O2 and 10% O2 air. For each inhalation gas, the OCTA measurements
were compared with peripheral capillary sO2 (spO2) provided by a pulse
oximeter. The retinal artery sO2 measurements corresponded well with spO2
reading as expected (R2 = 0.87). We found that both retinal and choroidal
circulation sO2 moderately increased when we supplied 5% CO2 air. 100%
O2 inhalation significantly increased both artery and vein oxygenation. On
the contrary, 10% O2 air could deplete the oxygen reservoir in the circulation
and lead to low sO2 readings.

9697-17, Session 3
Visible light optical coherence tomography measure retinal oxygen metabolic response to systemic oxygenation
Ji Yi, Boston Univ. (United States); Wenzhong Liu, Siyu
Chen, Vadim Backman, Northwestern Univ. (United States);
Nader Sheibani, Christine M. Sorenson, Univ. of Wisconsin-
Madison (United States); Amani A. Fawzi M.D., Robert A.
Linsenmeier, Hao F. Zhang, Northwestern Univ. (United States)

The lack of capability to quantize oxygen metabolism noninvasively impedes
both fundamental investigation and clinical diagnosis of a wide spectrum
of diseases including all the major blinding diseases such as age-related
macular degeneration, diabetic retinopathy, and glaucoma. Using visible light
optical coherence tomography (vis-OCT), we demonstrated accurate and
robust measurement of retinal oxygen metabolic rate (rMRO2)
noninvasively in rat eyes. The rMRO2 was calculated by concurrent
measurement of blood flow and blood oxygen saturation (sO2). Blood flow
was calculated by the principle of Doppler optical coherence tomography,
where the phase shift between two closely spaced A-lines measures the
axial velocity. The distinct optical absorption spectra of oxy- and deoxy-
hemoglobin provided the contrast for sO2 measurement, combined with
the spectroscopic analysis of vis-OCT signal within the blood vessels. We
continuously monitored the regulatory response of oxygen consumption to
a progressive hypoxic challenge. We found that both oxygen delivery, and
rMRO2 increased from the highly regulated retinal circulation (RC) under
hypoxia by 0.28±0.08 µL/min (p<0.001), and 0.20±0.04 µL/min (p<0.001)
per 100 mmHg systemic pO2 reduction, respectively. The increased oxygen
extraction compensated for the deficient oxygen supply from the poorly
regulated choroidal circulation (CC).

9697-18, Session 3
Noise-immune complex correlation for vasculature imaging based on standard and Jones-matrix optical coherence tomography
Shuichi Makita, Kazuhiro Kurokawa, Young-Joo Hong, En
Li, Univ. of Tsukuba (Japan); Masahiro Miura, Tokyo Medical
Univ. (Japan); Yosuno Yoshiaki, Univ. of Tsukuba (Japan)

A new optical coherence angiography (OCA) method, called correlation
mapping OCA (cmOCA), is presented by using the SNR-corrected
complex correlation. An SNR-correction theory for the complex correlation
calculation is presented. The method also integrates a motion-artifact
removal method for the sample motion induced decorrelation artifact.
The theory is further extended to compute more reliable correlation by
using multi-channel OCT systems, such as Jones-matrix OCT. The high
contrast vasculature imaging of in vivo human posterior eye has been
obtained. Composite imaging of cmOCA and degree of polarization
uniformity indicates abnormalities of vasculature and pigmented tissues
simultaneously.

9697-19, Session 3
Depth encoded three-beam swept source Doppler optical coherence tomography
Andreas Wartak, Richard Haindl, Wolfgang Trasicscher,
Bernhard Baumann, Michael Pircher, Christoph K.
Hitzenberger, Medizinische Univ. Wien (Austria)

A new technique to quantify human retinal and choroidal blood flow
by the means of a three-beam swept source Doppler optical coherence
tomography (SS-D-OCT) system is being developed. We present first in
vitro measurement results utilizing a perfused glass capillary as a flow
phantom. The three-dimensional velocity vector of moving scatterers ~ in
this case milk diluted with water ~ can be obtained without prior knowledge
of the sample geometry. Three differently angled beams focused onto the
same sample location suffice to quantify the absolute flow. In contrast to
previous spectral domain (SD-) D-OCT investigations, that already proved
the three-beam D-OCT approach to be suitable for in vivo retinal blood flow
evaluation, this current work aims for a similar functional approach with the
means of a differencing technique. Our SS-D-OCT system features a single high
speed broadband laser source (sweet rate: 100 kHz; central wavelength:
1060 nm; sweep range: 100 nm), a single mode fiber based Mach-Zehnder
interferometer setup as well as a balanced amplified photodetection
unit. The use of a swept source centered at 1060 nm promises several
advantages like reduced sensitivity roll-off, improved detection efficiency,
elimination of phase washout as well as an increased penetration depth in
comparison to the previous spectral domain approach at 840 nm. The in
vitro measurements regarding fluid motion inside the capillary show good
agreement between theoretically calculated and experimentally obtained
velocity vector components and flow values.

Return to Contents
Simultaneous and localized measurement of biofilm growth and flow in microfluidic channels using OCT

Nicolas Weiss, Academisch Medisch Centrum (Netherlands); Khalid El Tayeb El Obied, Univ. Twente (Netherlands); Jeroen Kalkman, Technische Univ. Delft (Netherlands); Rob G. H. Lammertink, Univ. Twente (Netherlands); Ton G. van Leeuwen, Academisch Medisch Centrum (Netherlands)

Bacteria communities have the ability to attach to a large variety of wet surfaces. Under favorable environmental conditions single individual bacteria cells can form large bacteria colonies called biofilms. For certain applications, such as e.g., water filtration and medical devices, the formation of biofilms can cause clogging of flow lines and spreading of infections. Besides nutrient supply and waste removal, it has been shown that the underlying flow conditions play an important role in biofilm growth dynamics.

In this work, we present simultaneous and localized measurements of axial and lateral flow velocities and biofilm growth using optical coherence tomography. We show that there is good agreement between velocity data measured by the OCT autocorrelation function and biofilm morphology measured by the magnitude of the OCT signal. We anticipate that the presented methodology will improve the quantification and understanding of the influence of flow parameters such as channel geometry and local flow velocity driving biofilm growth dynamics.

Miniaturized silicon photonic integrated swept source OCT receiver with dual polarization, dual balanced, in-phase and quadrature detection

Zhao Wang, Hsiang-Chieh Lee, Massachusetts Institute of Technology (United States); Long Chen, Diedrik Vermeulen, Torben Nielsen, Seo Yeon Park, Allan Ghaemi, Eric Swanson, Chris Doerr, Acacia Communications Inc. (United States); James G. Fujimoto, Massachusetts Institute of Technology (United States)

Miniaturization and cost reduction of OCT systems are important for enabling many new clinical applications as well as accelerating the development of existing applications. Silicon photonics is an important low-cost, high-volume, multi-functional platform for integrated optics because it can benefit from existing semiconductor fabrication techniques to integrate many advanced optical functions onto a single microchip. We present a miniaturized silicon photonic integrated swept source OCT receiver, measuring 374mm2, with advanced functionalities including dual polarization, dual balanced, in-phase and quadrature detection, essentially enabling the detection of the full vector field (amplitude, phase, and polarization) of the optical signal. With this integrated receiver, we demonstrate full-range OCT for complex conjugate artifact suppression, polarization diversity detection for removing polarization fading artifact, and polarization sensitive OCT for tissue birefringence imaging. The silicon photonic integrated receiver is a key advance towards developing a miniaturized, multi-functional swept source OCT system.

Simultaneous long-range and high-speed imaging with optically subsampled OCT

Meena Siddiqui, Massachusetts Institute of Technology (United States) and Harvard Univ. (United States) and Massachusetts General Hospital (United States); Serhat Tozburnur, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Benjamin J. Vakoc, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Current implementations of OCT can either image over long depth ranges with slower imaging speeds, or at high imaging speeds with more limited depth ranges. The simultaneous operation at multi-centimeter depth ranges and MHz-scale A-line rates is challenging due to limitations in the electronic bandwidths of current digitizers and data transfer buses. The lack of multi-cm depth range, MHz-speed OCT hinders the translation of the imaging technology to sites and organs with complex geometries and expansive fields. Here we describe a first demonstration of a simultaneous cm-scale depth range and MHz-scale A-line rate OCT platform. We describe the principles behind data compression by optically subsampled OCT, the development and performance of a novel subsampled OCT wavelength stepped source operating at 19 MHz A-line rates, the extension of passive quadrature demodulation architectures to GHz-scale acquisition bandwidths, and the first ever integration of these technologies into a subsampled OCT system capable of acquiring volume data at video-rates across multi-cm depth ranges. We use this platform to demonstrate depth resolved measurements over large fields that exhibit complex topography such as the face. The performance, limitations, and the next stages of technical development for this optically subsampled OCT platform are summarized. This platform may open new opportunities for camera-like OCT deployments in sites and organs that are inaccessible to current OCT technologies.

Electro-thermal MEMS fiber scanner for endoscopic optical coherence tomography

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This work report an electro-thermal micro-electro-mechanical system (MEMS) fiber scanner for endoscopic optical coherence tomography (OCT) imaging. The electro-thermal MEMS actuator is composed of a micro-platform, a group of bimorph actuators and a substrate. At first, a 40 mm long bare fiber was fixed on the actuator while keeping the distal end tip free. The micro-platform was then, attached with the fiber at 20 mm apart from the fixed end. Electro-thermal bimorph MEMS actuator with large vertical displacement realizes 1-D forward optical scanning up to 3 mm of scanning range with only 5 VAC p-p and 2 VDC operation voltages. The electro-thermal MEMS fiber scanner was combined with the high speed FDM-based swept-source OCT (SS-OCT) system and demonstrated its capability of performing cross-sectional imaging. The FDM laser source has a central wavelength of 1310 nm and a full wavelength sweeping range of ~ 150 nm, which provided an axial resolution of ~ 9.3 to 9.5 μm in air. The FDML sweeping frequency was 220 kHz, and the OCT imaging frame rate was synchronized with the resonant frequency of the MEMS fiber scanner (~88 frames per second). Due to the high actuation force of the electro-thermal actuation, proposed MEMS fibers scanner can scan the fiber tip to a millimeter range with low actuation voltages and thus may have potential of performing raster scan with non-resonant fiber cantilevers directly.
Noncontact phase-sensitive dynamic optical coherence elastography at megahertz rate

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Dynamic optical coherence elastography (OCE) techniques have shown great promise at quantitatively obtaining the biomechanical properties of tissue. However, the majority of these techniques have required multiple temporal OCT acquisitions (M-B mode) and corresponding excitations, which led to clinically unfeasible acquisition times and potential tissue damage. Furthermore, the large datasets and extended laser exposures hinder their translation to the clinic, where patient discomfort and safety are critical criteria. In this work we demonstrate noncontact true kilohertz frame-rate dynamic optical coherence elastography by directly imaging a focused air-pulse induced elastic wave with a home-built phase-sensitive OCE system based on a 4X buffered Fourier Domain Mode locked swept source laser with an A-scan rate of ~1.5 MHz. The elastic wave was imaged at a frame rate of ~7.3 kHz using only a single excitation. In contrast to previous techniques, successive B-scans were acquired over the measurement region (B-M mode) in this work. The feasibility of this method was validated by quantifying the elasticity of tissue-mimicking agar phantoms as well as of a porcine cornea ex vivo at different intraocular pressures. The results demonstrate that this method can achieve a depth-resolved elastogram in milliseconds. The reduced data set enabled a rapid elasticity assessment, and the ultra-fast acquisition speed allowed for a clinically safe laser exposure to the cornea.

Spectral estimation optical coherence tomography for axial super-resolution

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The sample depth reflectivity profile of Fourier domain optical coherence tomography (FD-OCT) is estimated from the inverse Fourier transform of the spectral interference signals (interferograms). As a result, the axial resolution is fundamentally limited by the coherence length of the light source. We demonstrate an axial resolution improvement method by using the autoregressive spectral estimation technique to instead of the inverse Fourier transform to analyze the spectral interferograms, which is named as spectral estimation OCT (SE-OCT). SE-OCT improves the axial resolution by a factor of up to 4.7 compared with the corresponding FD-OCT. Furthermore, SE-OCT provides a complete sidelobe suppression in the point-spread function. Using phantoms such as an air wedge and micro particles, we prove the ability of resolution improvement. To test SE-OCT for real biological tissue, we image the rat cornea and demonstrate that SE-OCT enables clear identification of corneal endothelium anatomical details ex vivo. We also find that the performance of SE-OCT is depended on SNR of the feature object. To evaluate the potential usage and define the application scope of SE-OCT, we further investigate the property of SNR dependence and the artifacts that may be caused. We find SE-OCT may be uniquely suited for viewing high SNR layer structures, such as the epithelium and endothelium in cornea, retina and aorta. Given that SE-OCT can be implemented in the FD-OCT devices easily, the new capabilities provided by SE-OCT are likely to offer immediate improvements to the diagnosis and management of diseases based on OCT imaging.

SNR of swept SLEDs and swept lasers for OCT

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A back-to-back comparison of a tunable narrow-band SLED (TSLED) and a swept laser are made for OCT applications. Both are 1310 nm sources sweeping at 50 kHz over a 100 nm tuning range and have similar coherence lengths. The TSLED consists of a seed SOA and two amplification SOAs. The ASE is filtered twice by a tunable MEMS Fabry Perot in a polarization multiplexed double-pass arrangement on either side of the middle SOA. This allows very long coherence lengths to be achieved. A fundamental issue with a SLED is that the RIN is proportional to 1/Linewidth, meaning that the longer the coherence length, the higher the RIN. High RIN also leads to increased clock jitter. Most swept source OCT calculations assume that the noise is independent of the amplitude of the signal light. The higher the signal, the higher the SNR. We show that in the case of the TSLED, that the high signal RIN and clock jitter give rise to additional noises that scale with signal power. This leads to an SNR limit in the case of the TSLED: The higher the signal, the higher the noise, so the SNR reaches a limit. While the TSLED has respectable sensitivity, the SNR limit causes noise streaks in an image where the A-line has a high reflectivity point. The laser, which is shot noise limited, does not exhibit this effect. This is illustrated with SNR data and side-by-side images taken with the two sources.

Flexible A-scan rate MHz OCT: computational downscaling by coherent averaging

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This work addresses the need for OCT systems where the imaging speed can be changed on the fly. In order to realize fast OCT systems with adjustable line rate, we investigate averaging of FDML based MHz OCT systems. The line rate can be reduced in software and traded in for increased system sensitivity and image quality. We compare coherent and incoherent averaging to effectively scale down the system speed of a 3.2MHz FDML OCT system to around 100 kHz in postprocessing. We quantify the effective sensitivity gain by incoherent and coherent averaging and observe a strong dependence on the system layout and settings. We compared averaging for a ~108dB sensitivity system and an 86dB one both running at 3.2MHz line rate. Our results indicate that low sensitivity systems benefit more from coherent averaging than one with high sensitivity. We demonstrate that coherent averaging is possible with MHz systems without special interferometer designs or phase calibration. We show OCT images of human finger in vivo with very high quality and deep penetration, comparable to images directly acquired with a line rate of several 10kHz. Our observations indicate that coherent averaging might be very useful for MHz retinal OCT engines.
The how and why of a $10 optical coherence tomography system

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Beyond the first world market for OCT, there are 6.5 billion people with similar eye and skin cancer care needs which cannot be met by current OCT systems due to cost. This paper will describe a means to manufacture a low cost, compact, multiple reference OCT (MR-OCT) system, using parts and a configuration similar to a CD-ROM or DVD pickup unit. Essentially, this is based on the use of a partial mirror in the reference arm of a time domain OCT system to provide multiple references and hence A-scans at several depths simultaneously. Data from MR-OCT systems in demonstration applications will be shown. Using this approach, a full depth scan of 1 to 2 mm can be achieved with a reference mirror movement of tens of microns. Since this can be achieved using voice coil similar to a DVD focus coil and, since the setup is similar to a CD-ROM pickup unit, we believe the entire system can be built for a cost of the order of $10. We have already shown that a system based on this configuration has achieved an SNR of greater than 90 dB which is sufficient for many applications. We will demonstrate that the system has the speed and sensitivity to acquire a full 3D fingerprint as an example.

9697-110, Session PMon

Fast subcellular optical coherence photoacoustic microscopy

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A fast subcellular optical coherence photoacoustic microscopy (FS-OCPAM) system was developed by combination two complementary imaging module of optical coherence microscopy (OCM) and photoacoustic microscopy (PAM). The system used optical scanning to realize fast imaging speed. For the melanin detection in the retinal pigment epithelium (RPE), the system provided high resolution of 1.24 μm and 0.59 μm for OCM and PAM, respectively. They both clearly showed the RPE cell morphology, and reflected the complementary optical properties of scattering and absorption. The morphology and the PA signal could be used to identify qualitatively and quantitatively the aging and healthy states of the RPE cells. The results show the potential applications in studying the real-time cellular response to external stimulations and the progress of aging and diseases at cellular level with FS-OCPAM.

9697-111, Session PMon

Cerebral metabolic rate of oxygen (CMRO2) assessed by combined Doppler and spectroscopic OCT

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A method of measuring cortical oxygen metabolism in the mouse brain that uses independent quantitative measurements of three key parameters: cerebral blood flow (CBF), arteriovenous oxygen extraction (OE), and hemoglobin concentration ([HbT]) is presented. Measurements were performed using a single visible light spectral/Fourier domain OCT microscope, with Doppler and spectroscopic capabilities, through a thinned-skull cranial window in the mouse brain. The system had a theoretical axial resolution in air of 1.05 μm, a lateral resolution of 22 μm, an A-scan rate of 90 kHz, and an imaging depth of 1.35 mm in air. The mean CBF was estimated by averaging total arteriolar and venular flows. These were calculated by summing the flows in all individual vessels (arterioles or venules, respectively). Flow in individual vessels was determined by integrating the Doppler velocity axial projection in the en face (xy) plane. Using spectroscopic fitting of the OCT dynamic signal, equivalent concentrations of oxyhemoglobin ([HbO2]) and deoxyhemoglobin ([Hb]) in microvasculature were estimated. Oxygen saturation (sO2) and hemoglobin concentration were determined as sO2 = [HbO2] / [HbT] and [HbT] = [HbO2] + [Hb]. Based on Fick’s principle, the cerebral metabolic rate of oxygen (CMRO2) was calculated by multiplying the CBF, OE and [HbT]. Baseline metabolic measurements in mice are shown to be consistent with literature values. Oxygen consumption, as measured by this method, did not change substantially during minor changes in either the fraction of inspired oxygen (FiO2) or in the fraction of inspired carbon dioxide (FiCO2), in spite of larger variations in sO2.

9697-112, Session PMon

Magnetic force optical coherence elastography at 1.5 million a-lines per second

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Optical Coherence Elastography (OCE) has been widely used to characterize the tissue elasticity. In this study, we introduced a new excitation method based on the magnetic field to induce the shear wave in phantoms and biological tissue to study elasticity in combination of Optical Coherence Elastography system at 1.5 million a-lines per second. Due to the electrical conductivity properties of biological tissues, it is possible to generate electrical current. When the current flows through the tissue with the presence of the magnetic field, magnetic force will be generated which in return leads to a localized tissue deformation and shear wave propagation. Compared to conventional OCE method (M-B acquisition), the advantage of the superfast OCE enables to record the shear wave propagation without synchronization and greatly reduces the time. In this work, the elasticities of different concentration of agar sampled were quantified with this method first, and then we estimated the biomechanical property of the porcine liver. The OCE results acquired from this magnetic force excitation were compared with the mechanical compressional tests in the end for validation purpose.

9697-113, Session PMon

Development of a whole model eye tissue phantom for swept-source optical coherence tomography (SS-OCT)

T. Scott Rowe, Rowe Technical Design (United States)

I document my latest work in developing a new model eye tissue phantom with a solid-state cornea and liquid filled anterior and posterior chambers designed for demonstrating, validating and comparing swept-source ophthalmic optical coherence tomography (SS-OCT) instruments. Whole Model Eye (WME) phantoms can serve a variety of purposes, including demonstrating instrument functionality and performance in the clinic and exhibit hall, validating axial length measurements from different commercial instruments and as an aide for the R&D engineer and field service technician in the development and repair of instruments, respectively. The ideal model eye for OCT would also have a retina phantom with volumetric morphology and scattering and absorption properties similar to that of normal human retina. These include a multi-layered structure of equivalent thickness to
nominal human retinal layers, including an inner limiting membrane (ILM) layer, neurosensory layers with appropriate backscattering properties, and a retinal pigment epithelium. A filled and sealed tissue phantom relieves the user of constant cleaning and maintenance associated with the more common water bath model eyes. Novel processes have been developed to create a crystalline lens phantom within the model eye that closely mimic the reflectance and scattering coefficients of the human crystalline lens, as imaged by the SS-OCT.

9697-114, Session PMon

In vivo Imaging of phonating larynx in awake patients using optical coherence tomography

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Determining the cross-sectional microanatomy of human larynx in vivo, particularly during phonation, may lead to a better understanding of vocal fold mechanics and provides a means to more reliably diagnose fold pathology without biopsy. Optical coherence tomography (OCT) can provide both structural and functional information of tissues, however, there have been limited developments in an office-based laryngeal OCT imaging system because of practical issues such as limited imaging range and speed, and subject head and physician hand motions. With the development of Vertical-Cavity Surface Emitting Lasers (VCSEL) based swept-source, many of the issues that impeded laryngeal OCT development were solved. We developed a gradient-index (GRIN) lens rod-based hand-held probe in conjunction with a 200 kHz VCSEL swept-source optical coherence tomography (SS-OCT) system for real-time imaging of the human larynx in an office setting. We were able to observe both the structure and motion of human vocal fold over a lateral scanning range of approximately 8.4mm at a nominal working distance of 6cm. Preliminary results showed vocal fold tissue structures and vibrational motion during subject phonation at 250Hz.

9697-115, Session PMon

Study of tailing effect in OCT angiography

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Optical coherence tomography (OCT) angiography (OCTA) is a new imaging modality providing visualization of three dimensional (3D) blood vessel networks, without a need of exogenous contrast agents. In part due to this attribute, OCTA promises an ideal imaging tool in the examination of pathological vascularization with respect to its localized depth information. However it is noticed that in OCTA there is notorious tailing effect from overlaying blood vessels that leads to strong image artifacts appearing in the results of blood vessel networks for example within outer retinal space and choroid in human eye imaging. These artifacts limit our ability to visualize and interpret 3D angiographic results. Despite the prevalence of tailing artifacts, the study regarding how and why they are created, to our knowledge, has not been reported so far.

In this work, the tailing effect in OCTA is examined and analyzed. Demonstrated through the data from in-vivo posterior eye angiographic imaging of human subject, it is found that tailing effect does not always lead to a noticeable OCT intensity decrease and is not due to multiple scattering which changes the optical pathlength. Instead it is caused by the induced Doppler Effect when photons pass through the blood vessels. Further based on the study, a simple practical approach is introduced to minimize these artifacts presented in the subretinal space, especially useful for examining clinical cases of choroidal neovascularization (CNV).

9697-116, Session PMon

Optical coherence tomography for pathological analysis of thyroid

Neda Haj-Hosseini, Pernilla Petersson, Oliver Gimm, Ivan Shabo, Linköping Univ. (Sweden)

Thyroid is mainly composed of follicles that produce hormones (thyroxine and triiodothyronine). In a normal thyroid, the follicles are expected to have identical shapes and sizes with an even spread in the tissue whereas in the majority of the diseases of the thyroid the follicles’ morphology are affected. This criterion may potentially be used for diagnosis during surgery. In this work, thyroid tissue specimens from patients with various diseases were studied using OCT. A clear morphological difference was visible in the images among different diseases. The images were analyzed using texture segmentation methods to derive quantitative information on the follicles’ cross-sectional area, number and density. The follicles had irregular shapes and various follicle diameters in the diseased tissues with. The density of the follicles showed correlation (r = 0.75) with the thyroid triiodothyronine hormone. Further work will comprise inclusion of more diseases and additional adaption of the image segmentation method to increase the analysis accuracy.

9697-117, Session PMon

Automated detection of inflammatory cells in whole anterior chamber of a uveitis mouse from swept-source optical coherence tomography images

Woo June Choi, Kathryn L. Pepple M.D., Ruikang K. Wang, Univ. of Washington (United States)

Cell grading in a rodent anterior chamber is essential for anterior inflammation evaluation in preclinical vision research. This paper describes a computerized method for three-dimensional (3D) detection and counting of the anterior chamber cells from swept-source optical coherence tomography (SS-OCT) images of a experimental rodent model of uveitis. The volumetric anterior segment OCT data is obtained from 100 kHz SS-OCT imaging of mouse eye in vivo. For the OCT crossections, each OCT structural image is despeckled and binarized. After removal of cornea, iris, and crystalline lens structures connected to the binary image border, an area thresholding is then employed for each labeled region to isolate only cell-like objects in the anterior chamber, followed by roundness estimation of the objects to identify potential cell candidates in the data. Finally, for 3D cell counting, an automatic cell tracking analysis is carried on the successive cell candidate images to eliminate redundancy in cell counting, allowing effective visualization and quantification of cell distribution in the
entire anterior chamber. The proposed method is demonstrated on anterior OCT images of normal and acute anterior uveitis of mice models, showing appreciable difference in their 3D cell density.

9697-118, Session PMon
Automatic three-dimensional segmentation combined with in vivo microvascular network imaging of human retina by intensity-based Doppler variance optical coherence tomography
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The vascular morphology of intraretinal layers allows earlier diagnosis and precise monitoring of several retinal diseases. Doppler optical coherence tomography (D-OCT) is a functional extension of OCT which can image not only structure but also blood flow and microvascular networks in the retina. Compared to the phase-resolved Doppler variance method, the amplitude variance method has the advantage of independence on phase stability. Intensity-based Doppler variance (IBDV) have been demonstrated in a phase instable situation, and the human choroidal blood vessel network was also achieved. Several segmentation methods have been successfully applied in two-dimensional (2-D) segment of intraretinal layer, but computation is time-consuming for segment in three-dimensional (3-D).

A fast 3-D dynamic programming expansion method was demonstrated for vessel boundary detection on magnetic resonance imaging sequences with improved efficiency and robustness, can be employed in retinal boundary detection. Here, we will report a method that combines the 3-D dynamic programming segmentation with IBDV based on swept-source OCT (SS-OCT) for visualizing microvascular morphology of intraretinal layers in vivo in a normal subject. This method was demonstrated by tested on 3-D data of a retina centered on the macular fovea with an image area of 2.57 mm x 2.57 mm. The seven surfaces of the intraretinal layers were successfully segmented automatically by using a 3-D expansion of a dynamic programming method and the macular retina can be decided into six layers, including NFL, GCL + IPL, INL, OPL, ONL + IS, and OS + RPE. The IBDV method was used to acquire the microvascular network of the macular retina. The IBFD method can be combined with 3-D surface of the segmented layers. The morphology of the microvascular network for the individual intraretinal layers can be visualized, and the segmentation method can be also used to enhance the contrast of the vascular images. This method has potential for earlier diagnosis and precise monitoring in retinal vascular diseases.

9697-120, Session PMon
Optimization of modified scanning protocol based correlation mapping optical coherence tomography at 200 kHz VCSEL source for in vivo microcirculation imaging applications
Cerine Lal, James McGrath, Hrebesh M. Subhash, Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Optical coherence tomography (OCT) based angiographic techniques that enables visualization of lumens of blood vessels at clinically relevant speed has been of high research interest in the recent past. Correlation mapping-OCT (cmOCT) is a magnitude based flow mapping technique which is based on the decorrelation between 2 adjacent B frames. In cmOCT technique, the adjacent B frames in areas of vascular region are decorrelated to the extent determined by the flow through the region. Previous studies using cmOCT have used a dense scan protocol to accomplish correlation between adjacent high density B-frames, however at the expense of longer scanning time. Compared to super luminescent diodes (SLDs) which were used in spectral domain OCT systems, swept source lasers offer higher scanning speeds and extended depth range imaging. Reduced scan time using a swept source OCT can provide better background suppression and wider field of view which can be used for real time clinical studies. In this study, we optimize the cmOCT angiographic algorithm using a 200 kHz VCSEL for in vivo microcirculation imaging applications by a modified scanning protocol which does not require high density B-frames. In order to demonstrate high speed cmOCT angiography, first we perform the experiments to detect the Brownian motion of intralipid particles in a translucent capillary tube. Further, it is extended to nail fold capillary imaging and to study corneal angiogenesis.
9697-121, Session PMon

**Ex vivo brain tumor analysis using spectroscopic optical coherence tomography**

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A big challenge during neurosurgeries is to distinguish between healthy tissue and cancerous tissue, but currently a suitable non-invasive real time imaging modality is not available. Optical Coherence Tomography (OCT) is a potential technique for such a modality. OCT has a penetration depth of 1-2 mm and a resolution of 1-15 μm which is sufficient to illustrate structural differences between healthy tissue and brain tumor. Therefore we investigated gray and white matter of healthy central nervous system and meningioma samples with a Spectral Domain OCT System (Thorlabs Callisto). Additional OCT images were generated after paraffin embedding and after the samples were cut into 10 μm thin slices for histological investigation with a bright field microscope. All samples were stained with Hematoxylin and Eosin. In all cases B-scans and 3D images were made. Furthermore a camera image of the investigated area was made by the built-in video camera of our OCT system. For orientation, the backsidies of all samples were marked with blue ink. The structural differences between healthy tissue and meningioma samples were most pronounced directly after removal. After paraffin embedding these differences diminished. A correlation between OCT en face images and microscopy images can be seen. In order to increase contrast, post processing algorithms were applied. Hence we employed Spectroscopic OCT, pattern recognition algorithms and machine learning algorithms such as k-means Clustering and Principal Component Analysis. These auspicious results will be investigated further and a system for in vivo measurements will be developed.

9697-122, Session PMon

**Comprehensive study of various amplitude-based angiography algorithms with CEECL and VCSEL based swept source optical coherence tomography systems**

James McGrath, Cerine Lal, Sean O’Gorman, Hrebesh M. Subhash, Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Optical coherence tomography (OCT) based flow quantification and microcirculation mapping has become an active area of research, which aims to visualize the lumens and architecture of blood vessels in many clinical and fundamental areas of research, including cardiology, dermatology, neurology, ophthalmology, small animal imaging studies, and so forth. The development of swept source OCT has brought many advantages over spectral domain OCT, including greater imaging depth, less depth-dependent signal roll-off and less motion-induced signal loss due to fringe washout. OCT amplitude based angiographic modalities and flow quantification techniques can provide non-invasive, label-free, high resolution depth resolved mapping of the vasculature. In this study we present a comprehensive comparison of various amplitude-based angiography algorithms such as correlation mapping OCT, speckle variance OCT, full spectrum amplitude decorrelation OCT and split-spectrum amplitude-decorrelation angiography using Fourier-Domain (FD) swept source OCT systems with two different optical source technologies, one at 16 kHz (CEECL-Cat-Eye External Cavity Laser) and another at 200 kHz (VCSEL-Vertical Cavity Surface Emitting Laser). The ability of the selected amplitude based angiography algorithms to visualize flow on these systems is presented. Additionally, a comprehensive computational performance analysis is presented for each selected angiographic algorithm. The angiogram SNR, depth of imaging, system sensitivity roll-off and fringe washout effect are also compared for these two systems. Further, to demonstrate in-vivo comparison of these modalities, measurements were taken from nail fold of a healthy adult volunteer.

9697-123, Session PMon

**OCT for blood glucose monitoring through signal attenuation**

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Optical Coherence Tomography (OCT) is an interferometry-based imaging technique that uses light to probe samples. As such, its signal is sensitive to the intensity of light returning from the sample and, through it, one can estimate the sample’s attenuation coefficient of light. The estimative can be performed by exponential fitting of the signal, following the Beer-Lambert’s law, and the coefficient may be related to sample-specific features. In this work, we aimed to explore the behavior of the attenuation coefficient of mouse’s blood under increasing glucose concentrations. Different samples were prepared in four glucose concentrations using a mixture of heparinized blood, PBS (Phosphate Buffer Saline) and glucose, with glucose concentrations being measured with a blood glucometer, for reference. The concentrations ranged from 230 mg/dL to 490 mg/dL.

The OCT system used was a commercial Spectral Radar OCT with 930 nm central wavelength and spectral bandwidth (FWHM) of 100 nm. The system proved to be sensitive for all blood glucose concentrations tested, with good correlations with attenuation coefficients. A trend was observed, with an increase in attenuation with higher values of glucose, in a linear fashion. Statistical difference was observed in all groups (p<0.001). This work opens the possibility towards a non-invasive diagnostic modality using OCT for glycemic control, which eliminates the use of analytes and/or test strips, as in the case with commercially available glucose meters.

9697-124, Session PMon

**Profilometry of the air-tissue interface by all-semiconductor anokinetic programmable swept-source with centimeters coherence length**

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

We report the possibility of providing 3-D profilometry of the air-tissue interface with micron-scale precision by using a novel, low cost, anokinetic swept source recently developed. Example OCT image taken from human finger skin is presented by using a home-built optical coherence tomography setup integrated with the swept source. Further work on provide 3D profilometry of the air-tissue interface on a TE wound model will be presented in the near future. This could provide a low cost, portable device that can reliably qulify the physical extent of skin wounds.

9697-125, Session PMon

**Nanoparticles displacement analysis using optical coherence tomography**

Marcin R. Strakowski, Maciej Kraszewski, Michal Trojanowski, Paulina Strakowska, Gdansk Univ. of
Technology (Poland)

Nanoparticles and nanotechnology play a very important role in modern science, technology, biology and medicine. Since the beginning of rapid growth of nanotechnology the new better and smart materials and more precise measurement techniques have been developed. Their applications can be found in modern medicine (nanoparticles as contrast agents in tomography imaging or drug delivery systems), biotechnology and industry. Despite of great progress in nanotechnology there is a lack of fast, reliable and nondestructive methods which can be applied in nanocomposites and nanostructure evaluation during manufacturing process and in their natural environment (e.g. spatial distribution of contrast agents in tumor). This is a challenging issue which has not been solved yet. For this purpose we propose to use the optical coherence tomography with polarization sensitive and spectroscopic analysis (PS-SOCT). The PS-SOCT delivers polarization sensitive and spectroscopic data simultaneously, which are useful for measured object characterization. Moreover, the light scattering properties of biomedical and technical objects depend on the type, size, shape, concentration, dispersion and alignment of nanoparticles inside. These features can be evaluated by the spatially resolved analysis of the backscattered light intensity, state of polarization and spectral characteristics. These data might be delivered by the PS-SOCT system which is one of the scopes of the presented research work. In this contribution we are going to present the developed PS-SOCT and also the algorithms and methods for quantitative characterization of the nanoparticles displacements in tested objects. The usefulness of the method is being confirmed by the measurements of TiO2 nanoparticles dispersed in PMMA matrix. We are also going to show measurements results taken from the nanocomposite materials having golden nanospheres and nanorods, which are widely used in medical treatment and diagnosis.

9697-126, Session PMon

Spectroscopic low coherence interferometry using a supercontinuum source and an ultra broadband spectrometer

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Spectroscopic optical coherence tomography (SOCT) combines the imaging capability of OCT with spectroscopic absorption information. We demonstrate a SOCT system working in the visible spectral range from 510 to 730 nm by combining a supercontinuum light source, a Linnik interferometer and a commercial available broadband spectrometer. This wavelength range is chosen because it covers a range of useful absorbers, including that of human proteins. SOCT requires a large bandwidth combined with a broadband spectrometer, due to the fact that reconstruction of SOCT data requires dividing the measured spectrum in spectral bands. The number of bands determines the spectral resolution, while the axial resolution depends on the spectral width of each window. A supercontinuum source with its broad spectrum allows a sufficient number of windows combined with a reasonable axial resolution.

The system is tested with a laser dye rhodamine B for calibration and verification. Rhodamine B is dissolved in ethanol in a cuvette and placed in one of the interferometer arms. It has an absorption peak at around 560 nm, which resembles the absorption spectrum of several proteins in the globin group. The results show that the absorption spectrum of rhodamine B can be reconstructed and show varying spectroscopic information retrieved from different concentration.

9697-127, Session PMon

Quantification of microvasculature of irradiated tumor tissue with optical coherence tomography

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We report the optical coherence tomography (OCT) quantitative assessment of early (up to 3 weeks) microvascular response of engrafted tumors in mouse dorsal skin window chamber model subjected to a high-dose single fraction radiation treatment. Optimized speckle variance OCT imaging of microvasculature was performed before and after treatment. Imaging was followed by OCT data pre- and post-processing to characterize the structure and function of tumor-associated and normal vasculatures.

Several pre-processing steps were necessary to prepare microvascular 3D datasets for quantification. These included noise and artifact removal, image registration, separation of tumor area relative to peritumoral and non-tumor areas, binarization of 3D images, and image skeletonization. Following these, several biological metrics were derived and then used to quantify early microvascular changes in response to radiotherapy. Vascular damage, angiogenesis, neovascularization and changes in vessel diameters were estimated with vascular volumetric and length densities. Average vessel length was used to quantify vessel/capillary pruning. Vessel tortuosity reflected efficiency of blood transport and vascular remodeling. Finally, microvasculature fractal dimension characterized changes in vascular space-filling properties and vascular network complexity through box-counting and sandbox methods.

This study demonstrates OCT’s potential to monitor and characterize early vascular changes in irradiated tissues. Obtained results are compared with those reported in the literature for other imaging modalities, and their interpretation in terms of underlying microvascular radiobiological changes is provided.

9697-128, Session PMon

Optical coherence tomography for longitudinal monitoring of tumor vascular permeability following radiation therapy

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Despite recent advances in radiation therapy and the emergence of imaging
A novel tri-band swept source optical coherence tomography (OCT) system is proposed and demonstrated for functional imaging. The tri-band source is composed of Fourier domain mode-locking laser and fiber optical parametric amplifier (FOPA). Two synchronized Fourier domain mode-locked laser cavities at 45 kHz repetition rate with the same delay fiber generate output over the spectrum windows centered at 1315 nm and 1605 nm, respectively. A booster optical amplifier is utilized for 1.3 μm wavelength band amplification; while a FOPA with a pump wavelength of 1555 nm is implemented for 1.6-μm band amplification, of which an idler band at 1.5 μm is generated with the same sweeping speed. It allows synchronized tri-band output with tens- to a hundred-nanometer sweeping range and up to ~35 mW output power for each band.

Lipid and artery functional imaging, as an important application field of spectroscopic OCT in coronary artery disease, is demonstrated with an in vitro artery model by processing the images obtained from the three bands. These images are post-processed by contrast stretching, intensity normalization, radiometric processing, and filtering. By differentiating the pixel intensities from 1.3/1.5 μm and 1.5/1.6 μm images, lipid and artery distribution can be visualized after processing the interferogram signals from these three bands. The functional imaging results coincide with the attenuation coefficients difference of lipid and artery between these wavelength bands. This tri-band system is more computationally efficient compared to single-band spectroscopic OCT that is based on low-contrast sub-band images, which has a potential to be utilized for quantitative spectroscopic OCT applications.

9697-129, Session PMon

In vivo imaging of melanoma tissues guided by magnetic nanoparticles augmented magneto-motive optical Doppler tomography system

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In this study, we demonstrated an in vivo experiment using a MM-ODT system that is capable of measuring real-time optical Doppler signals for the initial identification of melanoma cells. A magnetic field was externally applied to a sample containing injected SPIO magnetic nanoparticles to generate a phase shift in the nanoparticles. We performed an ex vivo MM-ODT experiment to verify the system response by injecting the nanoparticles into a pork belly sample. The mechanical movements of the nanoparticles in response to the magnetic field were identified, and it was also confirmed that the induced optical Doppler velocity is directly proportional to the concentration of the SPIO solution and the voltage applied to the solenoid.

The applicability and reliability of the proposed method for medical applications were tested by applying the system to obtain real-time MM-ODT images of in vivo melanoma tissue using a living mouse. The obtained results also confirmed that the suggested MM-ODT system is an effective means of in vivo imaging to observe living tissue augmented with magnetic nanoparticles. To the best of our knowledge, this is the first demonstration of in vivo melanoma tissue imaging in real time using an MM-ODT system.

9697-130, Session PMon

Tri-band optical coherence tomography for lipid and vessel spectroscopic imaging

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A novel tri-band swept source optical coherence tomography (OCT)
Adaptive optics (AO) is essential in order to visualize small structures such as cone and rod photoreceptors in the living human retina in vivo. By combining AO with optical coherence tomography (OCT) the axial resolution in the images can be further improved. OCT provides access to the phase of the light returning from the retina which allows a measurement of subtle length changes in the nanometer range. These occur for example during the renewal process of cone outer segments. We present an approach for measuring very small length changes using an extended AO scanning laser ophthalmoscope (SLO)/OCT instrument. By adding a second OCT interferometer that shares the same sample arm as the first interferometer, phase sensitive measurements can be performed in the en-face imaging plane. Frame averaging decreases phase noise which greatly improves the precision in the measurement of associated length changes.

9697-133, Session PMon

Application of deconvolution technique in optical coherent tomography for tear film measurement

Hui Lu, Kai Shen, Sarfaraz Baig, Michael R. Wang, Univ. of Miami (United States)

Dry eye debilitating the patients physically, limit their ability to perform daily tasks, cause clinical depression, and compromise quality of life. Accurate diagnosis of the dry eye and the research achievement on the pathological mechanism and treatment all depend largely on the available imaging techniques. The development of new imaging techniques helps better understanding of the disease and paves the way for accurate early diagnosis and better treatment. Optical coherence tomography can be used for tear film thickness determination, yet its limited axial resolution compromises its performance. In this paper, we applied deconvolution method to the optical coherence tomography system and improved the axial resolution more than two times. Tear film thickness distribution in an eye has been evaluated with resolution beyond the physical limit of the coherent length of the light source.

9697-134, Session PMon

Calculus diagnosis based on real-time polarization optical coherence tomography

Shang Ruei You, Chia-Wei Sun, Cheng-Han Huang?, National Chiao Tung Univ. (Taiwan)

Polarization-sensitive optical coherence tomography (PS-OCT) has been designed for clinical studies in the segment imaging of the eye because of light polarization, so we think that it might be used in teeth, too. Because the tooth enamel also has birefringence, its phase retardation imaging is more stable than the dental calculus. The dental calculus is composed of random pattern mineral, so its structure will cause high scattering and phase retardation. We use this property to distinguish dental calculus from the teeth.

9697-29, Session 5

Phase sensitive adaptive optics assisted SLO/OCT for retinal imaging

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Adaptive optics (AO) is essential in order to visualize small structures
narrow field of view (0.9 x 0.45 degree) images were acquired during an imaging session. Widefield images of the retina were used for visualizing the cross-sectional structures of the retinal layers, and for navigating across the retina in relation to anatomical landmarks, such as blood vessels. Narrowfield images were used for visualizing the photoreceptor mosaic en face at various retinal eccentricities from the fovea.

**9697-32, Session 5**

**Aberration-corrected high-speed full-field swept-source OCT**

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In ophthalmic and high resolution applications of optical coherence tomography (OCT), a limited focal range and aberrations can severely restrict imaging capabilities. Hence adaptive optics has to be used to obtain high-resolution data. An alternative solution is a computational correction of aberrations, which can also eliminate the limited focal depth of high resolution imaging. However, this requires phase-stable data and an aberration detection algorithm that works independently of sample structure. The former is problematic as for in vivo imaging applications the acquired phase data is usually dominated by sample motion and various sample structures occur. We show that full-field swept-source OCT obtains data with an equivalent of 38.6 MHz A-scan rate or 117 volumes per second. At these speeds sample motion has no significant impact on the obtained phases and the data is thus sufficiently phase-stable to correct aberrations and defocus even when imaging human retina in vivo. The aberrations of the data were determined and corrected by an optimization of image quality which worked well on different sample structures. We present near diffraction limited in vivo images of the nerve fiber layer, small capillaries, and photo receptor cells, all acquired from a single dataset.

**9697-33, Session 5**

**Adaptive optics full-field OCT: a resolution almost insensitive to aberrations**

Peng Xiao, Mathias Fink, Claude Boccara, Institut Langevin (France)

A Full-Field OCT (FFOCT) setup coupled to a compact transmissive liquid crystal spatial light modulator (LCSLM) is used to induce or correct aberrations and simulate eye examinations. To reduce the system complexity, strict pupil conjugation was abandoned. During our work on quantifying the effect of geometrical aberrations on FFOCT images, we found that the image resolution is almost insensitive to aberrations. Indeed if the object channel PSF is distorted, its interference with the reference channel conserves the main feature of an unperturbed PSF with only a reduction of the signal level. This unique behavior is specific to the use of a spatially incoherent illumination. Based on this, the FFOCT image intensity was used as the metric for our wavefront sensorless correction. Aberration correction was first conducted on an USAF resolution target with the LCSLM as both aberration generator and corrector. A random aberration mask was induced, and the low-order Zernike Modes were corrected sequentially according to the intensity metric function optimization. A Ficus leaf and a fixed mouse brain tissue slice were also imaged to demonstrate the correction of sample self-induced wavefront distortions. After optimization, more structured information appears for the leaf imaging. And the high-signal fiber-like myelin fiber structures were resolved much more clearly after the whole correction process for mouse brain imaging. Our experiment shows the potential of this compact AO-FFOCT system for aberration correction imaging. The presented approach that simulates eyes aberrations correction also opens the path to a simple implementation of FFOCT adaptive optics for retinal examinations.

**9697-34, Session 5**

**3D-Spectral domain computational imaging**

Trevor B. Anderson, Armin Segref, Grant Frisken, Dirk Lorenser, Herman Ferra, Steven Frisken, Cylite Pty Ltd. (Australia)

The transverse resolution of an OCT system is determined, for a given wavelength, by the numerical aperture of the objective lens. However, increasing the numerical aperture of the objective reduces the depth of field, resulting in a trade-off between transverse resolution and depth of field. Computational imaging offers a potentially cost effective means of overcoming this trade-off without the requirements for complex hardware. Importantly, it also offers a means to compensate for aberrations as recently described for high-resolution retinal imaging. A variety of computational imaging approaches have been demonstrated, from point scanning time domain systems to full field swept systems. The key requirement for all approaches is that they maintain lateral phase coherence. Full-field swept source demonstrations exploit their intrinsic spatial coherence, however they require very fast and expensive 2D sensors. In the paper we present a new spectral domain computational approach that, in contrast to point scanning, line field or full field approaches, captures 3D information with high transverse resolution by illuminating and scanning the sample with a small collimated beam (for example of 75x75 ?m2). The key benefits include the use of low cost sensor to capture the field with stable phase coherence over both the axial and lateral dimensions. Digital defocusing can thus be applied to a single frame and registration between scanned volumes enables 3D images to be reconstructed. We demonstrate a proof of principle with capable of maintaining of 5.1 um resolution over 300 um axial range.

**9697-35, Session 6**

**Towards low-risk in vivo diagnosis of pulmonary fibrosis with optical coherence tomography**

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Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal form of fibrotic lung disease, with a 3 year survival rate of 50%. Diagnostic certainty of IPF is essential to determine the most effective therapy for patients, but often requires surgery to resect lung tissue and look for microscopic honeycombing not seen on chest computed tomography (CT). Unfortunately, surgical lung resection has high risks of associated morbidity and mortality in this patient population. We aim to determine whether bronchoscopic optical coherence tomography (OCT) can serve as a novel, low-risk paradigm for in vivo IPF diagnosis without surgery or tissue removal. OCT provides rapid 3D visualization of large tissue volumes with microscopic resolutions well beyond the capabilities of CT. We have designed bronchoscopic OCT catheters to effectively and safely access the...
peripheral lung, and conducted in vivo peripheral lung imaging in patients, including those with pulmonary fibrosis. We utilized these OCT catheters to perform bronchoscopic imaging in lung tissue from patients with pulmonary fibrosis to determine if bronchoscopic OCT could successfully visualize features of IPF through the peripheral airways. OCT was able to visualize characteristic features of IPF through the airway, including microscopic honeycombing (< 1 mm diameter) not visible by CT, dense peripheral fibrosis, and spatial disease heterogeneity. These findings support the potential of bronchoscopic OCT as a minimally-invasive method for in vivo IPF diagnosis. However, future clinical studies are needed to validate these findings.

9697-37, Session 6

Super-achromatic microprobe for ultrahigh-resolution endoscopic OCT imaging at 800 nm

Wu Yuan, Milad Alemohammad, Xiaoyun Yu, Shaoyong Yu, Xingde Li, Johns Hopkins Univ. (United States)

In this paper, we report a super-achromatic microprobe made with fiber-optic ball lens to enable ultrahigh-resolution endoscopic OCT imaging. An axial resolution of -2.4 µm (in air) can be achieved with a 7-fs Ti:Sapphire laser. The microprobe has minimal astigmatism which affords a high transverse resolution of -5.6 µm. The miniaturized microprobe has an outer diameter of -520 µm including the encasing metal guard and can be used to image small luminal organs. The performance of the ultrahigh-resolution OCT microprobe was demonstrated by imaging rat esophagus, guinea pig esophagus, and mouse rectum in vivo.

9697-38, Session 6

Depth of focus extension for OCT by self-imaging wavefront division fiber optic probe

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Optical coherence tomography (OCT) has been a useful complement to our arsenal of medical imaging technologies owing to its capacity to acquire cross-sectional images from inside the body using scanning fiber optic probes. Conventional, cross-sectional OCT has nominal 10-7m axial resolution and 30-7m lateral resolutions, parameters that enable high quality imaging of microscopic architectural morphology. While this resolution is useful for many medical applications, it is insufficient for resolving the individual cells that are ultimately the root cause of many diseases. To address this gap, a super-continuum based spectral-domain OCT (SD-OCT) system (2-µm axial resolution in tissue) and an extended depth of focus fiber optic probe for endoscopic/intravascular imaging were designed and fabricated. In the fiber optic probe, a self-imaging wavefront division optical system was used to provide multiple circular propagation modes (fundamental mode, 1st, 2nd and 3rd order modes). Once transmitted through a relatively high NA lens (NA>0.1), these modes were projected as multiple coaxial foci (-3 µm FWHM) that spanned a greatly extended focal depth range. Using this concept, we fabricated a 500-µm-diameter self-imaging wavefront division fiber optic probe that can be inserted in any catheter or endoscope-scanning device. The probe also contained a common-path reference reflectance to ensure high axial resolution OCT imaging. Measurements showed that the probe provides a 20-fold depth of focus extension, maintaining a 3-5 µm lateral resolution (FWHWM of PSF) over a depth range of approximately 1 mm. These results suggest that this new optical configuration will be useful for achieving high-resolution, cross-sectional OCT imaging in medical imaging devices.

9697-39, Session 6

High speed, ultrahigh-resolution, distal scanning endoscopic OCT at 800 nm

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We present a high-speed, ultrahigh resolution, distal end scanning OCT endoscope of a small form factor operating at -825 nm. We were able to achieve an axial resolution of 2.6 µm with a home built 7 fs Ti:Sapphire source. Circumferential beam scanning was performed by a customized 900 µm diameter micromotor. We demonstrated the capability of this OCT endoscopy system on intraluminal imaging of guinea pig esophagus in vivo at a speed of 70 frames/second.
Rotational distortion and tissue deformation correction in catheter-based optical coherence tomography using a hybrid speckle-decorrelation and feature-tracking technique

Néstor Uribe-Patarroyo, Wellman Ctr. for Photomedicine (United States); Benedikt W. Graf, David A. Vader, NinePoint Medical (United States); Brett E. Bourn, Wellman Ctr. for Photomedicine (United States)

Optical coherence tomography (OCT) is increasingly used in gastrointestinal (GI) and intravascular clinical imaging due to its capability for high-speed optical sectioning, high resolution and moderate penetration depths. Catheter probes are commonly used when imaging luminal organs, providing angular scanning with a motor that rotates the optical assembly at the tip of the probe. The flexibility required by the catheter impacts the fidelity of the torque transfer and therefore the angle measured by the motor encoder does not match 1:1 the physical angular position of the probe tip. This creates non-uniform rotation distortion (NURD), deforming imaged structures. NURD generally changes frame to frame, and thus remains an important image artifact in catheter-based OCT diagnostics. Furthermore, tissue deformation due to patient motion introduces motion artifacts that cannot be measured with techniques that are sensitive only to the scanning speed of the probe. We present a new hybrid technique for NURD and tissue deformation correction in OCT which uses, first, measurements of the rotational speed during azimuthal scanning by means of the statistical fluctuations of speckle in tissue using intensity-based dynamic light scattering, and second, two-dimensional feature tracking for further NURD correction and tissue deformation compensation. We show that this technique outperforms speckle- and feature-tracking-only correction approaches. This technique does not require a reference frame with zero NURD, is apt for real-time implementation, and provides highly accurate NURD correction as seen in en face projections of clinical datasets.

High-resolution optical polarization tractography based on polarization-sensitive optical coherence tomography

Gang Yao, Univ. of Missouri (United States)

Fibrous tissues exist in many parts of the body such as muscles, neural fibers, dental tissues, skin, and cartilage. In these tissues, the directional fiber organization plays an essential role for their functions. Disruption of the normal fibrous structure is linked to tissue dysfunction. For example, the myocardial fibers form the unique “cross-helical” structure in heart which enables normal electric signal propagation and coordinated mechanical force production for efficient blood pumping. Changes in such a delicate heart structure are the most prominent pathological features in heart disease.

An imaging tool that can reveal the detailed fiber architecture will be valuable to study the structure-function relationship in fibrous tissues. Histological staining has been applied to reveal fiber orientation. However, histological examination is labor-intensive and only practical for imaging a very small area. Diffusion MRI based methods such as diffusion-tensor MRI (DTI) and diffusion spectrum MRI have been developed to image fiber orientation in brain and muscle. However, their spatial resolution is limited to 100-200 μm or worse.

Here, we described a new high-resolution tractography technology developed from Jones matrix polarization-sensitive optical coherence tomography (PSOCT). We developed a systematic methodology to extracted depth-resolved local optical axis which was then used to visualize the fiber orientation. We demonstrated the capability of this new method for visualization 3D fibrous tissue structure in heart, skeletal muscle and artery. In addition, we showed that the disruption of regular fiber orientation indicated tissue damage. The image results agreed well with histology.

Non-invasive, in vivo imaging of subcortical mouse brain regions with 1.7 ?m optical coherence tomography

Shau Poh Chong, Conrad W. Merkle, Tingwei Zhang, Harsha Radhakrishnan, Dylan F. Cooke, Leah Krubitzer, Vivek J. Srinivasan, Univ. of California, Davis (United States)

A spectral / Fourier domain OCT intravital microscope using a supercontinuum light source at 1.7 μm, with theoretical axial resolution of 7.5 μm in air (5.6 μm in tissue, not accounting for water absorption), was developed to study sub-cortical structures non-invasively in the living mouse brain. While increasing the excitation wavelength beyond 1.3 μm further reduces scattering, this benefit is potentially mitigated by increased water absorption. Recently, excitation wavelengths in the 1.7 μm water absorption spectral window were shown to optimally deliver light to a focal plane deep in brain tissue, when attenuation due to both single scattering and absorption were accounted for. Here, the benefits of 1.7 μm for deep tissue brain imaging are demonstrated by quantitatively comparing OCT signal attenuation characteristics of cortical tissue across visible and near-infrared wavelengths, measured at multiple co-registered locations in the same brain. Furthermore, imaging of hippocampal tissue architecture and white matter microvasculature are demonstrated in vivo through thinned-skull, glass coverslip-reinforced cranial windows in mice, up to a depth of about 2.2 mm. Layers or strata in the hippocampus proper are clearly visible, with contrasting reflectivity across different layers due to the underlying tissue types. In particular, the corpus callosum (cc) and alveus of hippocampus (alv) show high reflectivity due to the high degree of myelination in white matter. The stratum pyramidale (Py) contains the cell bodies of the pyramidal neurons and thus appears as a low scattering band. Applications of this novel platform include monitoring disease progression and pathophysiology in rodent models of Alzheimer’s disease and sub-cortical dementias, including vascular dementia.

Imaging of the stroke-related changes in mouse brain vascular system with the use of extended focus optical coherence microscopy

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Optical Coherence Microscopy is used for imaging of changes in the structure and dynamics of blood flow in small rodents’ brains in case of ischemic stroke. The stroke is induced by local production of clot using photothermolysis model. Bengal rose is utilized as phototoxic dye. The custom designed multimodal measurement platform consists of interferometer system combining extended focus Optical Coherence Microscope for both structural and angiographic imaging, the photothermolysis induction subsystem and brightfield microscope for control of the experiment and structural image reference. The extended focus is provided by the use of Bessel beam created with the use of axicon
9697-44, Session 7

Investigating alcohol-induced congenital heart defects using optical coherence tomography
Shi Gu, Lindsay M. Peterson, Pei Ma, Ganga Karunamuni, Michiko Watanabe, Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Fetal alcohol syndrome commonly results in neurological and craniofacial defects, additionally, as high as 54% of live-born children with this syndrome also possess cardiac abnormalities. We have previously shown that CNCC-ablated embryos exhibit similar structural and functional phenotypes as ethanol-exposed embryos. Here, we present progress on two fronts toward understanding the association between CNCC dysfunction and FAS-related CHDs. We have developed a technique for measuring the thickness of the cardiac cushions throughout the heart. These values were then mapped onto a surface mesh of the myocardial wall for 3-D visualization. The cushions were observed to be significantly reduced in the outflow tract of CNCC-ablated embryos. We also observed a correlation between abnormal pulsed Doppler waveforms and increased separation of the atrioventricular inferior and superior cushions. This correlation between function and structure will enable rapid phenotyping of perturbed embryos. Finally, we present our preliminary results using methyl donors to rescue ethanol-exposed embryonic CHDs. Betaine was administered along with the ethanol injection to embryos at 21 hours of development. The embryos were then analyzed at day 8 for survival and heart morphology. The administration of betaine resulted in a significant increase in survival and normalization of atrioventricular valve leaflet volume and interventricular septum thickness.

9697-45, Session 7

An integrated optical coherence microscopy imaging and optical stimulation system for optogenetic pacing in Drosophila melanogaster
Jing Men, Chao Zhou, Lehigh Univ. (United States)

Electrical stimulation is the clinical standard for cardiac pacing. Although highly effective in controlling cardiac rhythm, the invasive nature, non-specificity to cardiac tissues and possible tissue damage limits its applications. Optogenetic pacing of the heart is a promising alternative, which is non-invasive and more specific, has high spatial and temporal precision, and avoids the shortcomings in electrical stimulation. Drosophila melanogaster, which is a powerful model organism with orthologs of nearly 75% of human disease genes, has not been studied for optogenetic pacing in the heart. Here, we developed a non-invasive integrated optical pacing and optical coherence microscopy (OCM) imaging system to control the heart rhythm of Drosophila at different developmental stages using light. The OCM system is capable of providing high imaging speed (130 frames/s) and ultra-high imaging resolutions (1.5 μm and 5.9 μm for axial and transverse resolutions, respectively). A light-sensitive pacemaker was developed in Drosophila by specifically expressing the light-gated cation channel, channelrhodopsin-2 (ChR2) in transgenic Drosophila heart. We achieved non-invasive and specific optical control of the Drosophila heart rhythm throughout the fly’s life cycle (larva, pupa, and adult) by stimulating the heart with 475 nm pulsed laser light. Heart response to stimulation pulses was monitored non-invasively with OCM. This integrated non-invasive optogenetic control and in vivo imaging technique provides a novel platform for performing research studies in developmental cardiology.

9697-46, Session 7

Handheld OCT for longitudinal tracking of chronic middle-ear infection in response to therapeutic interventions
Guillermo L. Monroy, Ryan L. Shelton, Ryan M. Nolan, Paritosh Pande, Darold Spillman, Univ. of Illinois at Urbana-Champaign (United States); Daniel T. McCormick, AdvancedMEMS (United States); Michael Novak M.D., Ryan Porter M.D., Malcolm Hill M.D., Carle Foundation Hospital (United States); Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Middle ear infections, commonly referred to as otitis media (OM), affect up to 95% of children worldwide. Unfortunately, diagnostic equipment for this disease is limited to relatively simple illuminated magnifiers. Acute ear infections can be treated with antibiotics, although pathogenic antibiotic resistance due to over-prescription and misdiagnosis is a growing concern. Persistent infections are treated with a surgically invasive tympanostomy tube placement, one of the most common operations performed for children under anesthesia. In this study, a robust, portable, handheld imaging system that integrates optical coherence tomography (OCT) and video-based imaging within a MEMS-based handheld scanner was used to quantitatively characterize and assess the efficacy of therapeutic interventions to clear middle ear infection.

Patients were imaged when visiting ear specialists at Carle Foundation Hospital in Urbana, IL under an IRB-approved prospective study. The OCT system was transported to multiple clinical sites to image patients longitudinally through consecutive pre-operative, surgical, and post-operative visits. Subjects found to have a middle ear biofilm in OCT cross-sectional images at the pre-operative visit and surgical visit were subsequently imaged at the follow-up visit and had no visible biofilm. This finding suggests that therapeutic interventions, such as surgical tube placement, provide a means to clear the middle ear of infection-related components, including any middle ear biofilm. Furthermore, our robust, portable, handheld OCT-based system was successfully demonstrated as a rapid diagnostic tool to longitudinally follow patients in outpatient and surgical settings.

9697-47, Session 7

In-vivo cutaneous burn depth assessment and wound healing process observing with multi-functional optical coherence tomography
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Research about the cutaneous burn was separated by assessment of burn depth and development of wound healing therapy. Various in vivo optical techniques were used to determine burn depth and observe the wound healing process. In this paper, we report the usage of multimodal optical coherence tomography system, which containing angiographic and polarization sensitive OCT (PS-OCT) with conventional OCT system, at burn studies. Burn was induced at 4 different degrees by control the attachment time of 75 Celsius degree heated brass rod at dorsal skin of the rat. For the burn depth assessment, we imaged the different burn degrees area. Changes of polarization sensitive signal were providing burn depth
Development of a low-cost hand-held system for optical coherence tomography imaging

Paritosh Pande, Ryan L. Shelton, Guillermo L. Monroy, Ryan M. Nolan, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)
We report the development of a low-cost hand-held optical coherence imaging system. The proposed system is based on the principle of linear optical coherence tomography (Linear OCT), a technique which was proposed in the early 2000s as a simpler alternative to the conventional time-domain and Fourier-domain OCT. In our design, as in the traditional Michelson interferometer, light from a broadband source is split into sample and reference beams. Unlike in a Michelson interferometer though, upon return, a tilt is introduced to the reference beam before it is combined with the sample beam to illuminate a detector array. The resulting fringe pattern encodes information about the relative time-of-flight of photons between the sample and reference arms, which can be decoded by standard signal processing techniques to obtain depth resolved reflectivity profiles of the sample. The axial resolution and the SNR of our system was measured to be approximately 5.2 μm and 80 dB, respectively. The performance of the proposed system was compared with a standard state-of-the-art Fourier-domain low coherence interferometry (LCI) system by imaging several biological and non-biological samples. The results of this study indicate that the proposed low-cost system might be a suitable choice for applications where the imaging depth and SNR can be traded for lower cost and simpler optical design. Two potentially useful applications of the proposed imaging system could be for imaging the human tympanic membrane (TM) for diagnosing middle ear pathologies, and to visualize the sub-surface features of materials for non-destructive evaluation and quality inspection.

Needle-based polarization-sensitive OCT of breast tumor

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OCT imaging through miniature needle probes has extended the range of OCT and enabled structural imaging deep inside breast tissue, with the potential to assist in the intraoperative assessment of tumor margins. However, in many situations, scattering contrast alone is insufficient to clearly identify and delineate malignant areas. Here, we present a portable, depth-encoded polarization-sensitive OCT system, connected to a miniature needle probe. From the measured polarization states, we constructed the Mueller matrix at each sample location and improved the accuracy of the measured polarization states through incoherent averaging before retrieving the depth-resolved tissue birefringence. With the Mueller matrix at hand, additional polarization properties such as depolarization are readily available. We then imaged freshly excised breast tissue from a patient undergoing lumpectomy. The reconstructed local retardation highlighted regions of connective tissue, which exhibited birefringence due to the abundance of collagen fibers, and offered excellent contrast to areas of malignant tissue, which exhibited less birefringence due to their different tissue composition. Results were validated against co-located histology sections. The combination of needle-based imaging with the complementary contrast provided by polarization-sensitive analysis offers a powerful instrument for advanced tissue imaging and has potential to aid in the assessment of tumor margins during the resection of breast cancer.
ergonomic device that includes a collimator, two galvanometric scanning mirrors and an objective for focusing the light on the skin. The device is disinfectable and causes minimal discomfort to the patients. The system permits easy and fast in vivo acquisition of large three-dimensional datasets of human skin in a clinical environment. Jones formalism based processing of the data is used to extract the phase retardation image. Advanced coherent signal composition is implemented to enhance the signal-to-noise ratio.

High-resolution clinical PS-OCT videos of scars originated from burn wounds are shown. Histological slides of biopsies taken from the scanned area correlate well with the corresponding phase retardation images, demonstrating the diagnostic value of the technique.

9697-52, Session 8

Studying airway smooth muscle in vivo with PS-OCT

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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. We have developed novel techniques that we applied to Polarization Sensitive OCT (PS-OCT) in order to assess ASM, and validated our results with a substantial number of histological matches. In this work we employ our system in the study of ASM distributions in both asthmatic and non-asthmatic airways with data obtained in vivo from human volunteers. By isolating the ASM and performing volumetric analysis we obtain a variety of informative metrics such as ASM thickness and band width, and compare these quantities between subject types. Furthermore, we demonstrate that the degree of birefringence of the ASM can be associated with contractility, allowing us to estimate pressure exerted by ASM during contraction. We apply this technique to in vivo datasets from human volunteers as well.

9697-53, Session 8

Accurate and quantitative polarization-sensitive OCT by unbiased birefringence estimator with noise-stochastic correction

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We present an improved version of the MAP estimator of local phase retardation with noise stochastic correction. The estimator is designed for a Jones matrix OCT system with polarization diversity detection. The non-linear mathematical model of the effect of SNR in the local retardation measurement was implemented using Monte-Carlo simulation. A comparison of the performance of this new estimator with our previous estimator and the mean estimator is carried out using numerical simulation. Superior performance of the new estimator compared to the other estimators for the in-vivo measurement of anterior and posterior eye is also shown.

9697-54, Session 8

Jones-matrix estimation for quantitative birefringence analysis in polarization-sensitive OCT

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It has not been easy to estimate the true phase retardation quantitatively from noisy Jones matrices measured by PS-OCT, because a mean of measured phase retardation is not an estimate of the true value due to the asymmetric statistical distribution. Here, we show our novel method for this fundamental issue. We estimate Jones matrix by the maximum likelihood estimator of covariance matrix that is calculated from the measured Jones matrices, and obtain the best estimate of the phase retardation.

We validate our method using quarter waveplates at 1310 nm and 633 nm and a glass plate under low signal-to-noise ratio. The double-pass phase retardation was asymptotically unbiased and close to the true value by increasing number of pixels over -30 pixels, indicating the efficacy of our method. The new method is applied to images of the anterior eye segment as a spatial filter. The method using maximum likelihood estimation of the Jones matrix shows superior performance in polarimetric speckle reduction of local retardation images compared to coherent Jones-matrix averaging filter that was previously developed by our group.

We show the details of the theory and demonstrate the application for image processing of the anterior eye segment.

9697-55, Session 8

Spectral-domain, polarization-sensitive optical coherence tomography system insensitive to fiber disturbances

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This communication presents a spectral-domain, polarization-sensitive optical coherence tomography (PS-OCT) system based on a fiber interferometer using single-mode fibers and couplers. The two orthogonal polarization components are sequentially detected by a single line camera. Retardance measurements can be affected by polarimetric effects in fibers and couplers. This configuration bypasses such issues by performing polarization selection before the collection fiber, through the combination of a polarization rotator and a linear polarizer. Similar net retardance maps of a birefringent phantom are obtained for two different settings of induced fiber birefringence, demonstrating the tolerance of the configuration to fiber-based disturbances.
Polarization sensitive optical frequency domain imaging of plasmon-resonant nanoparticles using the depolarization index

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Nanoparticles have previously been proposed as exogenous contrast agents for optical coherence tomography (OCT). However, their scattering alone is generally insufficient to differentiate labeled from non-labeled biological structures with high sensitivity. To circumvent this limitation, we exploit the depolarization property of metallic nanoparticles (MMNs) with high aspect ratios. Imaged with polarization sensitive OCT, we reconstruct the sample Mueller matrix and calculate the depolarization index. Unlike the traditional degree of polarization uniformity (DOPU), the depolarization index is independent of the incident polarization state and more rigorously describes the depolarizing behavior of an optical system rather than the electric field. We demonstrate this depolarization contrast with imaging of nano-bio interactions in tumor-like spheroids, which mimic complex physiological conditions. The possibility to achieve contrast with molecular specificity in OCT by means of depolarization contrast would be enabling for a wide range of applications.

Towards intraoperative assessment of tumor margins in breast surgery using optical coherence elastography

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Surgical excision of tumor is a critical factor in the management of breast cancer. The most common surgical procedure is breast-conserving surgery. The surgeon’s goal is to remove the tumor and a rim of healthy tissue surrounding the tumor: the surgical margin. A major issue in breast-conserving surgery is the absence of a reliable tool to guide the surgeon intraoperatively assessing the margin. A number of techniques have been proposed; however, the re-excision rate remains high and has been reported to be in the range 30-60%. New tools are needed to address this issue. Optical coherence elastography (OCE) shows promise as a tool for intraoperative tumor margin assessment in breast-conserving surgery. Further advances towards clinical translation are limited by long scan times and small fields of view. In particular, scanning over sufficient areas to assess the entire margin in an intraoperative timeframe has not been shown to be feasible. Here, we present a protocol allowing ~75% of the surgical margins to be assessed within 30 minutes. To achieve this, we have incorporated a 65 mm-diameter (internal), wide-aperture annular piezoelectric transducer, allowing the entire surface of the excised tumor mass to be automatically imaged in an OCT mosaic comprised of 10 × 10 mm tiles. As OCT is effective in identifying adipose tissue, our protocol uses the wide-field OCT to selectively guide subsequent local OCE scanning to regions of solid tissue which often present low contrast in OCT images. We present promising examples from freshly excised human breast tissue.

Corneal elastic anisotropy and hysteresis as a function of IOP assessed by optical coherence elastography

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Several diseases may affect the elastic anisotropy of the cornea. This work evaluates the elastic anisotropy and hysteresis of in situ porcine corneas at different cycled intraocular pressures (IOPs) using a noncontact optical coherence elastography (OCE) technique. A focused air-pulse induced low amplitude (~10 µm) elastic waves in fresh porcine corneas in the whole eye-globe configuration. A phase-stabilized swept source optical coherence elastography (PhS-SSOCE) system imaged the elastic wave propagation at stepped radial directions. A closed-loop feedback system was utilized to artificially cycle the IOP from 15 to 30 and back to 15 mmHg while OCE measurements were performed. The elastic wave velocity was translated to Young’s modulus to determine the elastic anisotropy and hysteresis while cycling IOP. The OCE measurements demonstrated that the elastic anisotropy of the cornea became more pronounced at higher IOPs and that there were distinct radial angles of higher and lower stiffness. Furthermore, there was a measurable elastic anisotropic hysteresis while cycling IOP. This noncontact OCE method was capable of assessing corneal elastic anisotropy and hysteresis while cycling IOP. Due to the noninvasive nature and small amplitude of the elastic wave, this method may be useful for in vivo investigations of the elastic anisotropy of the cornea that could provide valuable information about corneal health and integrity.

Optical coherence elastography based on high speed imaging of single-shot laser-induced acoustic wave at 16 kHz frame rate

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Shear wave OCE (SW-OCE) is a novel technique that relies on the detection of the localized shear wave speed, to provide a quantitative elastography. In this study we demonstrate high speed shear wave imaging to capture single-shot transient shear wave propagation to perform SW-OCE. The fast imaging speed is achieved using Fourier domain mode-locked (FDML) high-speed swept-source OCT (SS-OCT) system. The frame rate of shear wave imaging is 16 kHz, at A-line rate of ~1.62 MHz, enabling the detection of high-frequency shear wave of up to 8 kHz. Several measures are taken to improve the phase-stability of the SS-OCT system, and the measured velocity sensitivity is ~10 nanometers. As an external stimulation to facilitate elastography, shear waves are generated with photo-thermal effect, by ultra-violet pulsed laser, which requires no contact to OCE subjects. High frequency shear waves launched by pulsed laser presents shorter wavelength and carries rich localized elasticity information. Benefiting from single-shot acquisition, each SWI scan only takes 2.5 milliseconds, and the reconstruction of elastography image can be performed real-time with ~20 Hz refresh rate. SW-OCE measurements are demonstrated on tissue-mimicking phantoms and porcine cornea. This study is the first demonstration of an all-optical method to perform real-time 3D SW-OCE. It is hoped that this technique will be applicable in clinical applications, to obtain high-resolution localized quantitative measurement of tissue biomechanical property.
Imaging shear wave propagation for the elastic measurement using OCT Doppler variance method

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In this study we develop an acoustic radiation force orthogonal excitation optical coherence elastography (ARFOE-OCE) method for the visualization of the shear wave and the calculation of the shear modulus based on the OCT Doppler variance method. The vibration perpendicular to the OCT detection direction is induced by the remote acoustic radiation force (ARF) and the shear wave propagating along the OCT beam is visualized by the OCT M-scan. The homogeneous agar phantom and two-layer agar phantom are measured using ARFOE-OCE system. The results show that the ARFOE-OCE system has the ability to measure the shear modulus beyond the OCT imaging depth. The OCT Doppler variance method instead of the OCT Doppler phase method are used for the vibration detection without the need of high phase stability and the phase wrapping correction. A M-scan instead of the B-scan for the visualization of shear wave also simplifies the data processing.

Ultra-high resolution optical coherence elastography using a Bessel beam for extended depth of field

Andrea Curatolo, The Univ. of Western Australia (Australia); Martin Villiger, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Dirk Lorenser, Philip Wijesinghe, Alex Fritz, Brendan F. Kennedy, David D. Sampson, The Univ. of Western Australia (Australia)

Visualizing stiffness within the local tissue environment at the cellular level has the potential to provide insight into the genesis and progress of disease. In this paper, we propose and demonstrate ultra-high resolution optical coherence elastography, a technique that produces three-dimensional images of the local axial strain that tissue experiences under compressive loading, using phase-sensitive detection of local tissue displacement. The technique employs a dual-arm extended focus optical coherence microscope (xf-FDOMC) to measure tissue displacement source under compression. The system uses a broadband supercontinuum laser diode for ultra-high axial resolution, Bessel beam illumination and Gaussian beam detection, maintaining sub-2 μm transverse resolution over nearly 100 μm depth of field (DOF), and spectral-domain detection allowing high displacement sensitivity. The signal processing chain involves calculating the phase difference of a pair of complex xf-FDOMC images of the uncompressed (pre-loaded) and compressed sample. Four A-scans are averaged for each transverse x-location to improve the displacement sensitivity. Phase unwrapping of the resulting phase difference allows the unambiguous calculation of the local displacement in the sample. Weighted-least squares (WLS) regression on the displacement map provides estimation of the local strain. We acquire two pairs of uncompressed – compressed B-scans per y-location, and average the resulting co-located strain B-scans. We present elastograms with resolution of 22715 μm (x,y,z), leading the way towards cellular biomechanics in situ. We demonstrate this record resolution in elastograms on a tissue phantom and freshly excised mouse aorta, revealing the mechanical heterogeneity of vascular smooth muscle cells and elastin sheets, otherwise unresolved in conventional lower resolution systems.

Single shot line-field optical coherence elastography

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Elastic wave imaging optical coherence elastography (EWI-OCE) is an emerging elastographic technique that can provide the local biomechanical properties of tissues based on analysis of the propagation of an externally induced elastic wave. The typical procedure for EWI-OCE involves acquisition of multiple M-mode images across the tissue surface (M-B mode) and requires repeated excitations at each OCE measurement position. This leads to long acquisition times that make this approach impractical for clinical use. In this work we present a non-contact single shot line-field optical coherence elastography system comprised of a line-field interferometer and an air-pulse delivery system. Spatial-temporal maps were acquired by a 128 kHz line-scan camera and precisely synchronized 8 kHz PZT phase modulator. The elastic wave propagation across the sample surface was acquired in the spatio-temporal domain with a temporal resolution of 62.5 μs by utilizing both forward and backward scans of the modulating reference mirror. Utilizing the Carré phase shifting algorithm, the elastic wave displacement profile was retrieved from the spatio-temporal interference patterns and used to compute the elastic wave group velocity. With this technique, the elastic wave propagation can be directly imaged along a single direction with only a single excitation. The OCE results on tissue-mimicking agar phantoms were in good agreement with uniaxial mechanical compression testing, demonstrating that the presented method can effectively reduce the acquisition time in EWI-OCE to milliseconds.
and RPE, a 2D projection map was created. The optic disc in the projection map was detected by the 2D graph search. The pre-defined optic disc information was then incorporated into the surface interaction constraints of the 3D graph search to obtain more accurate choroidal surfaces. Twenty SD-OCT images from 20 healthy subjects were used. The mean differences of the choroidal borders between the algorithm and manual segmentation were at a sub-voxel level, indicating a high level segmentation accuracy using this approach.

9697-64, Session 10

Motion correction in full-field swept-source OCT

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Lateral parallelization in full-field swept-source OCT (FF-SS-OCT) increases the acquisition speed compared to scanning OCT and provides inherent lateral phase stability, which enables new processing and contrast mechanisms. However, FF-SS-OCT obtains a single A-scan in the same time as an entire volume, which makes it especially sensitive to axial sample motion. FF-SS-OCT therefore requires expensive high-speed cameras that are operated with a restricted area of interest. Here we present two approaches to detect and correct axial motion from an acquired dataset: In the first approach, the axial sample motion is obtained from the acquired images by determining the phase shift between successive images. In the second approach, an iterative image quality optimization of the reconstructed volume determines the axial sample motion. The detected motion is then used to correct the acquired data. In FF-SS-OCT, this computational correction not only increases the area of interest and thus the field of view, but also makes the technique applicable with lower-cost cameras.

9697-65, Session 10

Optical coherence tomography noise modeling and fundamental bounds on human retinal layer segmentation accuracy

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The human retina is composed of several layers, visible by in vivo optical coherence tomography (OCT) imaging. To enhance diagnostics of retinal diseases, several algorithms have been developed to automatically segment one or more of the boundaries of these layers. OCT images are corrupted by noise, which is frequently the result of the detector noise and speckle, a type of coherent noise resulting from the presence of several scatterers in each voxel. However, it is unknown what the empirical distribution of noise in each layer of the retina is, and how the magnitude and distribution of the noise affects the lower bounds of segmentation accuracy. Five healthy volunteers were imaged using a spectral domain OCT probe from Bioptigen, Inc. centered at 850nm with 4.6μm full width at half maximum axial resolution. Each volume was segmented by expert manual graders into nine layers. The histograms of intensities in each layer were then fit to seven possible noise distributions from the literature on speckle and image processing. Using these empirical noise distributions and empirical

estimates of the intensity of each layer, the Cramer-Rao lower bound (CRLB), a measure of the variance of an estimator, was calculated for each boundary layer. Additionally, the optimum bias of a segmentation algorithm was calculated, and a corresponding biased CRLB was calculated, which represents the improved performance an algorithm can achieve by using prior knowledge, such as the smoothness and continuity of layer boundaries. Our general mathematical model can be easily adapted for virtually any OCT modality.

9697-66, Session 10

Development of a new robust and accurate spectroscopic metric for scatterer size estimation in optical coherence tomography (OCT) images

Michalis Kassinopoulos, Costas Pitris, Univ. of Cyprus (Cyprus)

The modulations appearing on the backscattering spectrum originating from a scatterer are related to its diameter as described by Mie theory for spherical particles. Many metrics for Spectroscopic Optical Coherence Tomography (SOCT) take advantage of this observation in order to enhance the contrast of Optical Coherence Tomography (OCT) images. However, none of these metrics has achieved high accuracy when calculating the scatterer size. In this work, Mie theory was used to further investigate the relationship between the degree of modulation in the spectrum and the scatterer size. From this study, a new spectroscopic metric, the bandwidth of the Correlation of the Derivative (COD) was developed which is more robust and accurate, compared to previously reported techniques, in the estimation of scatterer size. The self-normalizing nature of the derivative and the robustness of the first minimum of the correlation as a measure of its width, offer significant advantages over other spectral analysis approaches especially for scatterer sizes above 3 μm. The feasibility of this technique was demonstrated using phantom samples containing 6, 10 and 16 μm diameter microspheres as well as images of normal and cancerous human colon. The results are very promising, suggesting that the proposed metric could be implemented in OCT spectral analysis for measuring nuclear size distribution in biological tissues. A technique providing such information would be of great clinical significance since it would allow the detection of nuclear enlargement at the earliest stages of precancerous development.

9697-67, Session 10

Quantitative optical coherence tomography by maximum a-posteriori estimation of signal intensity

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Intensity averaging of optical coherence tomography (OCT) is frequently used for generating high contrast images. However, the averaged intensity is not quantitative and biased from the true OCT signal intensity especially at low intensities. Signal intensity threshold is sometimes applied to remove this bias, but it further reduces the quantitativeness and removes information of low-backscattering tissues. In this presentation, a maximum a-posteriori (MAP) estimation method for OCT signal intensity is developed. It has been developed for quantitative estimation of light scattering, based on a statistical model of signal formation. This method provides higher contrast, lower bias, and better quantification of scattering than standard multi-frame averaging. To examine the optical properties of the retina, attenuation coefficient values (based on a method originally developed by Vermeer et al) are estimated from MAP estimated intensity
data and averaged data. The performance of these methods, intensity and attenuation coefficient imaging, is evaluated by using ocular OCT images taken at the macular region of healthy subjects. This evaluation shows that MAP estimation provides higher contrast OCT images than standard averaging. In attenuation imaging, using the metrics of the Bhattacharyya distance, the Matusis distance and Fisher’s discriminant ratio, it is shown that the MAP estimator provides a larger histogram difference among different retinal tissue layers than an attenuation image computed from a standard averaged OCT image.

9697-68, Session 10
Rigorous simulation of OCT image formation using Maxwell’s equations in three dimensions
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Existing models of image formation in optical coherence tomography are based upon the extended Huygens-Fresnel formalism. These models all, to varying degrees, rely on scatterer ensemble averages, rather than deterministic scattering distributions. Whilst the former is sometimes preferable, there are a growing number of applications where the ability to predict image formation based upon deterministic refractive index distributions is of great interest, including, for example, image formation in turbid tissue.

A rigorous model based upon three-dimensional solutions of Maxwell’s equations offers a number of tantalising opportunities. For example, shedding light on features near or below the resolution of an OCT system and on the impact of phenomena usually described as diffraction, interference and scattering, but which more generally result from light scattering satisfying Maxwell’s equations. A rigorous model allows inverse scattering methods to be developed not requiring the first-order Born approximation. Finally, a rigorous model can provide gold standard verification of myriad quantitative techniques currently being developed throughout the field.

We have developed the first such model of image formation based upon three-dimensional solutions of Maxwell’s equations, which has vastly different properties to models based on two-dimensional solutions. Although we present simulated B-scans, this model is equally applicable to C-scans. This has been made possible by advances in computational techniques and in computational resources routinely available. We will present the main features of our model, comparisons of measured and simulated image formation for phantoms and discuss the future of rigorous modelling in optical coherence tomography research and application.

9697-69, Session 11
Three dimensional time lapse imaging of live cell mitochondria with photothermal optical lock-in optical coherence microscopy
Miguel Sison, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Sabyasachi Chakrabortty, Univ. Ulm (Germany); Jerome Extermann, Amir Nahas, Christophe Pache, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Tanja Weil, Univ. Ulm (Germany); Theo Lasser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

The photothermal optical lock-in optical coherence microscope (poli-OCM) introduced molecular specificity to OCM imaging, which is conventionally, a label-free technique. Here we achieve three-dimensional live cell and mitochondria specific imaging using ~4nm protein-functionalized gold nanoparticles (AuNPs). These nanoparticles do not photobleach and we demonstrate they’re suitability for long-term time lapse imaging. We compare the accuracy of labelling with these AuNPs using classical fluorescence confocal imaging with a standard mitochondria specific marker. Furthermore, time lapse poli-OCM imaging every 5 minutes over 1.5 hours period was achieved, revealing the ability for three-dimensional monitoring of mitochondria dynamics.

9697-70, Session 11
In vivo photothermal optical coherence tomography in the mouse eye
Maryse Lapierre-Landry, Andrew Y. Gordon, Jason R. Craft, Melissa C. Skala, Vanderbilt Univ. (United States)

OCT has become a standard in retina imaging at the pre-clinical and clinical level by allowing non-invasive, three-dimensional imaging of the tissue structure. However, OCT lacks specificity to contrast agents that could be used for in vivo molecular imaging. We have performed in vivo photothermal optical coherence tomography (PTOCT) of gold nanorods in the mouse retina after the mice were injected intravenously with the contrast agent. To our knowledge, we are the first team to perform PTOCT in the eye. Four lesions were induced by laser photocoagulation in each mouse retina (n=6 mice) and gold nanorods (untargeted and targeted with anti-mouse CD102 antibody, which labels neovascularule, peak absorption ~750nm) were injected intravenously by tail-vein injection five days later in four mice (two mice are controls). The mice were imaged with PTOCT the same day. Our instrument is a spectral domain OCT system (?=860nm) with a Titanium:Sapphire laser (?=750nm) added to the beam path using a 50:50 splitter to target the gold nanorods. We acquired PTOCT B-scans over one lesion per mouse eye. There was a significant increase in photothermal intensity at the center of the lesion in the gold nanorod group versus the control group. This experiment demonstrates the feasibility of PTOCT to image the distribution of contrast agents in the mouse retina. In the future we will use this method to optimize drug delivery to the retina in pre-clinical models.

9697-71, Session 11
Rhodopsin molecular contrast imaging by optical coherence tomography for functional assessment of photoreceptors
Zahra Nafra, Tan Liu, Shuliang Jiao, Florida International Univ. (United States)

Rhodopsin, the light-sensing molecule in the outer segments of rod photoreceptors, is responsible for converting light into neuronal signals in a process known as phototransduction. Rhodopsin is thus a functional biomarker for rod photoreceptors. We developed a novel technology based on visible-light optical coherence tomography (VIS-OCT) for in vivo molecular imaging of rhodopsin. The depth resolution of OCT allows the visualization of the location where the change of optical absorption occurs and provides a potentially accurate assessment of rhodopsin content by segmentation of the image at the location. A broadband supercontinuum laser, whose filtered output was centered at 520 nm, was used as the illuminating light source. To test the capabilities of the system on rhodopsin mapping we imaged the retina of albino rats. The rats were dark adapted before imaging. An integrated near infrared OCT was used to guide the alignment in dark. VIS-OCT three-dimensional images were then acquired under dark- and light- adapted states sequentially. Rhodopsin distribution was calculated from the differential image. The rhodopsin distributions can be displayed in both en face view and depth-resolved cross-sectional image. Rhodopsin OCT can be used to quantitatively image rhodopsin distribution and thus assess the distribution of functional rod photoreceptors in the
aqueous MB. As an initial demonstration the MB microspheres were imaged
microspheres were synthesized with an inner core containing 0.01% (w/v)
used in drug delivery and tissue engineering applications. 2.5 µm diameter
imaging. Both MB and PLGA are used clinically. PLGA is FDA approved and
without modifying the excited state dynamics of MB that enable PPOCT
MB offer several advantages over bare MB. The microsphere encapsulation
system. The simple addition of a dichroic mirror in the sample arm enabled
demonstrated in vivo molecular contrast imaging of methylene blue (MB)
the morphological context for the molecular information. We recently
provides detailed information on the local biochemistry and yield enhanced
retina. Rhodopsin OCT can bring significant impact into ophthalmic clinics
by providing a tool for the diagnosis and severity assessment of a variety of
retinal conditions.

9697-72, Session 11
Visible-light OCT to quantify retinal oxygen metabolism
Hao F. Zhang, Yi Ji, Siyu Chen, Wenzhong Liu, Brian T. Soetikno, Northwestern Univ. (United States)
We explored, both numerically and experimentally, whether OCT can be
acceptable candidate to accurately measure retinal oxygen metabolism. We
first used statistical methods to numerically simulate photon transport in the
retina to mimic OCT working under different spectral ranges. Then we
analyze accuracy of OCT oximetry subject to parameter variations such
as vessel size, pigmentation, and oxygenation. We further developed an
experimental OCT system based on the spectral range identified by our
simulation work. We applied the newly developed OCT to measure both
retinal hemoglobin oxygen saturation (sO2) and retinal flow. After
obtaining the retinal sO2 and blood velocity, we further measured retinal
vessel diameter and calculated the retinal oxygen metabolism rate (MRO2).
To test the capability of our OCT, we imaged wild-type Long-Evans rats
ventilated with both normal air and air mixtures with various oxygen
concentrations.

Our simulation suggested that OCT working within visible spectral range
is able to provide accurate measurement of retinal MRO2 using inverse
Fourier transform spectral reconstruction. We called this newly developed
technology vis-OCT, and showed that vis-OCT was able to measure the sO2
value in every single major retinal vessel around the optical disk as well
as in micro retinal vessels. When breathing normal air, the averaged sO2
in arterial and venous blood in Long-Evans rats was measured to be 95% and 72%, respectively. When we challenge the rats using air mixtures
with different oxygen concentrations, vis-OCT measurement followed analytical
models of retinal oxygen diffusion and pulse oximeter well.

9697-73, Session 11
Pump-probe optical coherence tomography using microencapsulated
methylene blue as a contrast agent
Wihan Kim, Erin Zebrowski, Hazel C. Lopez, Brian E. Applegate, Phapanin Charoenphol, Javier A. Jo, Texas A&M
Univ. (United States)
Molecular contrast imaging can target specific molecules or receptors to
provide detailed information on the local biochemistry and yield enhanced
visualization of pathological and physiological processes. When paired
with Optical Coherence Tomography (OCT) it can simultaneously supply
the morphological context for the molecular information. We recently
demonstrated in vivo molecular contrast imaging of methylene blue (MB)
using a 663 nm diode laser as a pump in a Pump-Probe OCT (PPoCT)
system. The simple addition of a dichroic mirror in the sample arm enabled
PPoCT imaging with a typical B30-nm band spectral-domain OCT system.
Here we report on the development of a microencapsulated MB contrast
agent. The poly lactic-co-glycolic acid (PLGA) microspheres loaded with
MB offer several advantages over bare MB. The microsphere encapsulation
improves the PPoCT signal both by enhancing the scattering and preventing
the reduction of MB to leucemethylene blue. The surface of the microsphere
can readily be functionalized to enable active targeting of the contrast agent
without modifying the excited state dynamics of MB that enable PPoCT
imaging. Both MB and PLGA are used clinically. PLGA is FDA approved and
used in drug delivery and tissue engineering applications. 2.5 µm diameter
microspheres were synthesized with an inner core containing 0.01% (w/v)
aqueous MB. As an initial demonstration the MB microspheres were imaged
in a 100 µm diameter capillary tube submerged in a 1% intralipid emulsion.

9697-74, Session 11
Interferometric near-infrared spectroscopy
Dawid Borycki, Oybek Kholiqv, Shau Poh Chong, Vivek J. Srinivasan, Univ. of California, Davis (United States)
We introduce and implement interferometric near-infrared spectroscopy
(NIRS), which simultaneously extracts the optical and dynamic properties
of turbid media from the analysis of the spectral interference fringe pattern.
The spectral interference fringe pattern is measured using a Mach-Zehnder
interferometer with a frequency swept narrow bandwidth light source
such that the temporal intensity autocorrelations can be determined for all
photon path lengths. This approach enables time-of-flight (TOF) resolved
measurement of scatterer motion, which is a feature inaccessible in well-
established diffuse correlation spectroscopy techniques. We prove this by
analyzing intensity correlations of the light transmitted through diffuse
fluid phantoms with photon random walks of up to 55 (approximately 110
scattering events) using laser sweep rates on the order of 100kHz. Thus, the
results we present here advance diffuse optical methods by enabling
simultaneous determination of depth-resolved optical properties and
dynamics in highly scattering samples.

9697-75, Session 11
Diffusion-sensitive optical coherence tomography for real-time monitoring
of mucus thinning treatments
Richard L. Blackmon, Patrick R. Sears, Lawrence E. Ostrowski, David B. Hill, The Univ. of North Carolina at
Chapel Hill (United States); Brian S. Chapman, Joseph B. Tracy, North Carolina State Univ. (United States); Silvia M.
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Chapel Hill (United States)
Mucus solid concentration (wt%) has become an increasingly useful
metric in real-time assessment of respiratory health in diseases like cystic
fibrosis and COPD, with higher wt% indicative of diseased states. However,
available in vivo rheological techniques are lacking. Gold nanorods (GNRs)
are ideal biological probes, whose diffusion through tissue is sensitive to
the correlation length of comprising biopolymers. Through employment
of dynamic light scattering theory on OCT signals from GNRs, we find that
weakly-constrained GNR diffusion predictably decreases with increasing
wt% (more disease-like) mucus. Here, we demonstrate that this method
is robust against movement of mucus during active transport on human
bronchial epithelial (hBE) air-liquid interface cultures (R' 2=0.976). We then
introduce diffusion-sensitive OCT (DS-OCT), where we collect Mmode image
ensembles, from which we derive depth and temporally-resolved GNR
diffusion rates. DS-OCT allows for real-time monitoring of changing GNR
diffusion in a location of interest, enabling monitoring of nanotopological
tissue dynamics never before seen. hBE cultures with a layer of endogenous
mucus, doped with topically deposited GNRs (80x22nm), were treated
with hypertonic saline (HS) or isotonic saline (IS). DS-OCT imaged up to
a depth of 600µm with 4.65µm resolution for up to 8min in increments
of 3sec or more. For both IS and HS conditions, DS-OCT captured mucus
hydration over time, with mucus layer height decreasing after IS treatment
and increasing after HS treatment. DS-OCT opens a new window into
understanding mechanisms of mucus thinning during treatment, enabling
real-time efficacy feedback needed to optimize and tailor treatments for
individual patients.

9697-76, Session 11
OCT based in vivo tissue injury mapping
Utku Baran, Yuandong Li, Ruikang K. Wang, Univ. of
In vivo methods for non-invasive characterization of tissue properties have started to draw attention in the community for the accurate determination of the extent and the spread of disease within tissue. Tissue characterization techniques often rely on the fact that the disease alters physical characteristics of the tissue and this alteration can cause observable changes in tissue properties (either optical or acoustic), primarily through absorption and scattering. Changes in the signal attenuation decay within tissue, measured as an attenuation coefficient, can be used to differentiate various tissue types with pathological conditions. We report a method called tissue injury mapping (TIM), which utilizes a non-invasive in vivo optical coherence tomography approach to generate optical attenuation coefficient and microvascular map of the injured tissue. We also propose a useful and yet simple algorithm called sorted average intensity projection (sAIP) for en face mapping of the reconstructed OACs belonging to different tissue types. Using sAIP method, without requiring pre-segmentation and complicated image processing algorithms, we are able to provide high contrast map of injured and healthy tissue regions for en face TIM applications. The infarct region development in mouse cerebral cortex during stroke is visualized using TIM. Moreover, we demonstrate the changes in human facial skin structure and microvasculature during an acne lesion development from initiation to scarring. The results indicate that TIM may be used to aid in the characterization and the treatment of various diseases by enabling a high resolution detection of tissue structural and microvascular changes.

### Dynamic full field OCT: metabolic contrast at subcellular level

Clement Apelian, Institut Langevin (France); Fabrice Harms, LLTech SAS (France); Olivier Thouvenin, Claude Boccara, Institut Langevin (France)

Cells shape or density is an important marker of tissues pathology. However, individual cells are difficult to observe in thick tissues frequently presenting highly scattering structures such as collagen fibers. Endogenous techniques struggle to image cells in these conditions. Moreover, exogenous contrast agents like dyes, fluorophores or nanoparticles cannot always be used, especially if non-invasive imaging is required. Scatters motion happening down to the millisecond scale, much faster than the millisecond OCT signal processing times, presents highly promising new opportunities. In this work, we have developed a method which allows us to perform high-speed image of subcellular structures. We have observed that TIM may be used to aid in the characterization and the treatment of various diseases by enabling a high resolution detection of tissue structural and microvascular changes.

### Longitudinal, 3D visualization of diabetes by functional optical coherence imaging

Corinne Berciaud, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Anja Schmidt-Christensen, Lund Univ. (Sweden); Daniel Szlag, Ecole Polytechnique Fédérative de Lausanne (Switzerland) and Nicolaus Copernicus Univ. (Poland); Jerome Extermann, Ecole Polytechnique Fédérative de Lausanne (Switzerland) and Univ. of Applied Science of Western Switzerland (Switzerland); Lisbeth Hansen, Lund Univ. (Sweden); Arno Bouwens, Martin Villiger, Joan Goulley, Ecole Polytechnique Fédérative de Lausanne (Switzerland); Frans Schuit, KU Leuven (Belgium); Anne Grapin-Botton, Univ. of Copenhagen (Denmark); Dan Holmberg, Lund Univ. (Sweden); Theo Lasser, Ecole Polytechnique Fédérative de Lausanne (Switzerland)

In diabetes, pancreatic ß-cells play a key role. These cells are organized within structures called islets of Langerhans inside the pancreas and produce insulin. Insulin is one of the main hormones contributing to glucose homeostasis. A decreased secretion of insulin leads to hyperglycemia, which is the hallmark of diabetes. Longitudinal high resolution tracking of the ß-cells over the disease progression is important to understand the mechanisms involved and to evaluate the efficiency of new treatments. We demonstrated that extended Optical Coherence Microscopy (xoC) has a specificity for the ß-cell due the high scattering properties of the insulin-zinc aggregates inside the ß-cell granules. In addition, xOIC offers a high resolution over an extended depth of field of 400 µm.

Longitudinal imaging of the islets is challenging due to the deep localization of the pancreas inside the abdominal cavity. In order to perform label-free non-invasive longitudinal imaging, we applied xOIC to image islets transplanted into the anterior chamber of the eye (ACE). We demonstrated quantification of the ß-cell volume and the vascular density over months. Using a mouse model of type I diabetes, we prove that xOIC can in addition detect the autoimmune inflammation inside the islets. In addition, we observed a reorganization of the vascular bed that correlates with the degree of inflammation. Finally, we imaged human islets grafted into the ACE of immunodeficient mice where fluorescence labelling is limited. We anticipate that this technique will become a powerful tool for the diabetes research community, drug discovery and testing.

### Depth-resolved nanoscale nuclear architecture mapping for early prediction of cancer progression

Shikhar Uttam, Hoa V. Pham, Justin LaFace, Univ. of Pittsburgh (United States); Douglas J. Hartman, Univ. of Pittsburgh School of Medicine (United States); Yang Liu, Univ. of Pittsburgh (United States)

Early cancer detection currently relies on screening the entire at-risk population, as with colonoscopy. Therefore, frequent, invasive surveillance of patients at risk for developing cancer carries financial, physical, and emotional burdens because clinicians lack tools to accurately predict which patients will actually progress into malignancy. We have developed a new method to image nuclear architecture on unstained tissue by quantifying the intrinsic depth-resolved density alteration of nuclear architecture, referred to as nanoscale nuclear architecture mapping (nanoNAM), rather than the amount of nuclear stain uptake. The image resolution of nuclear architecture map is still diffraction-limited, but the image contrast with nanoscale precision is produced by depth-resolved optical path-length difference.
ranging between 1 to 10 mm with a resolution of a few micrometers. To project tomography, has been demonstrated for imaging of specimens ill-suited for imaging of living samples. An interesting alternative, optical as well as the inherently slow 3D scan, these imaging schemes are often need for fluorescent labeling with ambiguous influence on cell functioning has prevailed for three-dimensional (3D) sub-cellular imaging. Due to the advantage of (a) requiring only a single modality, (b) being non-invasive in nature, and (c) being reflective of only the net power work generated by a ciliated surface. We demonstrate our all-optical approach to the estimation of force and power information from vectorial flow velocity fields obtained using OCT-based methods. We do so by (a) estimating the viscous stress tensor from flow velocity fields to estimate shearing force and (b) using the viscous stress tensor to estimate the power dissipation function to infer total mechanical power. These estimates have the advantage of (a) requiring only a single modality, (b) being non-invasive in nature, and (c) being reflective of only the net power work generated by a ciliated surface. We demonstrate our all-optical approach to the estimation of these parameters in the Xenopus animal model system under normal and increased viscous loading. Our preliminary data support the hypothesis that the Xenopus ciliated surface can increase force output under loading conditions.

In cilia-driven fluid flow physiology, quantification of flow velocity, shearing force, and power dissipation is important in defining abnormal ciliary function. The accurate quantification of flow can be robustly described in terms of shearing force. Dissipated power can be related to net ATP consumption by ciliary molecular motors. To date, however, only flow velocity can be routinely quantified in a non-invasive, non-contact manner. Additionally, traditional power-based metrics rely on metabolic consumption that reflects energy consumption not just from cilia but also from all active cellular processes. Here, we demonstrate the estimation of all three of these quantities (flow velocity, shear force, and power dissipation) using only optical coherence tomography (OCT). Specifically, we develop a framework that can extract force and power information from vectorial flow velocity fields obtained using OCT-based methods. We do so by (a) estimating the viscous stress tensor from flow velocity fields to estimate shearing force and (b) using the viscous stress tensor to estimate the power dissipation function to infer total mechanical power. These estimates have the advantage of (a) requiring only a single modality, (b) being non-invasive in nature, and (c) being reflective of only the net power work generated by a ciliated surface. We demonstrate our all-optical approach to the estimation of these parameters in the Xenopus animal model system under normal and increased viscous loading. Our preliminary data support the hypothesis that the Xenopus ciliated surface can increase force output under loading conditions.

Label-free three-dimensional imaging of C. elegans with visible wavelength extended-focus optical coherence microscopy


Over the past decades, confocal and two-photon fluorescence microscopy has prevailed for three-dimensional (3D) sub-cellular imaging. Due to the need for fluorescent labeling with ambiguous influence on cell functioning as well as the inherently slow 3D scan, these imaging schemes are often ill-suited for imaging of living samples. An interesting alternative, optical projection tomography, has been demonstrated for imaging of specimens ranging between 1 to 10 mm with a resolution of a few micrometers. To circumvent the drawbacks of fluorescence microscopy but yet maintain a sub-micrometric resolution, we introduce a fast, label-free 3D imaging system for sub-cellular imaging of live cells and small organisms. This is achieved by visible spectrum optical coherence microscopy (OCM). The broad visible wavelength range and the use of a high numerical aperture objective provide an axial and lateral resolution of 0.6 ?m and 0.4 ?m in water, respectively. Furthermore, the setup is decoupled into a Bessel illumination and a Gaussian detection mode, simultaneously offering an extended depth of field and a dark field configuration.

We apply our system to fast 3D sub-cellular imaging of living C. elegans worms. This simple model organism is extensively used in research because it is sufficiently complex to study many biological processes and diseases. We are interested to use the high resolution tomograms of whole C. elegans obtained with our platform for the study of aging and aging-related diseases. More specifically, we want to take advantage of the isotropic sub-micrometric resolution to observe the development of protein aggregates involved in neurodegenerative disorders.

Structural and functional measurements of fertilized mouse oocytes with combined high-resolution OCT and inverted microscope

Karol Karnowski, Nicolaus Copernicus Univ. (Poland); Anna Ajduk, Univ. of Warsaw (Poland); Maciej Wojtkowski, Maciej Szkulmowski, Nicolaus Copernicus Univ. (Poland)

We present a comprehensive imaging methodology for 3D structural and functional measurements of fertilized mouse oocytes. In contrary to methods used for mouse zygote imaging so far OCT provides 3D data without z axis movement of sample or objective lens. Furthermore, complex scanning protocols used in this study give access to different scales of repetition times and thus may become a tool for investigation of a different dynamic processes. Additionally, proposed scanning approach via variety of statistic operations can be used to enhance the quality of structural images. OCT system capabilities are presented and compared to standard microscopy. With a single 3D measurements one can extract 3D structure of the oocytes as well as en-face images that correspond to both bright and dark field microscopy. As an example of dynamic oocyte imaging pronuclei motion during development is presented. Limitations and possibilities of the new system are discussed.

Synchronous multimodal combination of full-field OCT and structured illumination fluorescence microscopy

Olivier Thouvenin, Mathias Fink, Claude Boccara, Institut Langevin (France)

FF-OCT is a full field high transverse resolution version of temporal domain OCT. It acquires En-face images with an isotropic 3D submicronic resolution deep inside a biological tissue. It can access an optical contrast at a given depth, meaning that FF-OCT is sensitive to variations of optical index. FF-OCT can thus probe the microarchitecture of a tissue without label. However, FF-OCT lacks of specific molecular contrast. On the contrary, Fluorescence microscopy can reveal labelled molecules with a very good specificity. Structured Illumination Microscopy (SIM) is a technique providing optical sectioning to fluorescence widefield microscopy. However, this technique can be complicated to implement in a tissue, and fails at providing environmental information. Therefore, combining FF-OCT and SIM has many advantages and adds a
specific molecular contrast to a microarchitecture image of a biological sample. Combining FF-OCT and SIM has already been reported in the literature.

Here, we report on the development of different ways to combine FF-OCT and SIM. On the contrary to previously described setups, our setup enables the synchronous detection of both modalities. We believe this is important to access to dynamical events that take place in tissues. With such a technique, we are able to detect fast changes happening both in the environment, and in the behavior of a specific molecule.

For now, we applied our technique to detect static structural information in the cornea. By the time of the conference, we expect to use our system to detect dynamical changes in a tissue.

9697-84, Session 12

2 μm axial resolution, fiber-optic SD-OCT operating at ~1300 nm for cellular resolution imaging of biological tissue

Kostadinka Bizheva, Bingyao Tan, Tyler Monahan, Julia Zangoulos, Mungo Marsden, Mojtaba Hajialamdari, Univ. of Waterloo (Canada)

Development of fiber optic based SD-OCT systems operating in the 1300 nm spectral region and capable of providing micrometer scale axial resolution in biological tissue is particularly challenging due to the large spectral bandwidth that has to be transmitted through the optical and fiber optic components of the imaging system with minimal spectral and power losses. Furthermore, the large spectral bandwidth requires cameras with larger number of pixels to achieve a decent scanning range. Here we present a novel fiber optic SD-OCT light source (NKT), a custom filter to select the necessary spectral bandwidth and a novel 146 kHz, 2046 pixel CCD (Sensors Unlimited). All optical and fiber optic components of the system were specifically selected to sustain a spectral bandwidth > 260 nm centered at ~1250 nm and to provide 2.9 μm axial resolution in free space, corresponding to 2.1 μm in biological tissue (n = 1.38). The axial resolution of the system was tested by imaging a pellicle with physical thickness of ~2 μm, as well the cellular structure of cucumber and healthy human skin. SNR > 95 dB was measured at ~100 μm depth with 5 mW optical power out of the imaging probe and at the full speed of the CCD camera. The SNR drop off over the scanning range of ~1.3 mm was ~10 dB. Currently the system provides scanning range of ~1mm in biological tissue and we are in the process of developing a full range OCT approach to double the scanning range.
9698-1, Session 1

**Time-resolved fluorescence spectroscopy for intraoperative assistance of thyroid surgery**

Luciano Bachmann, Mariana P. Brandão, Kaique Haleplian, Amando S. Ito, Ricardo Iwakura, Fagner S. Basilio, Luiz Carlos Conti de Freitas, Univ. de São Paulo (Brazil)

Searching for new methods to provide information of biochemical composition and structure is critical to improve the prognosis of thyroid diseases. The use of time-resolved fluorescence techniques to detect biochemical composition and tissue structure alterations could help develop a portable, minimally invasive, and non-destructive method to assist during surgical procedures. This research looks for employ a fluorescence technique based on lifetime measurements to differentiate healthy and benign lesions from malignant thyroid tissue. We employ a wide range of excitation and chose a more appropriate region for this work: 298-300 nm; and the fluorescence decay was measured at 340-450 nm. We observed fluorescence lifetimes at 340 nm emission of 0.80±0.26 and 3.94±0.47 ns for healthy tissue; 0.90±0.24 and 4.05±0.46 ns for benign lesions; and 1.21±0.14 and 4.63±0.25 ns for malignant lesions. For 450 nm emissions, we obtain lifetimes of 0.25±0.18 and 3.99±0.39 ns for healthy tissue, 0.24±0.17 and 4.20±0.48 ns for benign lesions, 0.33±0.32 and 4.55±0.55 ns for malignant lesions. We successfully demonstrated that fluorescence lifetimes at 340 nm emission can differentiate between thyroid malignant and healthy/benign tissues.

9698-2, Session 1

**Design and validation of a near-infrared fluorescence endoscope for detection of early oesophageal malignancy using a targeted imaging probe**

Dale J. Waterhouse, James Joseph, Univ. of Cambridge (United Kingdom) and Cancer Research UK Cambridge Institute (United Kingdom); Andre A. Neves, Cancer Research UK Cambridge Institute (United Kingdom); Massimiliano di Pietro, MRC Cancer Unit, Univ. of Cambridge (United Kingdom); Kevin M. Brindle, Cancer Research UK Cambridge Institute (United Kingdom) and Univ. of Cambridge (United Kingdom); Rebecca C. Fitzgerald, MRC Cancer Unit, Univ. of Cambridge (United Kingdom); Sarah E. Bohndiek, Univ. of Cambridge (United Kingdom) and Cancer Research UK Cambridge Institute (United Kingdom)

Barrett’s oesophagus is a condition that predisposes patients to oesophageal cancer. Early detection of cancer in these patients can be curative, but is confounded by a lack of contrast in white light endoscopy (WLE). Application of fluorescently-labelled lectins to the oesophagus during endoscopy can more accurately delineate dysplasia emerging within Barrett’s than WLE [1], but strong tissue autofluorescence has limited sensitivity and dynamic range of this approach. To overcome this challenge, we synthesized a near-infrared (NIR) fluorescent lectin and have constructed a clinically translatable endoscope for simultaneous WLE and NIR imaging. An imaging fibre bundle, shielded from patient contact using a disposable catheter (PolyDiagnost), delivers incoherent light and relays collected light into an optical path that splits the WL reflectance and NIR emission onto two cameras for simultaneous video-rate recording. The captured images are co-registered and the honeycomb artefact arising from the fibre bundle is removed using interpolation between image points derived from fibrelet centres.

Technical validation of the NIR imaging performance of this endoscope will be presented. Resolution was determined to be 200µm using a standard USAF test chart. A minimal detectable concentration of 60nM (12pmoles/cm²) was determined using a dilution series of IRDye800CW-lectin in black well plates. These results indicate the current design can image IRDye800CW-lectin at clinically relevant concentrations. Future work using ex vivo tissue specimens will determine safe illumination limits and sensitivity for dysplasia and adenocarcinoma in Barrett’s oesophagus, prior to commencing clinical trials.


9698-3, Session 1

**Real-time intraoperative multispectral fluorescence and color imaging platform for the visible and near infra-red region**

Nikolas Dimitriadis, Bart?omiej Grychtol, Martin Theuring, Tobias Behr, Nikolaos C. Deliolanis, Fraunhofer-Institut für Produktionstechnik und Automatisierung (Germany)

Both, multispectral (MS) reflectance and MS fluorescence imaging of tissue can reveal anatomical, functional, and pathological information. We present an imaging platform, which is based on a combined approach of temporal and spectral multiplexing. With the combination of two sensors, the system acquires MS fluorescence and reflectance images over the entire VIS/NIR spectrum with minimal spectral gaps. The images are unmixed to 6 fluorescence and 6 reflectance components. As an advantage, the system has no moving parts and runs at 30 fps in real-time. It can be easily combined with existing systems like surgical microscopes or rigid endoscopes.

9698-4, Session 2

**In vivo detection of oral epithelial cancer using endogenous fluorescence lifetime imaging: a pilot human study**

Javier A. Jo, Dae Yon Hwang, Jorge Palma, Shuna Cheng, Rodrigo Cuenga Martinez, Bilal H. Malik, Joey M. Jabbour, Yi-Shing L. Cheng, John Wright, Kristen C. Maitland, Texas A&M Univ. (United States)

Endogenous fluorescence lifetime imaging (FLIM) provides direct access to the concomitant functional and biochemical changes accompanying tissue transition from benign to precancerous and cancerous. Since FLIM can noninvasively measure different and complementary biomarkers of precancer and cancer, we hypothesize that it will aid in clinically detecting early oral epithelial cancer. Our group has recently demonstrated the detection of benign from premalignant and malignant lesions based on endogenous multispectral FLIM in the hamster cheek-pouch model. Encouraged by these positive preliminary results, we have developed a handheld endoscope capable of acquiring multispectral FLIM images in
real time from the oral mucosa. This novel FLIM endoscope is being used for imaging clinically suspicious pre-malignant and malignant lesions from patients before undergoing tissue biopsy for histopathological diagnosis of oral epithelial cancer. Our preliminary results thus far are already suggesting the potential of endogenous FLIM for distinguishing a variety of benign lesions from advanced dysplasia and squamous cell carcinoma (SCC). To the best of our knowledge, this is the first in vivo human study aiming to demonstrate the ability to predict the true malignancy of clinically suspicious lesions using endogenous FLIM. If successful, the resulting clinical tool will allow noninvasive real-time detection of epithelial precancerous and cancerous lesions in the oral mucosa and could potentially be used to assist at every step involved on the clinical management of oral cancer patients, from early screening and diagnosis, to treatment and monitoring of recurrence.

9698-5, Session 2

**Combined fiber probe for fluorescence lifetime and Raman spectroscopy**

Sebastian Dochow, Leibniz-Institut für Photonische Technologien e.V. (Germany) and Friedrich-Schiller-Univ. Jena (Germany); Dinglong M. Ma, Univ. of California, Davis (United States); Ines Latka, Leibniz-Institut für Photonische Technologien e.V. (Germany); Thomas W. Bocklitz, Friedrich-Schiller-Univ. Jena (Germany); Brad A. Hartl, Julien Bec, Hussain Fatakdaawa, Univ. of California, Davis (United States); Sebastian Wachsmann-Hogiu, Ctr. for Biophotonics, Univ. of California, Davis (United States); Eric T. Marple, Kirk Urmey, EmVision, LLC (United States); Michael Schmitt, Friedrich-Schiller-Univ. Jena (Germany); Laura Marcu, Univ. of California, Davis (United States); Jürgen Popp, Friedrich-Schiller-Univ. Jena (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany)

Raman spectroscopy has been proven to have tremendous potential as a biomedical analytical tool for spectroscopic disease diagnostics. The use of fiber optic coupled Raman spectroscopy systems can enable in-vivo characterization of suspicious lesions. However, Raman spectroscopy has the drawback of rather long acquisition times of several hundreds of milliseconds which makes scanning of larger regions quite challenging. By combining Raman spectroscopy with a fast imaging technique this problem can be alleviated in part. Fluorescence lifetime imaging (FLIM) offers a great potential for such a combination. FLIM can allow for fast tissue area pre-segmentation and location of the points for Raman spectra acquisition. Here, we introduce an optical fiber probe combining FLIM and Raman spectroscopy with an outer diameter of 2 mm. Fluorescence is generated via excitation with a fiber laser at 355 nm. The fluorescence emission is spectrally resolved using a custom-made wavelength-selection module (WSM). The Raman excitation power at 785 nm was set to 50 mW for the in-vivo measurements to prevent sample drying. The lateral probe resolution was determined to be <250 μm for both modalities. This value was taken as step size for several raster scans of different tissue types which were conducted to show the overlap of both modalities under realistic conditions. Finally the probe was used for in vivo raster scans of a rat’s brain and subsequently to acquire FLIM guided Raman spectra of several tissues in and around the craniotomy.

9698-6, Session 2

**Optical fiber Raman-based spectroscopy for oral lesions characterization: a pilot study**

Luís Felipe C. S. Carvalho D.D.S., Lázaro P. Medeiros Neto, Inajara P. Oliveira, João Lucas Rangel, Univ. do Vale do Paraíba (Brazil); Dácio Kitakawa, Prefeitura Municipal de São Paulo (Brazil); Airton A. Martin D.D.S., Univ. do Vale do Paraíba (Brazil)

In the clinical daily life various lesions of the oral cavity have shown different aspects, generating an inconclusive or doubtful diagnosis. In general, oral injuries are diagnosed by histopathological analysis from biopsy, which is an invasive procedure and does not give immediate results. In the other hand, Raman spectroscopy technique it is a real time and minimal invasive analytical tool, with notable diagnostic capability. This study aims to characterize, by optical fiber Raman-based spectroscopy (OFRS), normal, inflammatory, potentially malignant, benign and malignant oral lesions. Raman data were collected by a Holospec f / 1.8 spectrograph (Kayser Optical Systems) coupled to an optical fiber, with a 785nm laser line source and a CCD Detector. The data were pre-processed and vector normalized. The average analysis and standard deviation was performed associated with cluster analysis and compared to the histopathological results. Samples of described oral pathological processes were used in the study. The OFRS was efficient to characterized oral lesions and normal mucosa, in which biochemical information related to vibrational modes of proteins, lipids, nucleic acids and carbohydrates were observed. The technique (OFRS) is able to demonstrate biochemical information concern different types of oral lesions showing that Raman spectroscopy could be useful for an early and minimal invasive diagnosis.
Recently adipose-derived stem cell (ADSC) – based therapies have been compatible with established methods of fluorescent biomarker labelling. ADSC can yield identification result. A 10-fold cross-validation was performed on the obtained data are compared to a database so that machine learning can yield identification result. A 10-fold cross-validation was performed on a database over 8 species (7 strains, 1900 scatterograms), at 6h of incubation. It yielded a 94% discrimination rate between Gram+, Gram- and yeasts. Results can be improved by using a more relevant polynomial basis for projections, such as Fourier-Bessel functions. A fully integrated instrument system has been installed at the Grenoble hospital's laboratory of bacteriology and a validation campaign has been started for the early screening of SA (S. aureus) and MRSA carriers (6h of incubation).

Up to now, all the published studies about elastic scattering were performed in a forward mode, which is restricted to transparent media. However, in clinical diagnostics, most of media are opaque, such as blood-supplemented agar. That is why we propose a novel scheme capable of collecting back-scattered light which provides comparable results.

**Non-invasive monitoring of cytokine-based regenerative treatment of cartilage by hyperspectral unmixing**

Saabah B. Mahbub, Peter Sucker, Martin E. Gosnell, Ayad G. Anwer, Macquarie Univ. (Australia); Benjamin Herbert, The Univ. of Sydney (Australia); Graham Vesey, Regenesys Ltd. (Australia); Ewa M. Goldys, Macquarie Univ. (Australia)

Extracting biochemical information from tissue autofluorescence is a promising approach to non-invasively monitor disease treatments at a cellular level, without using any external biomarkers. Our recently developed hyperspectral unmixing techniques to study in-vitro the effects of ADSC-derived cytokine-rich secretions with the cartilage chip in both human and bovine samples. The study of metabolic effects of different cytokine treatment on different cartilage layers makes it possible to compare the merits of those treatments for repairing cartilage.

**Optical elastic scattering for early label-free identification of clinical pathogens**

Valentin Genuer, Olivier Gal, Jeremy Meteau, Pierre R. Marcoux, Emmanuelle Schultz, Commissariat à l’Énergie Atomique (France); Eric Lacot, Univ. Grenoble Alpes (France); Max Maurin, CHU Grenoble (France); Jean-Marc Dinten, Commissariat à l’Énergie Atomique (France)

We report here the ability of elastic scattering in discriminating Gram+, Gram- and yeasts at an early stage of growth (6h). Our technique is non-invasive, low cost and does not require neither skilled operators nor reagents. Therefore it is compatible with automation. It is based on the analysis of the scattering pattern (scatterogram) generated by a bacterial microcolony growing on agar, when placed in the path of a laser beam. Measurements are directly performed on closed Petri dishes.

The characteristic features of a given scatterogram are first computed by projecting the pattern onto the Zernike polygonal basis. Then the obtained data are compared to a database so that machine learning can yield identification result. A 10-fold cross-validation was performed on a database over 8 species (7 strains, 1900 scatterograms), at 6h of incubation. It yielded a 94% discrimination rate between Gram+, Gram- and yeasts. Results can be improved by using a more relevant polynomial basis for projections, such as Fourier-Bessel functions. A fully integrated instrument system has been installed at the Grenoble hospital’s laboratory of bacteriology and a validation campaign has been started for the early screening of SA (S. aureus) and MRSA carriers (6h of incubation).

Up to now, all the published studies about elastic scattering were performed in a forward mode, which is restricted to transparent media. However, in clinical diagnostics, most of media are opaque, such as blood-supplemented agar. That is why we propose a novel scheme capable of collecting back-scattered light which provides comparable results.

**Near infrared fluorescent image based evaluation of gastric tube perfusion after esophagectomy in preclinical model**

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This study was to evaluate the feasibility of near infrared (NIR) fluorescent images as a tool for evaluating the perfusion of the gastric tube after esophagectomy. In addition, we investigated the time required to acquire enough signal to confirm the presence of ischemia in gastric tube after injection of indocyanine green (ICG) through peripheral versus and central venous route. 4 porcine underwent esophagogastrectomy and their right gastric arteries were ligated to mimic ischemic condition of gastric tube. ICG (0.6mg/kg) was intravenously injected and the fluorescence signal-to-background ratios (SBR) were measured by using the custom-built intraoperative color and fluorescence imaging system (ICFIS). We evaluated perfusion of gastric tubes by comparing their SBR with esophageal SBR.

In ischemic models, SBR of esophagus was higher than that of gastric tube (2.8±0.54 vs. 1.7±0.37, p<0.05). It showed high esophagus-stomach signal to signal ratio (SSR, 1.8±0.76). We also could observe recovery of blood perfusion in few minutes after releasing the ligation of right gastric artery. In addition, in comparison study according to the injection route of ICG, The time to acquire signal stabilization was faster in central than in peripheral route (119 ± 65.1 seconds in central route vs. 295±130.4 in peripheral route, p<0.05).

NIR fluorescent images could provide the real-time information if there was ischemia or not in gastric tube during operation. And, central injection of ICG might give that information faster than peripheral route.

**Rapid diagnostic imaging and pathologic evaluation of surgical tissue using video rate structured illumination microscopy (VR-SIM)**

Mei Wang, David B. Tulman, Katherine N. Elfer, Andrew B. Sholl, Jonathon Q. Brown, Tulane Univ. (United States)

 Currently available pathology techniques for obtaining a rapid tissue diagnosis, or for determining the adequacy of specimens intended for downstream analysis, are too slow, labor-intensive, and destructive for point-of-care (POC) applications. We previously demonstrated video-rate structured illumination microscopy (VR-SIM) for accurate, high-throughput,
non-destructive diagnostic imaging of fluorescently-stained prostate biopsies in seconds per biopsy, with an area under the ROC curve of 0.82-0.88 after pathologist review. In addition, we have demonstrated that it is feasible to use VR-SIM to routinely image very large gross pathology specimens, such as entire prostate resection surfaces, in relatively short timeframes at subcellular resolution. However, our prior work has focused on applications in prostate cancer; the utility in other organ sites has not been explored.

Here we extended our technology to varying size kidney, liver, and lung biopsies. We conducted a validation study of VR-SIM against histopathology on a variety of human tissues, including both small biopsies and large slices of tissue. We conducted a blinded study in which the study pathologist accurately identified the organs based on VR-SIM images alone. The results were then used to create a clinical atlas between VR-SIM and H&E images for the different tissues of interest. This clinical atlas will be used to aid in pathologist interpretation in future POC clinical applications of VR-SIM in kidney, liver, and lung. Such applications could include on-site identification of the presence of kidney glomeruli to ensure successful downstream IHC analysis, or determination of the adequacy of lung cancer biopsies for genomic analysis.

9698-37, Session PSun
Development of single-channel stereoscopic imaging modality for real time retinal imaging
Edalat Radfar, Byungjo Jung, Jihoon Park, Sangyeob Lee, Myungjin Ha, Sungkon Yu, Seulgi Jang, Yonsei Univ. (Korea, Republic of)
Stereo images provide the depth perception and more spatial information from imaging target that is particularly useful and sometimes critical in retinal imaging during most of eye diagnosis and treatment operations. In this study, we developed a single-channel stereoscopic retinal imaging based on a transparent rotating deflector. Two different viewing angles are generated via imaging though a rotational optical deflector which is connected to a synchronized motor and is placed inside a lens system. According to eye structure and the function of objective lens in SSIM-TRD, the optical set up of imaging modality can compatible with retinal imaging when the cornea and eye lens are engaged in objective lens.

9698-39, Session PSun
A 2-axis Polydimethylsiloxane (PDMS) based electromagnetic MEMS scanning mirror for optical coherence tomography
Sehui Kim, Changho Lee, Jin Young Kim, Pohang Univ. of Science and Technology (Korea, Republic of); Jeehyun Kim, Kyungpook National Univ. (Korea, Republic of); Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of)
Optical coherence tomography (OCT) is a non-invasive imaging tool for visualizing cross-sectional images of biological tissues in a microscope. Various types of OCT systems have been applied to OCT for endoscopic catheters and handheld probes. Although these OCT scanners have several advantages of compact sizes and high speeds for real-time imaging, the complexities of the fabrication processes and relatively high-costs are the bottlenecks for fast clinical translation and each commercialization. To overcome these issues, we have developed a 2-axis polydimethylsiloxane (PDMS)-based electromagnetic MEMS scanning mirror based on flexible, cost-effective, and handle able PDMS. The size of this MEMS scanner is 15 x 15 x 15 mm. To realize the characteristics of the scanner, we obtained the DC/AC responses and scanning patterns. The measured maximum scanning angles are 6° and 11° along the X and Y axes, respectively. The resonance frequencies are 82 Hz and 57 Hz along the X axis and Y axis, respectively. The scanning patterns (a raster scan pattern and a Lissajous scan pattern) are also demonstrated by controlling the frequency and amplitude. Finally, we showed the in vivo 2D-OCT images of human fingers. The obtained field of view (FOV) is 8 x 8 x 8. The PDMS based MEMS scanning mirror has potential to
9698-40, Session PSun

**Detection of ictal and interictal migraine by using near-infrared spectroscopy**

Chao-Che Lee, Chia-Wei Sun, National Chiao Tung Univ. (Taiwan); Wei-Ta Chen, Neurological Institute, Taipei Veterans General Hospital (Taiwan)

Near-infrared spectroscopy (NIRS) is a low-cost, portable, and non-invasive optical sensing technology that can effectively measure the relative concentration of oxygenated hemoglobin (HbO2) and deoxygenated hemoglobin (Hb) in real time, providing a functional detail about the oxygenation of tissue.

9698-41, Session PSun

**Signal of prefrontal cortex with fibromyalgia based on NIRS method**

Chen Yu Lin, Chia Wei Sun, National Chiao Tung Univ. (Taiwan); Wei Ta Chen, Neurological Institute, Taipei Veterans General Hospital (Taiwan)

Monitor relative concentration in tissue oxygenated-hemoglobin (HbO2) and deoxygenated-hemoglobin (HHb). Both device set sample rate at 50 Hz with two wavelengths (760 nm and 850 nm) and the distances between one receiver and three light transmitters are 30 mm, 35 mm, 40 mm. The data measured from tissues used Bluetooth connection to computer for recording and analysis.

9698-42, Session PSun

**Application of the advanced technologies of laser diodes, LEDs and OLEDs for total jaundice management of newborn infants**

Mostafa Hamza, Mansoura Univ. (Egypt); Mohammad H. Sayed Elaahl, Military Medical Academy (Egypt); Ahmad M. Hamza, National Research Ctr. (Egypt); Aya M. Hamza, Yahya M. Hamza, Tabarak Children’s Hospital (Egypt)

The authors introduce the theory design and operating principles of new diagnostic and therapeutic systems for total management of neonatal hyperbilirubinemia using laser diodes, LEDs and OLEDs. Globally, neonatal jaundice is a major cause of newborn death and disability. The burden of brain injury due to jaundice remains a well-recognized threat in many countries in the world. It is important to detect jaundice in its early stages to prevent kernicterus in newborn infants. When jaundice is properly diagnosed, severe elevation of serum bilirubin can be prevented and effectively treated, preventing brain injury. However the accuracy and precision of the results obtained from conventional bilirubin meters have undesirable variability. Our new non-invasive transcutaneous bilirubin meters are implemented using laser diodes and LEDs. The operation principles of these novel compact and low-cost bilirubin meters are primarily based on the absorption characteristics of bilirubin in the visible region of the spectrum. Accurate measurement of bilirubin concentration is a major determination in the clinical management of neonatal hyperbilirubinemia. The management includes using blue laser diodes, LEDs and organic LEDs (OLEDs) for efficient phototherapy of jaundice. Non-invasive transcutaneous bilirubinometry was used in our clinics and hospitals for accurate and timely identification of neonates at risk of hyperbilirubinemia in addition to efficient phototherapy.

9698-43, Session PSun

**A scalable correlator for multichannel diffuse correlation spectroscopy**

Christopher J. Stapels, Noah J. Kolodziejski, Daniel McAdams, Matthew J. Podolsky, Daniel E. Fernandez, Radiation Monitoring Devices, Inc. (United States); Dana Farkas, Radiation Monitoring Devices, Inc. (United States) and Northeastern Univ. (United States); James F. Christian, Radiation Monitoring Devices, Inc. (United States)

Diffuse correlation spectroscopy is a technique which enables powerful and robust non-invasive optical studies of tissue micro-circulation and vascular blood flow. The technique amounts to autocorrelation analysis of coherent photons after their migration through moving scatterers and subsequent collection by single-mode optical detectors. A primary cost driver of DCS instruments are the commercial hardware-based correlators, limiting the proliferation of multi-channel instruments for validation of perfusion analysis as a clinical diagnostic metric. We present the development of a low-cost scalable correlator enabled by microchip-based time-tagging, and a software-based multi-tau data analysis method. We will discuss the capabilities of the instrument as well as the implementation and validation of 2 and 8-channel systems built for live animal and pre-clinical settings.

9698-44, Session PSun

**Raman spectroscopy and immunohistochemistry analysis for schwannoma characterization: a case study**

Lázaro P. Medeiros Neto, Maurilio J. das Chagas, Luis Felipe C. Carvalho, Isabelle Ferreira, Laurita dos Santos, Univ. do Vale do Paraíba (Brazil); Marcello H. Ribas, Instituto de Assistência Médica ao Servidor Público (Brazil); Vinicius de Almeida Loddi, Chagas Serviços Médicos (Brazil); Airton A. Martin D.D.S., Univ. do Vale do Paraíba (Brazil)

The schwannomas is a tumour of the tissue that covers nerves, called the nerve sheath. Schwannomas are often benign tumors of the Schwann cells, which are the principal glia of the peripheral nervous system (PNS). Preoperative diagnosis of this lesion usually is difficult, therefore, new techniques are being studied as pre surgical evaluation. Among these, Raman spectroscopy, that enables the biochemical identification of the tissue analyzed by their optical properties, may be used as a tool for schwannomas diagnosis. The aim of this study was to discriminate between normal nervous tissue and schwannoma through the confocal Raman spectroscopy and Raman optical fiber-based techniques combined with immunohistochemical analysis. Twenty spectra were analyzed from a normal nerve tissue sample (10) and schwannoma (10) by confocal Raman spectrometer Rivers diagnostic model 3510 (Netherlands), and the Holospec f / 18 (Kayser Optical Systems) coupled to an optical fiber, both with a 785nm laser line source. The data were pre-processed and normalized. The average analysis and standard deviation was performed associated with cluster analysis. AML, 1A4, CD34, Desmin and S-100 protein markers were used for immunohistochemical analysis. Immunohistochemical analysis was positive only for protein S-100 marker which confirmed the neural schwannoma origination. The immunohistochemistry analysis were important to determine the source of the injury, whereas Raman spectroscopy were able to differentiated tissues types indicating important biochemical changes between normal and benign neoplasia.
9698-45, Session PSun

Intraoperative autofluorescence imaging of parathyroid gland using DSLR camera

Yeh-Chan Ahn, Pukyong National Univ. (Korea, Republic of) and Ctr. for Marine-Integrated Biomedical Technology Research Ctr., Kosin Univ. (Korea, Republic of); Kang Dae Lee, Sung Won Kim, Hyoung Shin Lee, College of Medicine, Kosin Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Seo Hyun Song, Pukyong National Univ. (Korea, Republic of) and Ctr. for Marine-Integrated Biomedical Technology (Korea, Republic of) and Innovative Biomedical Technology Research Ctr., Kosin Univ. (Korea, Republic of); Chulho Oak, Kosin Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of)

Patients with thyroid cancer have not only thyroidectomy but also preclusive central compartment neck dissection, CCND to prevent from metastasis. During the operations, it is significant to preserve the parathyroid for calcium homeostasis because one of the functions of parathyroid glands is to maintain the body’s calcium levels. However, it is hard to distinguish the parathyroid from the surrounding tissues such as fat or connective tissues with the naked eye. Therefore, we present intraoperative near-infrared autofluorescence imaging system using that fluorescence intensity of parathyroid is higher than that of thyroid and all other tissues regardless of disease states. The NIR autofluorescence imaging system was composed of 780 collimated led light source with excitation filter, DSLR camera with emission filter. The system also included IR illuminator to check the anatomical positions around the parathyroid. We obtained 100% sensitivity and P/T ratio from 1.95 to 5.20. In conclusion, we could obtain the parathyroid autofluorescence images even it was covered by fat or connective tissues and even it was located at unusual locations.

9698-46, Session PSun

Evaluation of motion compensation method for assessing the gastrointestinal motility using three dimensional endoscope

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Functional gastrointestinal disorders (FGID) are the most common gastrointestinal disorders. The term “functional” is generally applied to disorders where there are no structural abnormalities. Gastrointestinal dysmotility is one of the several mechanisms that have been proposed for the pathogenesis of FGID and is usually examined by manometry, a pressure test. There have been no attempts to examine the gastrointestinal dysmotility by endoscopy. We have proposed an imaging system for the assessment of gastric motility using a three-dimensional endoscope. After we newly developed a three-dimensional endoscope and constructed a wave simulated model, we established a method of extracting three-dimensional contraction waves derived from a three-dimensional profile of the wave simulated model obtained with the endoscope. In the study, the endoscope and the wave simulated model were fixed to the ground. However, in a clinical setting, it is hard for endoscopists to keep the endoscope still. Moreover, stomach moves under the influence of breathing. Thus, three-dimensional registration of the position between the endoscope and the gastric wall is necessary for the accurate assessment of gastrointestinal motility. In this paper, we propose a motion compensation method using three-dimensional scene flow. The scene flow of the feature point calculated by obtained images in a time series enables the three-dimensional registration of the position between the endoscope and the gastric wall. We confirmed the validity of a proposed method first by a known-movement object and then by a wave simulated model.

9698-47, Session PSun

Imaging of the median nerve neuropathy by optical coherence tomography in rabbits

Yeh-Chan Ahn, Pukyong National Univ. (Korea, Republic of) and Ctr. for Marine Integrated Biomedical Technology (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Yu-Gyeong Chae, Ctr. for Marine Integrated Biomedical Technology (Korea, Republic of) and Pukyong National Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Young-Sik Kim, Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Dong-Kyu Kim, Innovative Biomedical Technology Research Ctr. (Korea, Republic of) and Kosin Univ. College of Medicine (Korea, Republic of); Eun-Kee Park, Kosin Univ. College of Medicine (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Sae Hyun Kim, Kosin Univ. College of Medicine (Korea, Republic of)

Optical coherence tomography (OCT) is based on backscattering of near infra-red light from tissue. It is a non-invasive and high-resolution technique for the evaluation of disease. OCT is used for the assessment of the microstructure of tissues and irregular textures corresponding to thickened walls. OCT findings, combined with electrodiagnostic study findings, showed comparative advantages in evaluating the microstructure of inflammation. OCT findings, combined with electrodiagnostic study findings, showed comparative advantages in diagnosis and treatment.

9698-48, Session PSun

Spectral aspects of noninvasive diagnostic melanoma imaging

Daniel S. Gareau, The Rockefeller Univ. (United States)

Melanoma is the most deadly skin cancer and early detection through screening saves lives. Technologies to aid screening seldom survive clinical trials because at high sensitivity, which is required to avoid the
false negative screening result, they typically achieve very low specificity. To test whether the clinical ABCD criteria are effective at identifying melanomas, we automated the approach in a computer vision application that operated on dermoscopic images of melanomas and nevi. We extended the diagnostic criteria beyond the standard ABCD criteria and found that there was evidence that particular standard criteria were indeed diagnostic but also that other standard criteria did not seem to possess the ability to discriminate melanomas from nevi. We achieved a several-fold improvement in diagnostic accuracy over standard practice. Additionally, new diagnostic criteria that we created were highly diagnostic. Spectral trends emerged in the diagnostic value of various criteria and ongoing work modeling the light/tissue interaction has begun to shed light on the interactions between particular wavelengths and particular malignant and common morphologies.

9698-49, Session PSun

A finger-free wrist-worn pulse oximeter for the monitoring of chronic obstructive pulmonary disease

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Current pulse oximeters are used to monitor the blood oxygen saturation of a patient. These pulse oximeters use a clip-like probe that measures the blood oxygen saturation from the fingertip, making it very inconvenient for the movement of fingers. Furthermore, it may cause finger pain when the probe is clamped on the finger for lengthy hours. Herein, a finger-free wrist-worn pulse oximeter is presented. This device allows patients to measure blood oxygen level and pulse rate without hindering their normal finger movement. This wrist-worn pulse oximeter is built with a reflectance oximetry sensor, which consists of light emitting diodes and more photodiode light detectors located side by side. This reflectance oximetry sensor is covered with an optical element with micro structured surface. This micro structured optical element is designed to modulate photon propagation beneath the skin tissue so that the photoplethysmogram signals of reflected lights or backscattered lights detected by the photodetector are therefore enhanced. Changes in light absorption during the pulsing cycle are measured by the PDs as scattered lights are reflected back from the pulsating arteriolar bed. By calculating the ratio of absorbance of oxygenated and deoxygenated hemoglobin at red and infrared beams during the arterial pulsing respectively, it determines the blood oxygen level and pulse rate from the wrist skin.

9698-15, Session 5

Three-dimensional ultrasonic needle tracking with a fiber-optic hydrophone and a custom 1.5D ultrasound imaging probe

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Precisely and efficiently guiding medical needles to procedure targets is critically important in minimally invasive procedures such as nerve blocks and fetal interventions. Ultrasound imaging is commonly used for device guidance. However, localizing the needle tip relative to the ultrasound imaging plane can be difficult when targets are deep and at steep insertion angles. This challenge is particularly acute with thin needles, which readily bend and deviate from the imaging plane during insertions. A reliable method to track the needle which is compatible with the current clinical workflow remains an ongoing challenge. Ultrasound tracking uses ultrasound to received and/or transmitted at the medical device tip to communicate with an external imaging probe. Subsequent processing of ultrasound transmissions locate the needle tip relative to ultrasound images in real-time. In this study, we integrated a broadband fiber-optic hydrophone into a needle to serve as the receiver and developed a custom external ultrasound imaging probe to allow for three-dimensional ultrasonic tracking. This probe had five rows of ultrasound transducer elements, with one acoustically-focused central array for in-plane tracking, and 4 side arrays for out-of-plane tracking. The performance of the system was evaluated using phantoms and ex vivo tissue samples. With high sensitivity observed at steep insertions angles (> 60 degrees) and large insertion depths (> 45 mm), and high tracking accuracy (< 1 mm), this system has strong potential to provide reliable device guidance for minimally invasive procedures.

9698-16, Session 5

Image-guided dynamic laser coagulation using a double-clad fiber-based system

Kathy Beaudette, Ecole Polytechnique de Montréal (Canada) and Massachusetts General Hospital (United States); William Lo, Martin Villiger, Milen Shishkov, 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)

Optical coherence tomography (OCT) used in combination with laser coagulation to mark selected regions of interest could efficiently guide the collection of biopsies. This would reduce false-negative findings, and have a significant impact on the management of Barrett’s esophagus. Here, we present a system based on double-clad fiber (DCF) capable of delivering marking laser light through the inner cladding while performing OCT through the core. A previously clinically validated commercial OCT system (NVisionVLE, Ninepoint Medical) was adapted in order to perform in vivo esophageal image-guided dynamic laser marking. A dedicated DCF coupler (core signal transmission: -95%; inner cladding coupling: -75%) was implemented into the system to combine both modalities into the DCF. The original rotary junction, necessary for helical scanning with a side-looking fiber probe, was replaced by a custom DCF-based optical rotary junction (Princetel Inc.). This rotary junction was optimized to provide low insertion loss and crosstalk to preserve imaging quality (effective dynamic range of up to 52dB). DCF-based OCT catheters were designed to have a beam waist diameter of 62±4µm at a working distance of 9.5±0.4mm, for use with a 17-mm diameter balloon sheath. Our previous ex vivo marking experiments demonstrate that, based on the characterization of these DCF components, the system is capable of single-pulse laser marking at 1436 nm. This enables further in vivo experiments to demonstrate image-guided dynamic laser marking and potentially other therapeutic applications.

9698-17, Session 5

A goggle navigation system for ultrasound and fluorescence dual-mode image-guided surgery

Ze Shu Zhang, Jin Pei, Dong Wang, Jian Ye, Qi Gan, Peng Liu, Jian Yue, Ben Zhong Wang, Peng Fei Shao, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Surgical resection remains the primary curative intervention for cancer treatment. However, the occurrence of a residual tumor after resection is very common, leading to the recurrence of the disease and the need for re-resection. We develop a surgical navigation system that combines near infrared fluorescent imaging and ultrasonography for intraoperative imaging.
return to contents
tissue structural organization (anisotropy) and can be used to diagnose myocardial infarct; circular birefringence (optical rotation) can measure glucose concentrations. The most comprehensive type of polarimetry analysis is to measure the Mueller matrix, a polarization transfer function that completely describes how a sample interacts with polarized light. To derive this 4x4 matrix it is necessary to observe how a tissue interacts with different polarizations. A well-suited approach for tissue polarimetry is to use photoelastic modulators (PEMs), which dynamically modulate the polarization of light. Previously, we have demonstrated a rapid time-gated Stokes imaging system that is capable of characterizing the state of polarized light (the Stokes vector) over a large field, after interacting with any turbid media. This was accomplished by synchronizing CCD camera acquisition times relative to two PEMs using a field-programmable gate array (FPGA). Here, we extend this technology to four PEMs, yielding a polarimetry system that is capable of rapidly measuring the complete sample Mueller matrix over a large field of view, with no moving parts and no beam steering. We describe the calibration procedure and evaluate the accuracy of the measurements. Results are shown for tissue-mimicking phantoms, as well as initial biological samples.

9698-21, Session 6

Optimal injection time of indocyanine green for intraoperative fluorescence image-guided thoracoscopic resection in rabbit model

Minji Kim, Korea Univ. (Korea, Republic of); Yuhua Quan, Korea Univ. Guro Hospital (Korea, Republic of); Byeong Hyun Choi, Korea Univ. (Korea, Republic of) and Korea Univ. Guro Hospital (Korea, Republic of); Yeonho Choi, Korea Univ. (Korea, Republic of); Hyun Koo Kim, Korea Univ. Guro Hospital (Korea, Republic of); Beop-Min Kim, Korea Univ. (Korea, Republic of)

Pulmonary nodule could be identified by intraoperative fluorescence imaging system from systemic injection of indocyanine green (ICG) which achieves enhanced permeability and retention (EPR) effects. This study was performed to evaluate optimal injection time of ICG for detecting cancer during surgery in rabbit lung cancer model.

VX2 carcinoma cell was injected in rabbit lung under fluoroscopic computed tomography-guidance. Solitary lung cancer was confirmed on positron emitting tomography with CT (PET/CT) 2 weeks after inoculation. ICG was administered intravenously and fluorescent intensity of lung tumor was measured using the custom-built intraoperative color and fluorescence merged imaging system (ICFIS) for 15 hours. Solitary lung cancer was resected through thoracoscopic version of ICFIS.

ICG was observed in all animals. Because Lung has fast blood pulmonary circulation, Fluorescent signal showed maximum intensity earlier than previous studies in other organs. Fluorescent intensity showed maximum intensity within 6-9 hours in rabbit lung cancer. Overall, Fluorescent intensity decreased with increasing time, however, all tumors were detectable using fluorescent images until 12 hours.

In conclusion, while there had been studies in other organs showed that optimal injection time was at least 24 hours before operation, this study showed shorter optimal injection time at lung cancer. Since fluorescent signal showed the maximum intensity within 6-9 hours, cancer resection could be performed during this time. This data informed us that optimal injection time of ICG should be evaluated in each different solid organ tumor for fluorescence image guided surgery.

9698-22, Session 7

Comparison of perfusion diagnostics with optical coherence tomography, sidestream-darkfield, incident darkfield and laser speckle contrast imaging in a tissue-like phantom

Sanne M. A. Jansen M.D., Daniel M. de Bruin, Dirk J. Faber, Ton G. van Leeuwen, Academisch Medisch Centrum (Netherlands)

There is no gold standard test for perfusion evaluation in surgery. Optical Imaging techniques are able to image tissue at high resolution and in real-time. Laser Speckle Contrast Imaging, Optical Coherence Tomography, Sidestream Darkfield and Incident Darkfield all use the interaction of light with tissue to create an image. To test their feasibility and explore validity in a controlled setting, we created a phantom with the optical properties of tissue and microvascular channels of 30-400 micrometer. With a Hamilton Syringe Pump we mimicked blood flow velocities of 0-20 mm/sec. Images of all different modalities at different blood flow velocities were compared in terms of imaging depth, resolution and hemodynamic parameters.

9698-23, Session 7

Fast ex-vivo wide-field OCT system for diagnostic and surgical guidance

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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. Previous clinical studies have shown the high efficiency of this imaging technique for the detection of cancer on various organs. This promises great potential of the technique for an ex-vivo quick analysis of surgical resections or biopsy specimens, in the aim to help the surgeon/radiologist decide on the course of action.

Here we will present some of the latest technical developments on a FFOCT system which can produce 1cm² images with 1 μm resolution in 1 minute. Larger samples, up to 50mm diameter, can also be imaged. Details on the large sample handling, high-speed image acquisition, optimized scanning, and accelerated GPU tiles stitching will be given. Results on the clinical applications for breast, urology, and digestive tissues will also be given. They highlight the relevance of the system characteristics for the detection of cancer on ex-vivo specimens.

FFOCT now appears clearly as a very fast and non-destructive imaging technique that provides a quick assessment of the tissue morphology. With the benefit of both new technical developments and clinical validation, it turned into a mature technique to be implemented in the clinical environment. In particular, the technique holds potential for the fast ex-vivo analysis of excision margins or biopsies in the operating room.

9698-24, Session 7

Quantification of NA-dependence of OCT signal attenuation

Liliana M. Peinado, Academisch Medisch Centrum (Netherlands); Paul R. Bloemen, Mitra Almasian, Ton G. van Leeuwen, Dirk J. Faber, Academisch Medisch Centrum (Netherlands)

There is no gold standard test for perfusion evaluation in surgery. Optical Imaging techniques are able to image tissue at high resolution and in real-time. Laser Speckle Contrast Imaging, Optical Coherence Tomography, Sidestream Darkfield and Incident Darkfield all use the interaction of light with tissue to create an image. To test their feasibility and explore validity in a controlled setting, we created a phantom with the optical properties of tissue and microvascular channels of 30-400 micrometer. With a Hamilton Syringe Pump we mimicked blood flow velocities of 0-20 mm/sec. Images of all different modalities at different blood flow velocities were compared in terms of imaging depth, resolution and hemodynamic parameters.
Despite the improvements in early cancer diagnosis, adequate diagnostic tools for early staging of bladder cancer tumors are lacking [1]. MEMS-probes based on optical coherence tomography (OCT) provide cross-sectional imaging with a high-spatial resolution at a high-imaging speed, improving visualization of cancerous tissue [2-3]. Additionally, studies show that the measurement of localized attenuation coefficient allows discrimination between healthy and cancerous tissue [4]. We have designed a new miniaturized MEMS-probe based on OCT that will optimize early diagnosis by improving functional visualization of suspicious lesions in bladder. During the optical design phase of the probe, we have studied the effect of the numerical aperture (NA) on the OCT signal attenuation. For this study, we have employed an InnerVision Santec OCT system with several numerical apertures (25mm, 40mm, 60mm, 100mm, 150mm and 200mm using achromatic lenses). The change in attenuation coefficient was studied using 15 dilutions of intralipid ranging between 6*10-5 volume% and 20 volume%. We obtained the attenuation coefficient from the OCT images at several fixed positions of the focuses using established OCT models (e.g. single scattering with known confocal point spread function (PSF)). As a result, a non-linear increase of the scattering coefficient as a function of intralipid concentration (due to dependent scattering) was obtained for all numerical apertures. For all intralipid samples, the measured attenuation coefficient decreased with a decrease in NA. Our results suggest a negligible influence of the NA on the measured attenuation coefficient.


6998-25, Session 7
Automated 3D segmentation of oral mucosa from wide-field OCT images
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Optical Coherence Tomography (OCT) can discriminate morphological tissue features important for oral cancer detection such as the presence or absence of basement membrane and epithelial thickness. We previously reported an OCT system employing a rotary-pullback catheter capable of in vivo, rapid, wide-field (up to 90 x 2.5mm2) imaging in the oral cavity. Due to the size and complexity of these OCT data sets, rapid automated image processing software that immediately displays important tissue features is required to facilitate prompt bed-side clinical decisions.

We present an automated segmentation algorithm capable of detecting the epithelial surface and basement membrane in 3D OCT images of the oral cavity. The algorithm was trained using volumetric OCT data acquired in vivo from a variety of tissue types and histology-confirmed pathologies spanning normal through cancer (8 sites, 21 patients). The algorithm was validated using a second dataset of similar size and tissue diversity. We demonstrate application of the algorithm to an entire OCT volume to map epithelial thickness, and detection of the basement membrane, over the tissue surface. These maps may be clinically useful for delineating pre-surgical tumor margins, or for biopsy site guidance.

9698-26, Session 7
Fully automatic segmentation and characterization of in vivo esophageal tissue by optical coherence tomography
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Background - Optical coherence tomography (OCT) is an imaging modality that acquires cross-sectional images of the microscopic structure of the esophagus, including conditions such as Barrett’s Esophagus and dysplasia in the same. Tethered capsule OCT endomicroscopy (TCE) device has the potential to become a population based screening method that identifies patients with abnormal esophagus that can be further referred for an upper endoscopy.

Current limitation - A TCE dataset is typically made by >1,000 images and data analysis for the characterization of esophageal wall is currently performed manually, resulting in a time-consuming and thus inefficient procedure. Additionally, since the capsule optics is optimally focused approximately 500 μm outside the capsule wall, the best quality images are obtained when the tissue is in full contact with the capsule. As such, it is crucial to provide feedback for the operator about tissue contact during the imaging procedure to acquire best quality data.

Methods - In this study we developed an automatic algorithm for the segmentation and characterization of the esophageal wall. An adaptive binarization technique followed by morphological operations and spline fitting was used to achieve 3D fully automatic segmentation of esophageal tissue. A layer detection procedure was then applied for the characterization of normal (i.e. squamous) esophagus vs. abnormal tissue.

Validation - The proposed algorithm has been validated over a total of 150 images randomly selected from 4 different patients (including both normal and abnormal esophagus) by comparing automatic to manual image analysis (gold standard).

Results - A 2D Dice correlation coefficient of 0.97 ± 0.2 and a correlation r=0.98 (over individual A-scan lines) has been found by comparing manual vs. automatic segmentation. Tissue analysis validation over 20 images (10 normal esophagus and 10 abnormal tissue) showed an A-scan line classification accuracy of 87%.

Discussion – The proposed methods automatically provides two-dimensional representations of both the “contact map” from the data collected in a human pilot study, and a “tissue map” depicting areas of abnormal tissue through an entire acquisition. Our initial results suggest that this algorithm has the potential to improve the current data acquisition procedure and provide an efficient, automatic characterization of diseased esophageal wall.
A thermal-magnetic biosensor for point of care applications

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We developed a novel thermal-magnetic biosensor (TMAGS) for point of care applications. The magnetic part of the biosensor is a planar device composed by a primary micro-coil able to generate an induced magnetic field which induced a voltage equal and opposite in the two secondary coils. The presence of magnetic material on one of the two secondary coils causes a variation of induced magnetic field especially in the area where the magnetic material is located. Consequently will change the amount of magnetic flux linked with the two secondary coils and this results in a total output voltage different from zero. The voltage variation, therefore, is a measure of the amount of magnetic material present in the active zone. The differential approach is used here in order to filter out undesired effects related to interfering signals. A device sensitivity of 5.5mV/ng and a resolution of 0.008ng have been observed. The biosensor also presents a heater and a thermal sensor respectively to set and read-out the chip temperature. To integrate a micro-heater allows establishing either a uniform temperature or a constant gradient in a given region can be also confined in a particular region of the biological system with elevated control and accuracy of the temperature in the reaction chamber. This aspect enable the device to be used for several biochemical analysis that need temperature control and activation, i.e. nucleic acids amplification (rt-PCR), antigen-antibody detection (immune-assay), SNP detection, etc..

Multimodal optical biopsy probe to improve the safety and diagnostic yield of brain needle biopsies

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Brain needle biopsy (BNB) is performed to collect tissue when precise neuropathological diagnosis is required to provide information about tumor type, grade, and growth patterns. The principal risks associated with this procedure are intracranial hemorrhage (due to clipping blood vessels during tissue extraction), incorrect tumor typing/grading due to non-representative or non-diagnostic samples (e.g. necrotic tissue), and missing the lesion. We present an innovative device using sub-diffuse optical tomography to detect blood vessels and Raman spectroscopy to detect molecular differences between tissue types, in order to reduce the risks of misdiagnosis, incorrect tumour grading, and non-diagnostic samples. The needle probe integrates optical fibers directly onto the external cannula of a commercial BNB needle, and can perform measurements for both optical techniques through the same fibers. This integrated optical spectroscopy system uses diffuse reflectance signals to perform a 360-degree reconstruction of the tissue adjacent to the biopsy needle, based on the optical contrast associated with hemoglobin light absorption, thereby localizing blood vessels. Raman spectra measurements are also performed intermittently for tissue characterization. A detailed sensitivity of the system is presented to demonstrate that it can detect absorbers with diameters >300 μm located up to 72 mm from the biopsy needle core, for bulk optical properties consistent with brain tissue. Results from animal experiments are presented to validate blood vessel detection and Raman spectrum measurement without disruption of the surgical workflow. We also present phantom measurements of Raman spectra with the needle probe and a comparison with a clinically validated Raman spectroscopy probe.

An optical spectroscopy instrument designed for in-vivo use in a primary care clinical setting

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While there are a plethora of in-vivo spectroscopic techniques that have demonstrated the ability to detect a number of diseases in research trials, very few techniques have successfully become a fully realized clinical technology. This is primarily due to the stringent demands on a clinical device for widespread implementation. Some of these demands include: simple operation requiring minimal or no training, safe for in-vivo patient use, no disruption to normal clinic workflow, tracking of system performance, warning for measurement abnormality, and meeting all FDA guidelines for medical use. Previously, our group developed a fiber optic probe-based optical sensing technique known as low-coherence enhanced backscattering spectroscopy (LEBS) to quantify tissue ultrastructure in-vivo. Now we have developed this technique for the application of prescreening patients for colonoscopy in a primary care (PC) clinical setting. To meet the stringent requirements for a viable medical device used in a PC clinical setting, we developed several novel components including an automated calibration tool, optical contact sensor for signal acquisition, and a contamination sensor to identify measurements which have been affected by debris. The end result is a state-of-the-art medical device that can be realistically used by a PC physician to assess a person’s risk for harboring colorectal precancerous lesions. The pilot study of this system shows great promise with excellent stability and accuracy in identifying high-risk patients. While this system has been designed and optimized for our specific application, the system and design concepts are universal to most in-vivo fiber optic based spectroscopic techniques.

High definition Mueller polarimetric endoscope

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The mechanism of most medical endoscopes is based on the interaction between light and biological tissue, inclusive of absorption, elastic scattering and fluorescence. In essence, the metrics of those interactions are obtained from the fundamental properties of light as an electro-magnetic waves, namely, the radiation intensity and wavelength. As another fundamental property of light, polarisation can not only reveal tissue scattering and absorption information from a different perspective, but is also able to provide a fresh insight into directional tissue birefringence properties induced by birefringent compositions and anisotropic fibrous structures, such as collagen, elastin, muscle fibre, etc at the same time. Here we demonstrate a low cost high definition Muller polarimetric endoscope with minimal alteration of a rigid endoscope. By imaging birefringent tissue mimicking phantoms and a porcine bladder, we show that this novel endoscopic imaging modality is able to provide different information of interest from unpolarised endoscopic imaging, including linear depolarization, circular depolarization, birefringence, optic axis orientation
and dichroism. This endoscope can potentially be employed for better tissue visualisation and benefit endoscopic investigations and intra-operative guidance.

9698-31, Session 9
Which blood oxygen index by NIRS is sensitive to shock severity?
Ting Li, Kai Li, Univ. of Electronic Science and Technology of China (China)

Clinical shock-monitoring mainly depends on measuring oxygen saturations by blood-gas analyzer from the central internal jugular vein blood samples invasively and discontinuously. The golden standard indicator is the central internal jugular vein oxygenation (SjvO2). Our previous study revealed that near-infrared spectroscopy (NIRS) is capable of measuring shock noninvasively and continuously by placing the probe in shock-sensitive sites of the human body. Various blood oxygen indices at different sites measured by NIRS in monitoring shock have been reported, but no quantitative comparison among these NIRS-measured blood oxygen indices in sensitivity of telling the shock severity is provided. Here we collected data by NIRS on 25 shock patients from the intensive care unit of the hospital. The blood oxygen indices included cerebral venous oxygen saturation (ScvO2), tissue oxygen saturation of internal jugular area (StO2), and tissue oxygen saturation of extremities areas (SteO2). The golden standard SjvO2 and the diagnosis on shock severity based on the trained doctors’ observation on patients are also recorded. The full comparison analysis showed ScvO2 and StO2 are more sensitive to shock severity than other indices, while SteO2 is the earliest indicator for shock diagnosis.

9698-32, Session 9
Diagnosis potential of near infrared Mueller Matrix imaging for colonic adenocarcinoma
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Mueller matrix imaging along with polar decomposition method was employed for the diagnosis of colonic adenocarcinoma by polarized light in the near-infrared spectral range (850–1100 nm). A high-speed (≤10 ms) Mueller matrix imaging system with dual-rotating waveplates was developed. 16 (4 by 4) full Mueller matrices of the colonic tissues (i.e., normal colon and colonic adenocarcinoma) were acquired. Polar decomposition was further implemented on the 16 images to derive the diattenuation, depolarization, and the retardance images. The decomposed images showed clear margin between the normal colon and colon adenocarcinoma tissue samples. The work shows the potential of near-infrared Mueller matrix imaging for the early diagnosis and detection of malignant lesions in the colon.

9698-33, Session 9
Investigation of oxygenation dynamics of sepsis patient using far-infrared intervention with near-infrared spectroscopy measurement
Kuei Hung Chuang, Chia-Wei Sun, National Chiao Tung Univ. (Taiwan)

Near-infrared Spectroscopy (NIRS) is a noninvasive technique to monitor tissue oxygenation dynamics and microcirculation. Because of the advantage, sepsis has been investigated with NIRS for many years including severe sepsis and sepsis shock. However, it is necessary to involve a physical method, which is often venous occlusion to change hemodynamics. There are some drawbacks of vessel-occlusion method that include skin contact, uncomfortable and microcirculation block of patients. In order to improve the user experience from venous occlusion, we developed a new method which is far-infrared illumination. In this study, we investigated sepsis patient with far-infrared illumination and NIRS. The result release that this method could recognize severe sepsis and sepsis shock with good user experience. We hope this method can be used for clinical diagnosis of other peripheral arterial disease.

9698-34, Session 9
A novel method to estimate oxygen saturation of the internal jugular vein blood
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Internal jugular vein oxygen saturation (SjvO2) provides an indirect evaluation of cerebral oxygen and is used to guide physiologic management decisions in a variety of clinical paradigms. There is few articles to introduce measuring SjvO2 though noninvasive approach. Here we describe a novel method based on postural change to estimate SjvO2 by using Near Infrared spectroscopy (NIRS). Postural changes affect the cross-sectional area (CSA) of the internal jugular vein (IJV) and cause the sectional change of the IJV blood volume. When patients were in the supine and 80° head-up tilting (HUT) position respectively, the CSAs were imaged by the use of ultrasound. Then using digital image processing technology, we compared the CSAs quantitatively. CSA change raised light absorption change. SjvO2 had been determined from light absorption measurements in two wavelength, before and after the position changes, using a modified algorithm of a reflectance-based oximeter. The method had been applied to the vertical area over the IJV of 11 patients who were placed a central venous catheter for medical uses. The oxygen saturation for IJV blood noninvasively measured by NIRS (SjvO2NIRS), was found to be highly correlated (r=0.812, p<0.001) and fairly agreed at discordance coefficient of 0.735 with SjvO2BGA measured by a blood-gas analyzer.

9698-35, Session 10
3D shape reconstruction for minimally invasive surgical interventions using OFDR in optical fibers
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Many research groups are working on image-guided medical interventions using minimally invasive instruments such as catheters or needles in order to reach a targeted area with greater precision. 3D real-time tracking of these instruments can be of clinical value for monitoring these remotely controlled instruments by providing retroactive feedback to the user. Many imaging systems such as CT, MRI or ultrasound are also able to provide such information. Fluoroscopic imaging requires contrast injections, and CT and MRI are acquired intermittently, therefore it remains challenging to obtain a precise 3D representation with a sequence of images. Also, the procedures can be time consuming and exposure to radiation can be harmful for patients. Here, we propose a new alternative, simpler and safer, using only optical fibers inserted inside these surgical instruments. Using optical frequency domain reflectometry (OFDR) to obtain distributed measurements of strain along the fibers, it is possible to reconstruct the three-dimensional shape of catheters or needles. To do so, three fibers...
are glued in a specific geometry for the three degrees of freedom, leading to real time 3D images with fast processing. We present here results demonstrating the promising aspects of this approach. By comparing the results using fibers with different Rayleigh scattering coefficients, the advantages as well as the limitations of using the very simple OFDR technique as a high-precision truly-distributed strain sensor are discussed.

9698-36, Session 10

The joint analysis of perfusion and coenzymes NADH and FAD in diabetes

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Blood circulation and the state of oxidative phosphorylation were studied in the tissues of patients with type II diabetes and the presence of “diabetic foot” syndrome. During joint studies, the tissue perfusion and the content of coenzymes NADH and FAD were evaluated using laser Doppler flowmetry (LDF) at 1064 nm and fluorescence spectroscopy (FS) with wavelength of excitation at 365 nm and 450 nm, respectively. Registration of perfusion parameters and the amplitude of the fluorescence coenzymes were simultaneously made using a “LAKK-M” system (SPE “LAZMA” Ltd, Russia) in the same tissue volume through one optical fibre probe with a 3 mm diameter.

The experimental study involved 25 patients with type II diabetes and 12 patients with neuroischaemic form of “diabetic foot” syndrome. In clinical cases, a balance and a correlation in the “delivery-consumption” system were observed during the compensation of microcirculatory-tissue relationships. Thus, an elevated level of nutritive blood flow was accompanied by elevated levels of NADH, which may indicate the activation of the oxidative metabolic processes.

Presence of the “diabetic foot” syndrome often revealed more prevalent signs of imbalance in the “delivery-consumption” system. In some cases of coarse ischaemic lesions, decreases in the oxidative metabolism with activation of glycolysis were detected. This required enhanced delivery of the substrate and, as a result, blood flow in the microvasculature. A simultaneous increase in NADH and FAD was also observed in these cases. Thus, the proposed method allows for the identification of violations in microcirculation and metabolism during diabetes.

9698-38, Session 10

Real-time wide-field metabolic imaging achieved through coherent spatial frequency domain imaging (cSFDI)

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Reduced oxygen metabolism is a well-established adaptation to chronic tissue hypoxia, seen in numerous forms of cancer and cardiovascular disease. Measuring the metabolic rate of oxygen consumption could yield new insights into the disease process and provide clinicians with novel tools for early diagnosis, staging and monitoring of therapeutic interventions. Spatial frequency domain imaging (SFDI) is a wide-field, noncontact, optical imaging modality capable of quantitatively assessing tissue concentrations of oxy- and deoxyhemoglobin. Laser speckle imaging (LSI) is a related wide-field optical imaging tool capable of measuring relative tissue perfusion. LSI and SFDI can be combined through the integration of spatial frequency domain techniques into core LSI technologies, in so-called coherent spatial frequency domain imaging (cSFDI). cSFDI is capable of recovering intrinsically coregistered absorption coefficient (\(\mu_a\)), reduced-scattering coefficient (\(\mu_s'\)), and optical property-corrected speckle flow index (SFI). Having access to \(\mu_a\) at multiple wavelengths enables the recovery of oxy- and deoxyhemoglobin, which can be combined with SFI to estimate the metabolic rate of oxygen consumption. Real-time cSFDI is achieved through advanced demodulation techniques that reduce the burden of data acquisition. Here we present a high-speed, portable cSFDI system capable of gathering data at 25 Hz. We demonstrate the performance of this system in dynamic studies of healthy subjects undergoing a variety of physiological challenges. In particular, we explore metabolism during post occlusive reactive hyperemia in the lower extremity during. The data gathered in these studies is then used to synthesize a metric for oxygen metabolism.
9699-1, Session 1
Digital detection of biomarkers for high-sensitivity diagnostics at low-cost (Invited Paper)

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We have demonstrated Interferometric Reflectance Imaging Sensor (IRIS) with the ability to detect single nanoscale particles. In single-particle modality of IRIS (SP-IRIS), the interference of light reflected from the sensor surface is modified by the presence of particles producing a distinct signal that reveals the size of the particle. In our approach, the dielectric layered structure acts as an optical antenna optimizing the elastic scattering characteristics of nanoparticles for sensitive detection and analysis. By extending single-particle IRIS to in-liquid dynamic imaging, we demonstrated real-time digital detection of individual viral pathogens as well as single molecules labeled with Au nanoparticles. With this technique we demonstrate real-time simultaneous detection of multiple targets in a single sample, as well as quantitative dynamic detection of individual biomolecular interactions for reaction kinetics measurements. This approach promises to simplify and reduce the cost of rapid diagnostics at very high sensitivity and specificity.

We have also demonstrated the utility of DNA-conjugated antibodies on SP-IRIS platform. Utilizing a DNA surface for conversion to an antibody array offers an easier manufacturing process by replacing the antibody printing step with DNA printing. DNA-directed immobilization technique also has the added advantages of programmable sensor surface generation based on the need, and resistance to high temperatures required for microfluidic device fabrication. These capabilities improve the existing SP-IRIS technology, resulting in a more robust and versatile platform, ideal for point-of-care diagnostics applications.

9699-2, Session 1
Single DNA imaging and length quantification through a mobile-phone microscope

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The development of sensitive optical microscopy methods for the detection of single DNA molecules has become an active research area which cultivates various promising applications including point-of-care (POC) genetic testing and diagnostics. Direct visualization of individual DNA molecules usually relies on sophisticated optical microscopes that are mostly available in well-equipped laboratories. For POC DNA testing, there is an increasing need for the development of novel single DNA imaging and sensing methods that are field-portable, cost-effective, and accessible for diagnostic applications in resource-limited or field-settings. To this end, we developed a mobile-phone integrated fluorescence microscopy platform that allows imaging and sizing of single DNA molecules that are stretched on a chip. This handheld device contains an opto-mechanical attachment integrated onto the existing smartphone camera module, which creates a high signal-to-noise ratio dark-field imaging condition by using an oblique illumination/excitation configuration. Using this device, we demonstrated imaging of individual linearly stretched lambda DNA molecules (48 kilobase-pair, kbp) over a 2 mm2 field-of-view. We further developed a robust computational algorithm and a smartphone app that allowed the users to quickly quantify the length of each DNA fragment imaged using this mobile interface. The cellphone based device was tested by five different DNA samples (5, 10, 20, 40, and 48 kbp), and a sizing accuracy of <1 kbp was demonstrated for DNA strands longer than 10 kbp. This mobile DNA imaging and sizing platform can be very useful for various diagnostic applications including the detection of disease-specific genes and quantification of copy-number-variations at POC settings.

9699-3, Session 1
NutriPhone: smartphone platform for vitamin B12 quantification in point-of-care settings

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Vitamin B12 deficiency is the leading cause of cognitive decline in the elderly and is associated with increased risks of several acute and chronic conditions including anemia. The deficiency is prevalent among the world population, most of whom are unaware of their condition due to the lack of a simple diagnostics system. Recent advancements in the smartphone-enabled mobile health can help address this problem by making the deficiency tests more accessible. Previously, our group has demonstrated the NutriPhone, a smartphone platform for the accurate quantification of vitamin D levels. The NutriPhone technology comprises of a disposable test strip that performs a colorimetric reaction upon collecting a sample, a reusable accessory that interfaces with the smartphone camera, and a smartphone app that stores the algorithm for analyzing the test-strip reaction. In this work, we show that the NutriPhone can be expanded to measure vitamin B12 concentrations by developing a lateral flow assay for B12 that is compatible with our NutriPhone system. Our novel vitamin B12 assay incorporates blood sample processing and key reagent storage on-chip, which advances it into a sample-in-answer-out format that is suitable for point-of-care diagnostic applications. In order to enable the detection of pM levels of vitamin B12 levels, silver amplification of the initial signal is used within the total assay time of less than 15 minutes. We demonstrate the effectiveness of our NutriPhone system by deploying it in a resource-limited clinical setting in India where it is used to test tens of participants for vitamin B12 deficiency.

9699-4, Session 1
Cellphone-based colorimetric microplate reader for point-of-care testing

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Enzyme-linked immunosorbent assay (ELISA) in a microplate format has been a gold standard first-line clinical test for diagnosis of various diseases including infectious diseases. However, this technology requires a relatively large and expensive multi-well scanning spectrophotometer to read and quantify the signal from each well, hindering its implementation in resource-limited settings. Here, we demonstrate a cost-effective and handheld smartphone-based colorimetric microplate reader for rapid digitization and quantification of immunoserology-related ELISA tests in a conventional 96-well plate format at the point of care (POC). This device consists of a bundle of 96 optical fibers to collect the transmitted light from each well of the microplate and direct all the transmission signals from the wells onto the camera of the mobile-phone. Captured images are then transmitted to a remote server through a custom-designed app, and both quantitative and qualitative diagnostic results are returned back to the user within ~1 minute per 96-well plate by using a machine learning algorithm. We tested this mobile-phone based micro-plate reader in a clinical microbiology lab using FDA-approved mumps IgM, measles IgG, and herpes simplex virus IgG (HSV-1 and HSV-2) ELISA tests on 1138 remnant patient samples (roughly 50% training and 50% testing), and achieved an overall accuracy of ~99% or higher for each ELISA test. This handheld and cost-effective platform could be immediately useful for large-scale vaccination monitoring in low-infrastructure settings, and also for other high-throughput disease screening applications at POC.

9699-5, Session 3

Low-cost flatbed scanner label-free biosensor

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Low-cost, portable, and easy-to-use label-free biosensors have high potential to be used in low resource settings and point-of-care applications. Here, we demonstrate utilization of a flatbed document scanner as a label-free interferometric biosensor for imaging DNA and protein microarrays on Si/SiO2 layered substrates. The interference-based sensing relies on changes in optical path length upon biomass accumulation on the top surface. The added biomass results in a quantifiable shift in spectral reflectivity, which is sampled at different wavelengths to determine its thickness.

We use a contact image sensor (CIS) based flatbed scanner, which sequentially illuminates the chip surface with red, green and blue LEDs. To capture specularly reflected light from the layered surface, we re-align the scanner head that consists of an LED waveguide, a GRIN lens array, and a detector. By inserting a 3D-printed wedge beneath the moving head, we achieve an optimal tilt to capture the light reflected off the chip surface. To bring the chip surface to focus, we replace the scanner glass with a custom-made chip holder of varying heights. We use the scanner software from manufacturer to capture images, and MATLAB for data analysis. With the scanner-based biosensor, we demonstrate detection of DNA hybridization in microarray format, and use a state-of-the-art label-free biosensor, Interferometric Reflectance Imaging Sensor (IRIS), as a benchmark. With a cost as low as 100USD, and ability to scan a 4mmx4mm area in 30 seconds with ~8um lateral resolution, our USB-powered biosensor offers a low-cost, easy-to-use alternative to commercially available label-free systems.

9699-6, Session 3

Development and bench testing of a multi-spectral imaging technology built on a smartphone platform

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Cervical cancer screening presents a great challenge for clinicians across the developing world. In many countries, cervical cancer screening is done by visualization with the naked eye. Simple brightfield white light imaging with photo documentation has been shown to make a big impact on cervical cancer care. Adoption of smartphone based cervical imaging devices is increasing across Africa. However, advanced imaging technologies such as multi-spectral imaging systems, rarely make it to the field clinic in low-resource settings, where they are needed most. To address this challenge, the technology of a smartphone-based mobile colposcopy imaging system was refined, integrating components required for low cost, portable multi-spectral imaging of the cervix.

This paper describes the refinement of the mobile colposcope to enable it to acquire images of the cervix at multiple illumination wavelengths, including modeling and laboratory validation (clinical testing is presented in a separate paper). Wavelengths were selected to enable quantifying the main absorbers in tissue (oxy- and deoxy-hemoglobin, and water), as well as scattering parameters that describe the size distribution of scatterers. The necessary hardware and software modifications are reviewed. The white light illumination was replaced with a series of LEDs that were mounted on a printed circuit board. A lens array was used to co-register the beam from the various LEDs on a target (the cervix) that is 30 cm away. The app was refined to integrate and synchronize multi-spectral illumination and image capture. Initial testing suggests the multi-spectral mobile colposcope holds promise for use in low-resource settings.

9699-7, Session 3

Mechanical and optical behavior of a tunable liquid lens using a variable cross section membrane

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The use of constant thickness membranes in optofluidic lenses has been extensively studied; however, the information about liquid lenses with membranes of varying thickness is limited. We present simulation and experimental results of the mechanical and optical behaviors of a tunable liquid lens with a variable cross section membrane, by impinging collimated light in the system. The liquid lens consists of three elements: a thin flat glass, a silicone rubber membrane (plane – concave) and some liquid between them. The volume and pressure of the liquid are increased inside the lens cavity, causing membrane deformation, and thus, variations in the focal length of the lens. Experimental data were obtained from stress–strain tests of silicone rubber according to the ASTM D638 standard, to perform the simulation of the membrane mechanical behavior. We obtained the profiles and the algebraic expressions of the surface of the membrane for different pressures and the values of spherical aberration vs. pressure. The membrane design and its mechanical simulation were developed using Solidworks software; the spherical aberrations and optical characteristics of the lens were obtained with the OSLO software.

9699-8, Session 3

Development of a miniature multiple reference optical coherence tomography imaging device

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Multiple reference optical coherence tomography (MR-OCT) is a new technology ideally suited to low-cost, portable OCT imaging. This
technology is an extension of time-domain OCT with the addition of a partial mirror in front of the reference mirror. This enables extended, simultaneous depth scanning with the relatively short sweep of a miniature voice coil motor on which the scanning mirror is mounted. Applications of this technology include biometric security, ophthalmology, personal health monitoring and non-destructive testing.

This work details early-stage development of the first iterations of a miniature MR-OCT device. The initial device utilized a fibre-coupled input from an off-board superluminescent diode (SLD), while TO can-mounted and chip-on-submount SLD designs are also being investigated. Typical dimensions of the module are 40 mm by 60 mm, but future designs are expected to be more compact. Off-the-shelf miniature optical components, voice-coil motors and photodetectors are used, with the complexity of design depending on specific applications.

The photonic module can be configured as either polarized or non-polarized and can include balanced detection. The device also has an on-board transimpedance amplifier with complimentary outputs. Results show comparisons of the devices to bench-top MR-OCT and commercial SS-OCT systems.

Assembly of the photonic modules require extensive planning. In choosing the optical components, Zemax simulations are performed to model the beam characteristics. The layout procedure is modelled using Solidworks and each component is placed and aligned via a well-designed alignment procedure involving an active-alignment ‘pick-and-place’ automation system.

9699-9, Session 3

Wavelength scanning achieves pixel super-resolution in holographic on-chip microscopy

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We report, for the first time, a wavelength scanning based pixel super-resolution technique in lensfree holographic imaging to generate high-resolution reconstructions of the specimen from low-resolution (i.e., under-sampled) diffraction patterns recorded at multiple wavelengths within a narrow spectral range (e.g., 15-20 nm). Our wavelength scanning super-resolution approach can also be integrated with multi-height and/or multi-angle on-chip imaging techniques to obtain even higher resolution reconstructions, with an effective numerical aperture of 1. This new wavelength scanning based pixel super-resolution approach can provide competitive microscopy solutions for high-resolution and field-portable imaging needs, potentially impacting tele-pathology applications in resource-limited-settings.

9699-10, Session 4

Design of miniature mobile phone based cell incubator microscope for real-time fluorescence detection

Taerim Yoon, Kyujung Kim, Pusan National Univ. (Korea, Republic of)

The purpose of the cell culture is to look for a response to a variety of drugs and analysis biological phenomena via the studies of the cells. An incubator create an environment to growth and maintenance of biological samples. This device provides a suitable environment for the growth of biological samples through the control of the room temperature and CO2 concentration. In order to maintain this environment, an incubator is closed for biological samples are not affected by outer environment. A closed cell incubator is limited to real-time observation through the microscope because cell incubator is necessary to frequently open and close. Here we present a microscope for real-time observation based on the smartphone. The miniature mobile phone based cell incubator microscope was fabricated by using 3D design tools and 3D printer. Mimpi microscope is cost-effective compared to conventional microscope and this size is smaller than that. This smartphone-based microscope install in the incubator. Thus, Mimpi microscope and sample are not interference of the external environment during cell observation. Thus, we can be obtained a highly reliable measurement result of the sample. In this experiment, we can obtain high resolution real-time sample images. An obtain image is shown to Mimpi microscope user by external device which is connected to smartphone via wireless communication.

9699-11, Session 4

Difference among human normal, Barrett’s Dysplasia and Adenocarcinoma revealed by autofluorescence spectroscopy

Kenneth J. Zhou, Stony Brook Univ. (United States); Jun Chen, Tianjin Medical Univ. General Hospital (China)

Previous study of optical screening of cancer has shown that difference of native fluorescence spectra can be used to distinguish cancer tissue from normal tissue. The native fluorophores, such as NADH and FAD, are involved in the oxidation of fuel molecules, and therefore, direct monitoring of NADH fluorescence dynamically can interpret the metabolic activity of cells. Usually the metabolic rate of advanced metastatic cancer cells is greater than that of less advanced cancer cells, causing the effect known as hypoxia, which was found by Warburg.

The aim of the present research is to determine if the native fluorescence spectroscopy approach is effective to detect changes of fluorophore compositions among different types of human Esophagus tissues. Human Esophagus tissues, such as normal, Barrett’s, Dysplasia and Adenocarcinoma, were excited by the selective excitation wavelength of 351 nm laser. The contributions of principle biochemical components to fluorescence spectra from the tissue samples were investigated using the different non-negative constraint blind source separation methods. The higher relative content of NADH and lower collagen contents were observed in the Adenocarcinoma in comparison with the normal and Dysplasia tissues. This work shows the changes of relative contents of collagen and NADH obtained using native fluorescence spectroscopy may present potential criteria - for detecting different types of human Esophagus tissues.

9699-12, Session 4

Monitoring bacterial metabolic activity for food hygiene detection using NADH fluorescence

Kenneth J. Zhou, Stony Brook Univ. (United States); Jun Chen, Tianjin Medical Univ. General Hospital (China)

Food safety is a global health goal. Therefore, detection of food hygiene is the solution to the prevention and recognition of problems related to health and safety. For this purpose, a non-contact optical method to monitor bacterial metabolic activity to detect microbial pathogens in food using NADH fluorescence was proposed.

The native fluorescence (FL) spectra of different muscle foods stored at 4 °C (refrigerated) and 25 °C (at room temperature) were measured with the selected excitation wavelength of 340 nm as a function of storage time. The contributions of NADH and collagen to the FL spectra were unmixed using Spectral Fitting. The change of NADH contents was found that can be used to detect different types of human Esophagus tissues.
Circularly polarizer partially-coherent light is used to illuminate the synovial objective-lens FOV for point-of-care diagnostics of gout and pseudogout. This study presents the possibility that the change of NADH determined by native FL spectroscopy may be used as a “fingerprint” or criterion for monitoring the spoilage status of muscle foods by measures of bacterial metabolic activity based on the fluorescence of NADH.

Evaluation of a polarization sensitive multiple reference optical coherence tomography system

Sean O’Gorman, Paul M. McNamara, Roshan I. Dsouza, Kai Neuhaus, Hrebesch M. Subhash, National Univ. of Ireland, Galway (Ireland); Josh Hogan, Carol J. Wilson, Compact Imaging, Inc. (United States); Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Multiple reference optical coherence tomography (MR-OCT) is a new compact optical sensor platform based on a small form factor recirculating reference arm scanning optical delay, which promises a robust, cost-effective semi-solid state design for integration with next generation mobile devices. The miniature re-circulating optical delay is based on a voice coil motor (VCM) actuator and a partial mirror. Imaging capability of MR-OCT has been recently demonstrated using a non-polarizing bulk optics design and an unbalanced detection system with a sensitivity of up to 98dB at an axial rate of 1200 A-scans per second. In this study, we implemented a polarization based MR-OCT with balanced detection system and have gathered preliminary results in comparison with a non-polarizing MR-OCT with balanced detection. The polarization based MR-OCT can suppress the surface reflection from the sample and provide higher contrast, moreover the balanced detection scheme can improve the sensitivity by reducing common-mode noise of the system.
spectroscopic characteristics of transmitted light through relatively-pure plasma with high spatial and time resolution. Moreover, it is expected to improve quantitativity by integrating spectral characteristics obtained from interferograms with high visibility. We report measurement results of blood glucose level of rat’s whole blood with a prototype apparatus at the near-infrared region (wavelength : 900-1700 nm).

9699-17, Session 5

Phase-sensitive multiple reference optical coherence tomography
Roshan I. Dsouza, Hrebesh M. Subhash, Kai Neuhaus, National Univ. of Ireland, Galway (Ireland); Josh Hogan, Carol J. Wilson, Compact Imaging, Inc. (United States); Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Multiple reference OCT (MR-OCT) is a recently developed novel time-domain OCT platform based on a miniature reference arm optical delay, which utilizes a single miniature actuator and a partial mirror to generate recirculating optical delay for extended axial-scan range. MR-OCT technology promises to fit into a robust and cost-effective design, compatible with integration into consumer-level devices for addressing wide applications in mobile healthcare and biometry applications. Using conventional intensity based OCT processing techniques, the high-resolution structural imaging capability of MR-OCT has been recently demonstrated for various applications including in vivo human samples. In this study, we demonstrate the feasibility of implementing phase based processing with MR-OCT for various functional applications such as Doppler imaging and sensing of blood vessels, and for tissue vibrography applications. The MR-OCT system operates at 1310nm with a spatial resolution of ~26 µm and an axial scan range of 600Hz. Initial studies show a displacement-sensitivity of ~20 nm to ~120 nm for the first 1 to 9 orders of reflections, respectively with a mirror as test-sample. The corresponding minimum resolvable velocity for these orders are ~2.3 µm/sec and ~15 µm/sec respectively. Data from a chick chorioallantoic membrane (CAM) model will be shown to demonstrate the feasibility of MR-OCT for imaging in-vivo blood flow.

9699-18, Session 5

The impact of relative intensity noise on the signal in multiple reference optical coherence tomography
Kai Neuhaus, Hrebesh M. Subhash, Roshan I. Dsouza, National Univ. of Ireland, Galway (Ireland); Josh Hogan, Carol J. Wilson, Compact Imaging, Inc. (United States); Martin J. Leahy, National Univ. of Ireland, Galway (Ireland); Svetlana Slepneva, Guillaume Huyet, Centre for Advanced Photonics & Process Analysis (Ireland)

Multiple reference optical coherence tomography (MR-OCT) applies a unique low-cost solution to enhance the scanning depth of standard time domain OCT by inserting an partial mirror into the reference arm of the interferometric system. This novel approach achieves multiple reflections for different layers and depths of an sample with minimal effort of engineering and provides an excellent platform for low-cost OCT systems based on well understood production methods for micro-mechanical systems such as CD/DVD pick-up systems. The direct integration of a superluminescent light-emitting diode (SLED) is a preferable solution to reduce the form-factor of MR-OCT because of their compact size (high throughput with many imagers per incubator) and sub-micron resolution over cm². Several presented systems uses digital holography to recover phase and amplitude of a sample. To avoid speckle, most of the systems use incoherent light sources positioned many centimeters away from the sample and camera chip to record interferograms.

Here, we demonstrate an ultra-compact illumination system for a lensless phase imager. The device is composed of a side illumination system that uses a Dove prism onto which a photopolymer film is laminated on one side. An array of light sources is set along one slanted side of the prism and several analog hologram gratings are recorded in the photopolymer film to redirect the light out of the prism with different specific beam angles. The sample can thus be illuminated with different directions and a camera placed behind the sample records the inline holograms. For each illumination direction, one digital inline hologram is recorded with the camera. The stack of images is then processed in a reconstruction using Gerchberg-Saxton and tomographic type algorithm. The quantitative phase and amplitude of the samples are reconstructed with subpixel resolution. The side illumination system effectively folds one axis (z axis) which creates a flat lensless imaging system.

9699-19, Session 5

Flat lensless phase imager
Manon Rostykus, Christophe Moser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Compact lensless optical imaging systems are mainly applied in microscopy for biological samples. Indeed, they are well suited as imagers inside cell incubators because of their compact size (high throughput with many imagers per incubator) and sub-micron resolution over cm². Several presented systems uses digital holography to recover phase and amplitude of a sample. To avoid speckle, most of the systems use incoherent light sources positioned many centimeters away from the sample and camera chip to record interferograms.

Here, we demonstrate an ultra-compact illumination system for a lensless phase imager. The device is composed of a side illumination system that uses a Dove prism onto which a photopolymer film is laminated on one side. An array of light sources is set along one slanted side of the prism and several analog hologram gratings are recorded in the photopolymer film to redirect the light out of the prism with different specific beam angles. The sample can thus be illuminated with different directions and a camera placed behind the sample records the inline holograms. For each illumination direction, one digital inline hologram is recorded with the camera. The stack of images is then processed in a reconstruction using Gerchberg-Saxton and tomographic type algorithm. The quantitative phase and amplitude of the samples are reconstructed with subpixel resolution. The side illumination system effectively folds one axis (z axis) which creates a flat lensless imaging system.

9699-20, Session 6

A mobile telemedicine approach for early diagnosis of oral cancer in resource limited settings (Invited Paper)
Radhika Chigurupati D.D.S., Boston Univ. (United States)

Oral Cancer is major public health problem in the world, with the Indian sub-continent accounting for one-quarter of the new incident cases (80,000 of 270,000) annually. Over 60% of the oral cancer cases in Low and Middle-Income Countries (LMIC) are diagnosed at an advanced stage. Therefore, the overall survival rates of oral cancer patients in LMIC is abysmal at 20-25% lower than that reported for patients in developed countries. The reasons for delay in diagnosis are complex and affected by numerous factors including the provider, the patient and the healthcare infrastructure and delivery model. Early diagnosis of oral cancer requires an effective screening method. At present, the most cost-effective method for screening of oral cancer is by visual inspection of the oral cavity by a community health workers or oral healthcare providers. To make this method more effective, we proposed the diagnosis of oral cancer by harnessing the potential of a smart phone application. A custom application for oral cancer screening on a mobile device has been programmed based on clinical characteristics and behavioral risk factor history to aid health workers and dental students to diagnose premalignant and malignant oral lesions. The eMOCHA framework supports, images, location-based data, text via a client smartphone. A web-based application allows a remote specialist to retrieve the patient data sent by the health worker to make a diagnosis and further recommendations regarding the need for biopsy or follow up or intervention by a specialist. As a next step, we propose to refine the mobile application for diagnosis of oral cancer using an intelligent image analysis algorithm to make the recommendations at the point of care.
Comparison of performance of mobile and traditional colposcopy in high- and low-resource settings

Octavio A. Villalobos-Méndez, Centro Médico Pro Salud (Mexico); Bruce Kahn M.D., Scripps Clinic Medical Group (United States); Sonia Contreras, International Community Foundation (United States); Marta M. Madiedo-Camargo, Centro Médico Pro Salud (Mexico); David Levitz, MobileODT Ltd. (Israel)

Cervical cancer is the leading cause of cancer death for women in the developing world. Much of this tragedy results from a lack of resources needed to implement the standard of care in OECD countries of screening, followed by colposcopy and guided biopsy. One key bottleneck in the process is the lack of available coloscopes. Another issue is uncertainty about the reliability of colposcopy in low resource settings. Unfortunately, well-controlled trials that compare colposcopy at high resource and low resource settings are lacking.

A smartphone-based mobile colposcope was recently developed for use in low resource settings. The mobile colposcope is undergoing clinical validation in a multi-center non-inferiority trial that is taking place in a high resource site (Scripps clinic, San Diego, CA) and a low resource site (ProSalud clinic, Tijuana, Mexico). In this paper, the performance of both the mobile colposcope and traditional colposcope from the high resource site are compared to the performance at the low resource site.

The trial is designed as a paired, multi-reader multi-case non-inferiority trial, with n=50 patients at each site (100 total), and m=8 reviewers. Patients referred to colposcopy following abnormal screening tests were recruited, and imaged with both a mobile colposcope and a traditional colposcope. Excisional biopsies were taken from suspect sites; if no suspect sites were found, a random biopsy or endocervical curettage were taken. Biopsy results were considered as truth. Images were analyzed by reviewers, and were rated both in terms of lesion severity, and in terms of sites suitable for biopsy.

Field-testing of a cost-effective mobile-phone based microscope for screening of Schistosoma haematobium

Hatice Ceylan Koydemir, Univ. of California, Los Angeles (United States); Isaac I. Bogoch, Univ. of Toronto (Canada); and Toronto General Hospital (Canada); Derek Tseng, Univ. of California, Los Angeles (United States); Richard K. D. Ephraim, Evans Duah, Univ. of Cape Coast (Ghana); Joseph Tee, Volta River Authority (Ghana); Jason R. Andrews, Stanford Univ. (United States) and Stanford School of Medicine (United States); Aydogan Ozcan, Univ. of California, Los Angeles (United States) and California NanoSystems Institute (United States)

Schistosomiasis is a parasitic and neglected tropical disease, and affects >200-million people across the world, with school-aged children disproportionately affected. Here we present field-testing results of a handheld and cost effective smartphone-based microscope in rural Ghana, Africa, for point-of-care diagnosis of S. haematobium infection. In this mobile-phone microscope, a custom-designed 3D printed opto-mechanical attachment (~150g) is placed in contact with the smartphone camera-lens, creating an imaging-system with a half-pitch resolution of ~0.87µm. This unit includes an external lens (also taken from a mobile-phone camera), a sample tray, a z-stage to adjust the focus, two light-emitting-diodes (LEDs) and two diffusers for uniform illumination of the sample. In our field-testing, 60 urine samples, collected from children, were used, where the prevalence of the infection was 72.9%. After concentration of the sample with centrifugation, the sediment was placed on a glass-slide and S. haematobium eggs were first identified/quantified using conventional benchtop microscopy by an expert diagnostician, and then a second expert, blinded to these results, determined the presence/absence of eggs using our mobile-phone microscope. Compared to conventional microscopy, our mobile-phone microscope had a diagnostic sensitivity of 72.1%, specificity of 100%, positive-predictive-value of 100%, and a negative-predictive-value of 57.1%. Furthermore, our mobile-phone platform demonstrated a sensitivity of 65.7% and 100% for low-intensity infections (≤50 eggs/10 mL urine) and high-intensity infections (>50 eggs/10 mL urine), respectively. We believe that this cost-effective and field-portable mobile-phone microscope may play an important role in the diagnosis of schistosomiasis and various other global health challenges.
The study of biological specimens onboard compact Lab-on-a-Chip (LoC) platforms with embedded label-free, quantitative, 3D imaging functionalities is highly demanded. Here we introduce a novel imaging modality, named Space-Time Scanning Interferometry (STSI), which synthesizes space-time interferograms with intriguing features. Indeed, a single linear sensor array is sufficient to build up a synthetic interferogram with unlimited Field of View (FoV) along the scanning direction and reduced noise. If a small subset of detector lines are selected, synthetic interferograms can be obtained, shifted each other of the desired phase step allowing phase retrieval. The STSI principle is well-suited to be applied in all the cases where the object motion is an intrinsic feature of the system, e.g. in case of microfluidics, so that the advantages of STSI have no cost associated with. Starting from these considerations, we applied the STSI method to in-flow on-chip microscopy of biological samples. Out-of-focus recordings are performed using a single line detector and polymeric micro-lenses embedded onboard chip, in order to synthesize a Space-Time Digital Hologram (STDH) carrying full-field, 3D information of the flowing samples. We discuss the method and prove that a STDH still maintains all the advantageous capabilities of DH microscopy. The throughput of the imaging system is dramatically increased as STDH provides unlimited FoV, refocusable imaging of samples flowing inside a liquid volume with no need for hologram stitching. The trade-off between sample magnification and FoV is thus overcome and becomes possible to move a huge step toward the integration of the imaging functionalities onboard chip for high-throughput rapid diagnostics.

**Initial clinical testing of a multi-spectral imaging system built on a smartphone platform**

Jonah Mink, MobileODT Ltd. (Israel) and Univ. of Pennsylvania (United States); Bruce Kahn M.D., Scripps Clinic Medical Group (United States); Charles Hummel, Danielle Burkland, Univ. of Pennsylvania (United States); Leigh Cataldo, Scripps Clinic Medical Group (United States); David Levitz, MobileODT Ltd. (Israel)

Multi-spectral imaging systems are often expensive and bulky. As such, they seldom reach the patients who need it most, many of which live outside OECD countries where there is little access to advanced biophotonics imaging systems. An innovative multi-spectral imaging system was fitted onto a mobile colposcope, an imaging system built around a smartphone in order to image the uterine cervix from outside the body. The multi-spectral mobile colposcope (MSMC) acquires images at different wavelengths. Those frames are then processed with a set of transformations and by curve fitting to a theoretical model, resulting in maps of the main absorbers and scatterers in the imaged tissue.

This paper presents the clinical testing of MSMC imaging (the technical validation of the MSMC system is presented in another paper). Patients who were referred to colposcopy following abnormal screening test (Pap or HPV DNA test) according to the standard of care were enrolled. Multi-spectral image sets of the cervix were acquired, consisting of images from the various wavelengths. Image acquisition took 1-2 sec. Areas suspected for dysplasia under white light imaging were biopsied, according to the standard of care. Biopsied sites were recorded on a clockface map of the cervix. Following the procedure, MSMC data was processed from the sites of biopsied sites. The histopathological assessment was correlated to parameters measured from the spectral fit, including oxy- and deoxy-hemoglobin concentrations, and scatter size distribution parameters. The result suggests MSMC holds promise for cervical imaging in low resource settings.

**A game-based crowd-sourcing platform for rapidly training middle and high school students to perform biomedical image analysis**

Steve W. Feng, Min-jae Woo, Hannah Kim, So Jung Ki, Lei Shao, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

We have developed an easy-to-use and widely accessible crowd-sourcing tool for rapidly training humans to perform biomedical image diagnostic tasks and demonstrated this platform's ability on middle and high school students in South Korea to diagnose malaria infected red blood cells (RBCs) using Giemsa-stained thin blood smears imaged under light microscopes. We previously used the same platform (i.e., BioGames) to crowd-source diagnostic performances of individual RBC images, marking them as malaria positive (infected), negative (uninfected), or questionable (insufficient information for a reliable diagnosis). Using a custom-developed statistical framework, we combined the diagnoses from both expert diagnosticians and the minimally trained human crowd to generate a gold standard library of malaria-infection labels for RBCs. Using this library of labels, we developed a web-based training and educational toolset that provides a quantified score for diagnosticians/users to compare their performance against their peers and view misdiagnosed cells. We have since demonstrated the ability of this platform to quickly train humans without prior training to reach high diagnostic accuracy as compared to expert diagnosticians. Our trial group of middle and high school students has collectively played more than 170 hours, each demonstrating significant improvements after only 3 hours of training games, with diagnostic scores that match expert diagnosticians'. We plan to further demonstrate this tool's effectiveness for other diagnostic tasks involving image labeling and aim to provide an easily-accessible and quickly adaptable framework for online training of new diagnosticians.
StressPhone: smartphone enabled detection of stress related salivary biomarkers

Aadhar Jain, Elizabeth Rey, Seoho Lee, Dakota O'Dell, David Erickson, Cornell Univ. (United States)

Anxiety disorders are estimated to be the most common mental illness in US affecting around 40 million people and related job stress is estimated to cost US industry up to $300 billion due to lower productivity and absenteeism. A personal diagnostic device which could help identify stressed individuals would therefore be a huge boost for workforce productivity. We are therefore developing a point of care diagnostic device that can be integrated with smartphones or tablets for the measurement of cortisol - a stress related salivary biomarker, which is known to be strongly involved in body’s fight-or-flight response to a stressor (physical or mental). The device is based around a competitive lateral flow assay whose results can then be read and quantified through an accessory compatible with the smartphone. In this presentation, we report the development and results of such an assay and the integrated device. We then present the results of a study relating the diurnal patterns of cortisol levels and the alertness of an individual based on the circadian rhythm and sleep patterns of the individual. We hope to use the insight provided by combining the information provided by levels of stress related to chemical biomarkers of the individual with the physical biomarkers to lead to a better informed and optimized activity schedule for maximized work output.

Evaluation of PpIX formation in Cervical Intraepithelial Neoplasia I (CIN) using widefield fluorescence images

Fernanda M. Carbinatto, Natâlia M. Inada, Thereza C. Fortunato, Univ. de São Paulo (Brazil); Wellington Lombardi M.D., Eduardo V. da Silva, UNIARA (Brazil); José D. Vollet-Filho, Cristina Kurachi, Sebastião Pratavieira, Vanderlei S. Bagnato, Univ. de São Paulo (Brazil)

Optical techniques has been described as auxiliary technology for screening of neoplasia because shows the potential for tissues differentiation in real-time and it is a noninvasive and safe. However, only endogenous fluorophores present in the lesion may be insufficient and needed of the administration of the fluorophores synthesized, such as, the precursor molecule of protoporphyrin IX (PpIX) induced by 5-aminolevulinic acid (ALA) and your derivatives (MAL). Topical application of MAL, induces formation of PpIX through of the endogenous photosensitizer PpIX in tissues where carcinogenesis has begun. The PpIX tend to accumulate in premalignant and malignant tissues and the illumination with light with appropriate wavelength beginning to excitation of PpIX fluorescence, which helps to localize PpIX-rich areas and identify potentially malignant tissues. The aim of the study is to evaluate the production of PpIX in the cervix with Cervical Intraepithelial Neoplasia grade I (CIN I) through of the fluorescence images captured after an hour of cream application. It was possible to visualize PpIX fluorescence in cervix and to observe the selectivity in fluorescence in squamocolumnar junction, which a pre-cancerous condition (CIN) usually is localized. Through the image processing it was possible to identify potentially malignant tissues by difference of fluorescence by intensity of the matrix pixels. Fluorescence images obtained allowed the differentiation in vivo of the fluorescence of the PpIX in cervix with CIN.

Melanoma detection using a mobile phone app

Luciano Elias-Diniz, Karin M. Ennser, Swansea Univ. (United Kingdom)

According to the American Cancer Society, the number of melanoma cases has greatly increased in the last 30 years. In 2015, it is estimated that it will cause 9,440 deaths and 73,870 new cases will be diagnosed in the USA. As other types of cancer, the efficiency of the treatment and the survivability is heavily dependent on the stage it is discovered. One method of diagnosis, the ABCDE signs (asymmetry, border irregularity, color, diameter and evolution) can be very helpful in detecting melanin moles. Even with their recognition depending on subjective patterns, a computer can be used to detect them. With this aim, a lightweight Android app was devised to identify melanoma signs in moles photos and help doctors in isolated areas, with no access to sophisticated equipment, to diagnosis this disease. Basically, the software classifies the moles into benign or malign using a score based system. It first filters part of the hair in the image, detect the nevus border and then proceed to calculate its area, perimeter, shape factor (which is related to border irregularity) and colours present. This data is then compared with expected values, obtained from a melanoma database from Pedro Hispano Hospital. Finally, the app displays the results in the screen, indicating whether the mole is malign and showing the corresponding binary image, which can be saved for monitoring the case. When used on a test group, the software could detect all malign cases correctly, and showed an accuracy of 80% for benign cases.

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Multiple reference optical coherence tomography (MR-OCT) is a unique low-cost version of time-domain OCT (TD-OCT) based on a miniature reference arm optical delay, which utilizes a single miniature actuator and a partial mirror to generate recirculating optical delay for extended axial-scan range. Although the signal-to-noise ratio for TD-OCT was shown not to be better compared to spectral-domain OCT or swept-source OCT systems, reconstructed images recorded with MR-OCT suggest sufficient or even competitive image quality comparable to commercial systems. In this manuscript a signal processing system is presented that allows for easy evaluation of multiple processing modules, such as filters, to be able to quantify systematically the effect on the image quality.

The results of the evaluation of different filter types and the effects due to limiting parameters such as amount of filter orders or taps, and reduced sample frequency are compared and discussed. Reducing the demand in hardware resources is a desirable goal for low-cost user platforms, such as smart-phones or tablet computers, and improved understanding of algorithms provide the potential to achieve real-time processing for MR-OCT image reconstruction.

9699-33, Session PSun

**Potential applications of Near Infrared auto-fluorescence spectral polarized imaging for assessment of food quality and safety**

Kenneth J Zhou, Stony Brook Univ. (United States); Jun Chen, Tianjin Medical Univ. General Hospital (China)

The current growing low production costs and high efficiency in the food industry need for maintenance of high-quality standards and assurance of food safety while avoiding liability issues. Quality and safety of food depend on physical (texture, color, tenderness etc.), chemical (fat content, moisture, protein content, pH, etc.), and biological (total bacterial count etc.) features.

There is a need for a rapid (less than a few minutes) and accurate detection system in order to optimize quality and assure safety of food. The fluorescence ranges for known fluorophores are limited to ultraviolet emission bands, which are not in the tissue near infrared (NIR) “optical window”. Biological tissues excited by far-red or NIR light would exhibit strong emission in spectral range of 650-950 nm although no characteristic peaks show the emission from which known fluorophores. The characteristics of the auto-fluorescence emission of different types of tissues were found to be different between different tissue components such as fat, high quality muscle food.

In this paper, NIR auto-fluorescence emission from different types of muscle food and fat was measured. The differences of fluorescence intensities of the different types of muscle food and fat emissions were observed. These can be explained by the change of the microscopic structure of physical, chemical, and biological features in meat. The difference of emission intensities of cancerous and normal prostate tissues was applied to monitor food quality and safety using spectral polarized imaging, which can be detect deep depth fat under the muscle food up to several centimeter.
Development of a reference platform for the characterization of liquid phantoms (Invited Paper)

Paul Lemailliet, David W. Allen, Jeeseong C. Hwang, National Institute of Standards and Technology (United States)

Liquid biomedical phantoms are commonly used as reference for the biomedical optics community. The National Institute of Standards and Technology (NIST) is currently developing a reference instrument aimed at measuring the optical properties of solid and liquid reference phantoms from the visible to near infrared. The instrument is a double-integrating sphere (DIS) setup with tunable light source composed of a broadband laser-driven light source coupled to a monochromator. The optical properties of the sample are computed from the measurements of the total reflectance and total transmittance in the continuous wave domain. The data is analyzed by a custom inversion procedure based on Prahl’s adding-doubling algorithm allowing the computation of the uncertainty budget of the measurement results. In this talk we will present the measurements of the optical properties of liquid phantoms composed of Intralipid and India ink. The phantoms are contained in a liquid cell equipped with optical flats. The optical properties of the phantoms are compared to measurements of identical phantoms by dark field microscopy.

Development of breast cancer tissue phantoms for terahertz imaging

Alec Walter, Tyler Bowman, Magda El-Shenawee, Univ. of Arkansas (United States)

The aim of this work is to find robust phantoms which would be viable for use in pulsed terahertz imaging of breast cancer margins. While most frequency ranges have a robust number of proven imaging phantoms, the terahertz range is fairly new and lacks proven phantoms for a majority of tissue types. In order to accurately mimic tumor margins, phantoms have been developed for the three main breast tissue types: fatty, fibrous and cancerous. Water dispersed emulsions consisting of varying amounts of olive oil and a neutral detergent are solidified using a common phantom compound known as TX151. These compounds are characterized using terahertz transmission spectroscopy to obtain the frequency dependent refractive index and absorption coefficient over a range from 0.15 to 2 terahertz. These properties have been compared to known values for each of the three tissue types obtained from freshly excised breast cancer tissue reported in the literature. Using this method, phantoms which closely matched their respective fresh tissues were developed for both cancerous and fibrous tissues. Since both the refractive index and absorption coefficient of the fatty tissue are relatively low, this emulsion method was unable to be used. Instead, commercially available emulsions were tested out of which one was found whose optical properties allowed it to work as a phantom for fatty breast tissue. These three phantoms have allowed for a progression in terahertz assessment of breast tumor margins in controlled lab experiments.

Characterization of homogeneous tissue phantoms for performance tests in diffuse optics

Heidrun Wabnitz, Dieter Richard Taubert, Physikalisch-Technische Bundesanstalt (Germany); Tsukasa Funane, Masashi Kiguchi, Hitachi, Ltd. (Japan); Hideo Eda, The Graduate School for the Creation of New Photonics Industries (Japan); Antonio Pifferi, Politecnico di Milano (Italy) and CNR-Istituto di Fotonica e Nanotecnologie (Italy); Alessandro Torricelli, Politecnico di Milano (Italy); Rainer Macdonald, Physikalisch-Technische Bundesanstalt (Germany)

Solid turbid phantoms with homogeneous optical properties play an important role in performance assessment, validation and standardization of instruments for diffuse optical imaging and spectroscopy. Apart from serving as test objects for the quantification of the reduced scattering and absorption coefficients, they can mimic the diffuse attenuation in tissues. Basic requirements are a realistic attenuation for a given source-detector geometry as well as a nearly Lambertian angular characteristic of the outgoing radiation.

In this contribution we focus on homogeneous slab phantoms of a few cm thickness that are employed, in particular, to assess the responsibility of the detection system of instruments in diffuse optics and to support standardized performance tests of functional near-infrared spectroscopy devices. We present three methods to quantify the wavelength-dependent diffuse transmittance, relying on (1) measurement of radiance exiting the phantom by a detector far from the exit aperture, (2) simple recording of radiance by a power meter close to the exit aperture and correction for the finite distance between phantom surface and detector, (3) determination of the reduced scattering and absorption coefficients by time-resolved diffuse transmittance and reflectance measurements and forward calculation of the time-integrated diffuse transmittance based on the diffusion model. The implications of the different approximations related to these approaches are discussed. First experimental results were found to be consistent. All methods were applied to characterize slab phantoms made of various materials, including epoxy resin with added scattering and absorbing materials as well as polyoxymethylene (POM) materials.

Low-cost tissue simulating phantoms with tunable, wavelength-dependent scattering properties

Rolf B. Saager, Alan Quach, Rebecca A. Rowland, Melissa L. Baldado, Adrien Ponticorvo, Anthony J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

Tissue-simulating phantoms provide the opportunity to evaluate the performance of optical and spectroscopic instruments under controlled experimental conditions. Recent efforts have advanced phantom fabrication methods to provide more tissue realistic phantoms, both in terms of a) incorporating absorbing agents that more faithfully mimic in vivo tissue chromophores spanning visible and near infrared regimes and b) accounting for multi-layer tissue structures with distinct optical properties. The spectral scattering properties in these phantoms, however, are typically based only on a single scattering agent, thereby lacking the spectral scattering
3D-printed phantom for the characterization of non-uniform rotational distortion

Geoffrey Hohert, Hamid Pahlevaninezhad, Anthony Lee M.D., Pierre M. Lane, BC Cancer Agency Research Ctr. (Canada)

Endoscopic catheter-based imaging systems that employ a 2-dimensional rotary or 3-dimensional rotary-pullback scanning mechanism require constant angular velocity at the distal tip to ensure correct angular registration of the collected signal. Non-uniform rotational distortion (NURD) – often present due to a variety of mechanical issues – can result in inconsistent position and velocity profiles at the tip, limiting the accuracy of any measurements. Since artifacts like NURD are difficult to identify and characterize during tissue imaging, phantoms with well-defined patterns have been used to quantify position and/or velocity error. In this work we present a fast, versatile, and cost-effective method for making fused deposition modeling 3D printed phantoms for identifying and quantifying NURD errors along an arbitrary user-defined pullback path. Eight evenly-spaced features are present at the same orientation at all points on the path such that deviations from expected geometry can be quantified for the imaging catheter. The features are printed vertically and then folded together around the path to avoid issues with printer head resolution. This method can be adapted for probes of various diameters and for complex imaging paths with multiple bends. We demonstrate imaging using the 3D printed phantom with a 1mm diameter rotary-pullback OCT catheter and system as a means of objectively evaluating the mechanical performance of similarly constructed probes.

9700-7, Session 2

Customized three-dimensional printed optical phantoms with user-defined absorption and scattering

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The use of reliable tissue-simulating phantoms spans multiple applications in optical spectroscopy and imaging, including device calibration and testing of new imaging procedures and modalities. Three-dimensional (3D) printing may significantly expand the ability to fabricate optical phantoms with arbitrary geometries and spatially varying optical properties, allowing for increasingly realistic and physiologically relevant optical phantoms. We demonstrate here 3D printed tissue-simulating phantoms with customized absorption (\(\mu_a\)) and reduced scattering (\(\mu_s'\)) coefficients. This was accomplished through the incorporation of nigrosin, an absorbing dye, and titanium dioxide (TiO2), a scattering agent, to the 3D printable thermoplastic acrylonitrile butadiene styrene (ABS). A physiologically relevant range of \(\mu_a\) and \(\mu_s'\) was demonstrated through a 3D-printed matrix of cubes, each made with customized filament with different concentrations of nigrosin and TiO2. Repeatability was demonstrated within 11.2% for \(\mu_a\) and 70.4% for \(\mu_s'\). Additionally, a tissue-simulated mouse phantom, which mimicked the geometry and optical properties of a live mouse with an implanted xenograft tumor, was printed using two different custom filaments and dual extrusion 3D printing. One filament had optical properties corresponding to normal tissue and the other corresponded to tumor tissue; the 3D printed tumor’s absorption and reduced scattering matched the live tumor within 4% at wavelengths of 659nm and 731nm. Inexpensive hobby-level 3D printers and filament extruders were used for all experiments. 3D printing with custom filaments and user defined optical properties may allow for more complex and physiologically relevant optical phantoms over multiple spatial scales.
A three-dimensional printed phantom for conjoined twins separation surgery

Shuwei Shen, Zhu Hua Zhao, Yilin Han, Guang Li Liu, Erbao Zhang, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Surgical planning is critical for the successful outcome in complicated surgeries, such as the separation of conjoined twins. This article introduces a method to fabricate a conjoined twins phantom mimicking the structure and mechanical properties of the twins’ organs, which can help doctors to develop a better separation surgery plan. 3D models were reconstructed from CT scans data of conjoined twins and these models were used as the source file to fabricate organs. The skeletons were fabricated using a 3D printer using translucent light-curable materials, while other organs were fabricated in a pouring method with silica in different proportions, and using UV-curable ink based phantoms and PDMS based phantoms. Different material properties of the phantoms are adjusted by adding titanium dioxide powder and carbon black particles, respectively. Different material proportions of CT contrast materials were added when pouring. CT scans of the fabricated phantom were compared with that of the source model to verify the structure and position accuracy. The mechanical properties of the organs in the phantom were also tested and modified to bring them closer to the corresponding parts in body. The phantom tissues were transparent, to help doctors clearly get a view of the internal distribution of different organs and helping doctors determine the separation path. Meanwhile, mechanical similarity could help doctors get a more realistic feeling during surgery exercises to improve the success rate of the surgery.

A vertical double integrating sphere system for optical characterization of phantom materials in 3D printing

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Accurate detection of material optical properties plays a key role in many biomedical optical applications. Characterization of absorption and scattering properties of biologic tissue enables 3D printing of standard tissue-simulating phantoms for medical spectral device calibration. Conventional double integrating sphere systems have several limitations and are not suitable for optical characterization of liquid or soft materials used in 3D printing. We propose a vertical double integrating sphere system and the associated reconstruction algorithm for optical characterization of phantom materials that simulate different human tissue components. The system characterizes absorption and scattering properties of silicone based phantoms, UV-curable ink based phantoms and PDMS based phantoms in the wavelength range from 400 nm to 1200 nm. The absorption and scattering properties of the phantoms are adjusted by adding titanium dioxide powder and carbon black particles, respectively. Different material compositions are added in the phantoms and characterized by the vertical double integrating sphere system in order to simulate the human tissue properties. Our preliminary test results suggest that the vertical integrating sphere system is able to characterize optical properties of 3D printing materials without precipitation effect of the liquid samples or wrinkling effect of the soft phantoms during the optical measurement.
Quantitative assessment of hyperspectral imaging in detection of plasmonic nanoparticles: a modified contrast-detail analysis approach

Jianting Wang, U.S. Food and Drug Administration (United States); Yu Chen, Univ. of Maryland, College Park (United States); T. Joshua Pfefer, U.S. Food and Drug Administration (United States)

Hyperspectral reflectance imaging (HRI) is an emerging imaging modality being applied for clinical indications such as tissue oximetry and cancer detection based on endogenous biological constituents including plasmonic nanoparticles. However, there is currently a lack of standardized test methods for objective, quantitative evaluation of HRI systems. Contrast-detail analysis (CDA) is a phantom-based test method commonly used to evaluate medical imaging devices (e.g., mammography systems). We investigated a modified CDA method to quantify the detectability of gold nanoparticles by HRI systems. Silicone-based turbid phantoms containing micro-fluidic channels were developed for the CDA tests. Polydimethylsiloxane (PDMS) phantom materials were doped with chromophores and scatterers to achieve biologically relevant optical properties. Molds were used to produce cylindrical channels of diameters 0.3 to 1.65 mm and depths of 0.2 to 2 mm inside the phantoms. Channels were filled with a mixture of hemoglobin and concentrations of gold nanorods (GNR) and measured with our HRI system and the quantity of GNRs was solved with a spectral unmixing algorithm from the reflectance spectra. The lowest detectable concentration was determined as a function of inclusion size and depth. Results indicated nonlinear increases in estimated GNR quantity with channel diameter and actual GNR concentration, and a significant decrease in estimated GNR quantity with increasing channel depth. It is demonstrated that our modified CDA test method involving turbid microchannel phantoms can help to elucidate the combined performance of imaging devices and plasmonic nanoparticle contrast agents. This approach may be useful for performing clinical trial standardization and device re-calibration, thus ensuring quality control and clinical performance.

The component validation of direct diode 488nm lasers in BD AccuriTM flow cytometers

Wei P. Chen, BD Biosciences (United States); Ningyi D. Luo, Pavilion Integration Corp. (United States)

Background: The 488nm laser is the most important excitation light source of flow cytometry. The frequency-doubled diode 488nm lasers are used in this excitation in BD Accuri. For using cost effective lasers, we issue the test protocol to validate the component test for test of “BD AccuriTM Flow Cytometers 488nm using direct diode lasers to replace frequency-doubled diode laser.”

Methods: BD Bioscience issued the protocols of the laser component test on wavelength, power, power variation, noise, and polarization at the operation temperature range of cytometer. Pavilion Integration Corporation validated the test direct diode 488nm lasers based on the protocols of BD Biosciences. BD Bioscience test one of laser samples to further validate the test of Pavilion Integration Corporation.

Results: All pass (only show the results of power in this Abstract)

spec/SN [Test to PIC] (Test in BD)

power (mW) [16 C(31C)] (16 C(31C))
spec (>=) [20 (20)] (20 (20))

SN1858 [20.39 (20.26)] (20.56 (20.37))

Conclusion: The test results of the 488nm 20mW laser met the component requirements for BD AccuriTM Cytometer.

Dynamic thermal effects of epidermal melanin and plasmonic nanoparticles during photoacoustic breast imaging

Pejghan Ghassemi, Quanzeng Wang, T. Joshua Pfefer, U.S. Food and Drug Administration (United States)

Photoacoustic Tomography (PAT) employs high-power near-infrared (NIR) laser pulses to generate structural and functional information about deep subsurface chromophores in tissue. Such insights may facilitate detection of breast cancer – the most common cancer in women. PAT mammography has been the subject of extensive research, including techniques based on exogenous agents for PAT contrast enhancement and molecular specificity. However, photothermal safety risks of PAT due to strong absorptive chromophores such as epidermal melanin in highly pigmented skin and plasmonic nanoparticle agents have not been rigorously studied. We have used computational and experimental approaches to elucidate highly dynamic optical-thermal processes during PAT. A Monte Carlo model was used to simulate light propagation at 800 and 1064 nm in a multi-layer breast tissue geometry with a spherical tumor inclusion incorporating nanoparticles. Energy deposition results were then used in a bioheat transfer model to simulate temperature transients. Experimental measurements involved multi-layer hydrogel phantoms with inclusions incorporating gold nanoshells and nanorods. Phantom optical properties were measured using the inverse-adding-doubling technique. Thermal imaging was performed as phantoms were irradiated with an NIR optical parametric oscillator emitting 10 ns pulses. Scenarios using 10 Hz pulsed laser irradiation of breast tissue containing various nanoparticles concentrations were implemented experimentally and computationally. Laser exposure levels were based on ANSI/IEC limits. Surface temperature measurements were compared to corresponding simulation data. In general, the effect of highly pigmented skin showed greater effect on temperature rise than nanoparticles. Results provide key initial insights into light-tissue interactions impacting the safety and effectiveness of PAT.

Margin assessment of three-dimensional breast cancer phantoms using terahertz imaging

Tyler Bowman, Alec Walter, Magda El-Shenawee, Univ. of Arkansas (United States)

This work focuses on pulsed terahertz imaging for the application of surgical margin assessment of breast cancer. The process of a breast cancer patient undergoing partial mastectomy or lumpectomy in order to remove a tumor from the breast has become much more common. While a lumpectomy can have the same long-term survival rate as a mastectomy when successful, the assessment of the outside edge, or margin, of the removed tissue lump is critical. Terahertz imaging shows great potential for rapid margin assessment due to its ability to differentiate between cancerous and non-cancerous tissue and reasonable penetration depth. However, access to fresh samples in order to test an imaging methodology for terahertz is limited, thus the use of phantoms mimicking fresh breast cancer tissue is ideal. In this work, various tissue types and orientations are tested using
breast cancer phantoms to refine an imaging methodology that can detect breast cancer up to 0.5-1.0 mm from the edge of the sample. The depth of the cancer within the sample is estimated using time of flight analysis of the reflected peaks in the pulsed time domain signal. Breast tissue phantoms have been designed to resemble fresh infiltrating ductal carcinoma, fibroglandular tissue, and fatty tissue of the breast to accomplish this work. By using the phantoms in order to create ideal cases where the geometry of the sample can be controlled, it will then be possible to develop the imaging methodology to be utilized for fresh surgical sections.

9700-15, Session 4

From theory to practice: the broadening role of polydimethylsiloxane phantoms as an intermediary between model validation and instrument performance testing (Invited Paper)

Rolf B. Saager, Alan Quach, Gordon T. Kennedy, Bruce J. Tromberg, Anthony J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

Polydimethylsiloxane (PDMS) has been a popular medium to fabricate tissue simulating optical phantoms. Recently, its use has significantly expanded in instrument calibration and performance testing, validation of advanced models of light transport of complex tissue geometries and evaluation of novel measurement modalities. To meet these demands, fabrication methods of these optical phantoms have become more refined and its structure and constituent components (i.e. dyes and scattering agents) have evolved to better mimic optical properties of tissue spanning both visible and near infrared regimes. We present efforts at the Beckman Institute that address these challenges through PDMS phantoms.

9700-16, Session 4

Tissue-simulating phantoms for testing pretreatment planning of photodynamic therapy with optical flap

Gal Shafirstein, Emily Oakley, Brian Wrazen, Juliann Lajoie, Tyger Howell, David A. Bellnier, Roswell Park Cancer Institute (United States)

Background and Objective: Delivering a uniform and well controlled light dose to large and curved surfaces is a technical challenge in photodynamic therapy (PDT). Several groups have demonstrated that woven layers of optical fibers can be used to uniformly illuminate complex surfaces. However, in many cases there is a requirement to deliver a high PDT light dose while limiting exposure to nearby tissues, thus minimizing the adverse effects of treatment. For that reason, we modified a flexible, mesh-style brachytherapy applicator (Freiburg FlapTM) to deliver PDT light, i.e. “optical flap”. Pretreatment dosimetric verification of our optical flap required that we develop stable, large and flexible tissue-mimicking phantoms. Methods: Several pre-treatment planning schemas were devised for the optical flap. Flexible phantoms with different optical properties were created. The light dose distributions within the phantoms were characterized with digital imaging and dosimetry measurements. The deposited PDT dose distribution was evaluated by overlaying the optical flap with Photofrin-containing Lewis Lung carcinoma cultures in 96-well plates. Results: The tissue-mimicking phantoms can be used to quantify the light dose distribution from the optical flap. The pattern of tumor cell killing corresponded to light intensity measured using digital image analysis. Conclusions: The optical flap pretreatment plans can be tested in flexible phantoms mimicking optical properties of human tissue. In-vivo studies are warranted, to test the impact of blood flow and tissue oxygenation on the ability to deliver prescribed PDT dose to the target tissue.

9700-18, Session 4

Novel organosilicon phantoms as testing material for photoacoustic imaging

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Photoacoustic (PA) imaging systems are commercially available, and the biomedical applications based on photoacoustic effects are emerging in different fields. Indeed, there is a lack of reference protocols, based on dedicated and well characterized phantoms designed for semi-quantitative evaluation of photoacoustic system performance and for the assessment of the contrast enhancement provided by different contrast agents. Thus we designed, prepared and tested a polydimethylsiloxane (PDMS) phantom for photoacoustic applications with tuned properties and well defined geometry. The approach is straightforward and the system is particularly promising since it is stable for months, durable (reusable), and non-toxic during preparation. Furthermore, it did not show a signal response to the laser irradiation when filled with water and it allowed reaching lower detection limits than standard agar phantoms. Moreover, this phantom, with its exceptionally fine geometry (of the order of tens of micrometers), enables the estimation of the PA dynamic range and Signal to Noise ratio for specific contrast media and the intrinsic resolution achievable. By modifying the properties of the material, adding TiO2 and black ink, the optical absorption and scattering coefficients were tuned in order to be similar to those of biological tissues. Our results confirm that the PDMS phantoms can mimic the tissue properties within the scope of PA imaging. The PDMS phantom can become a particularly promising tool in the field of photoacoustics for the evaluation of the performance of a PA system and as a model of the structure of vascularized soft tissues.
Changes based on the intensity of the reflected light over a wide range of bacterial colonies. Spectral measurement can be useful to identify these or absence of the functional groups or due to new interactions in various under different conditions. These changes can happen due to the presence signatures which will vary with different types of bacteria and yeasts, and using optical measurement methods. Microbes are known to carry spectral Microbes exhibit various spectral characteristics which can be analyzed and correlate with different scatter sources in this end, we have developed a hyperspectral dark-field scatter imaging system to enable wide-field imaging of the sample’s scatter properties as a function of the illumination wavelength. Hyperspectral imaging techniques were implemented to image the spatial distribution of characteristic scattering spectra and to correlate them with different scatter sources in the sample. We will discuss promises and challenges of the hyperspectral dark-field scatter technique in assessing the properties of meso- and micro-scale scatter sources in biomimetic phantoms and tissues. We will also present progress in developing a simulation algorithm to evaluate scatter parameters from experimental results.

Hyperspectral scatter microscopy of tissue optical properties in support of quantitative clinical applications

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Spectral analysis of different bacteria and yeast colonies

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Microbes exhibit various spectral characteristics which can be analyzed using optical measurement methods. Microbes are known to carry spectral signatures which will vary with different types of bacteria and yeasts, and under different conditions. These changes can happen due to the presence or absence of the functional groups or due to new interactions in various bacterial colonies. Spectral measurement can be useful to identify these changes based on the intensity of the reflected light over a wide range of wavelengths from ultraviolet, to visible and infra-red. Recent studies have reported the use of the multi-spectral measurement to identify different bacterial colonies in food surfaces. However, the spectral signatures vary in each study as the experiments that are conducted have used different light sources and evaluated the reflected light at different exposure levels. It is important to accurately identify the presence of microbes for non-invasive and in-vivo diagnostic applications. This research study analyzed the spectral characteristics of Escherichia coli, Staphylococcus aureus and the yeast Candida albicans in petri dishes using a high resolution VIS-NIR spectrometer. The results show that there are unique spectral signatures for the microorganisms, probably reflecting the difference between prokaryotic and eukaryotic biology. The identification of spectral signatures will be useful in various applications such as clinical diagnosis and food quality assessment.

Spatially resolved diffuse reflectance spectroscopy of two-layer turbid media using a densely packed multi-pixel photodiode probe

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Diffuse reflectance spectroscopy is a well-established technique that quantifies tissue optical properties and enables non-invasive medical diagnostics [1]. Spatially resolved diffuse reflectance (SRDR) spectroscopy systems incorporate multiple detectors positioned at multiple distances with respect to the illumination source, which can be used to interrogate biological tissues with layered structures, such as the skin and cervix [2]. Each detector selectively collects diffusely back-reflected photons penetrating to different depths within the tissue, thus enabling depth-resolved optical tissue characterization [3]. Typical SRDR probes are fiber-based and incorporate 3-8 source-detector pairs, but lack dense (< 250 μm spatial resolution) measurement capability.

In this work, we conducted SRDR measurements on bi-layer PDMS skin tissue mimicking phantoms of varying top layer thicknesses (200 μm-1000 μm) and varying optical properties using our previously reported [4] concentric multi-pixel photodiode array (CMPA) probes. The CMPA probe was fabricated using standard microfabrication technology, with 24 semi-annular Si photodiodes (PD) positioned around a central illumination aperture. The illumination aperture center to PD center distances range from 1.4 mm to 3.5 mm. The CMPA is the most densely packed and semiconductor SRDR probe reported to date.

SRDR measurements were conducted on six bi-layer phantoms for wavelengths ranging from 350-850 nm with 20 nm step size. The SNRs of the measurements are above 40 dB. Signal contrasts between the single layer phantom and bi-layer phantoms with varying top layer thicknesses are as high as 80%. The signal contrast as a function of photodiode position informs about the layer thicknesses. The mean error between the Monte Carlo simulations and the experiment is less than 6.2%.

References:

9700-25, Session 6

Fiber optic microprobes with rare-earth-based phosphor tips for proton beam characterization

Arash Darafsheh, Andrew Soldner, Alireza Kassaei, Jarod C. Finlay, Univ. of Pennsylvania (United States)

We investigated feasibility of using fiber optics probes with rare-earth-based phosphor tips for proton beam radiation dosimetry. We designed and fabricated a fiber probe with submillimeter resolution based on TbF3 phosphors and evaluated its performance for measurement of proton beam including profiles and range. The fiber optic probe, embedded in tissue-mimicking phantoms, was irradiated with a clinical proton beam with energy of 180 MeV and the luminescence spectroscopy was performed by a CCD-coupled spectrograph to analyze the emission spectra of the fiber tip. In order to measure the spatial beam profile and percentage depth dose, we used singular value decomposition method to spectrally separate the phosphors ionoluminescence signal from the background Čerenkov radiation signal. The spectra of the TbF3 fiber probe showed characteristic ionoluminescence emission peaks at 489, 542, 586, and 620 nm. By using singular value decomposition we found the contribution of the ionoluminescence signal to measure the percentage depth dose in phantoms and compared that with measurements performed with ion chamber. We observed quenching effect at the spread out Bragg peak region, manifested as under-responding of the signal, due to the high LET of the beam. However, the beam profiles were not affected by the quenching effect. The experimental apparatus and spectroscopy method developed in this work provide a robust platform for characterization of proton-irradiated nanophosphor particles for ultralow fluence photodynamic therapy or molecular imaging applications.

9700-26, Session 6

A wearable infrared videopupillography with multi-stimulation of consistent illumination for binocular pupil response

Ou-Yang Mang, National Chiao Tung Univ. (Taiwan); Yi-Chun Tsai, Jin-Chern Chiou, Ting-Wei Huang, National Chiao Tung Univ. (Taiwan); Mei-Lan Ko, National Taiwan Univ. Hospital, Hsin-Chu Branch (Taiwan)

The pupil response to light can reflect various kinds of diseases which are related to physiological health. Pupillary abnormalities may be influence on people by autonomic neuropathy, glaucoma, diabetes, genetic diseases, and high myopia. In the early stage of neuropathy, it is often asymptomatic and difficult to detect even by ophthalmologists. In addition, the position of injured nerve can lead to unsynchronized pupil response for human eyes. In our study, we design the pupilometer to measure the binocular pupil response simultaneously. It uses the different wavelength of LEDs such as white, red, green and blue light to stimulate the pupil and record the process. Therefore, the pupilometer mainly contains two systems. One is the image acquisition system, it use the two cameras modules with the same external triggered signal to capture the images of the pupil simultaneously. The other one is the illumination system. It use the boost converter ICs and LED driver ICs to supply the constant current for LED to maintain the consistent luminance in each experiments for reduced experimental error. Furthermore, the four infrared LEDs are arranged nearby the stimulating LEDs to illuminate eyes and increase contrast of image for image processing. In our design, we success to implement the function of synchronized image acquisition with the sample speed in 30 fps and the stable illumination system for precise measurement of experiment.

9700-27, Session 6

Illumination-parameter adjustable and illumination-distribution visible LED helmet for low-level light therapy on brain injury

Pengbo Wang, Yuan Gao, Univ. of Electronic Science and Technology of China (China); Xiao Chen, Huazhong Univ. Of Science And Technology (China); Ting Li, Univ. of Electronic Science and Technology of China (China)

Low-level light therapy (LLLT) has been clinically applied. Recently, more and more cases are reported with positive therapeutic effect by using transcranial light emitting diodes (LEDs) illumination. Here, we developed a LLLT helmet for treating brain injuries based on LED arrays. We designed the LED arrays in circle shape and assembled them in multi-layered 3D printed helmet with water-cooling module. The LED arrays can be adjust to touch the head of subjects. A control circuit was developed to drive and control the illumination of the LLLT helmet. The software portion provides the control of on and off of each LED arrays, the setup of illumination parameters, and 3D distribution of LLLT light dose in human subject according to the illumination setups. This LLLT light dose distribution was computed by a Monte Carlo model for voxelized media and the Visible Chinese Human head dataset and displayed in 3D view at the background of head anatomical structure. The performance of the whole system was fully tested. One stroke patient was recruited in the preliminary LLLT experiment and the following neuropsychological testing showed obvious improvement in memory and executive functioning. This clinical case suggested the potential of this illumination-parameter adjustable and illumination-distribution visible LED helmet as a reliable, noninvasive, and effective tool in treating brain injuries.

9700-28, Session 6

Modelling and design of modified Wollaston prisms and the application in differential interference contrast microscopy

Site Zhang, Huying Zhong, Frank Wyrowski, Friedrich-Schiller-Univ. Jena (Germany)

Wollaston prisms and modified Wollaston prisms, which is interesting for various applications like optical metrology, topography of surfaces and biological imaging, has been theoretically studied and also been practically applied. The previous studies are mostly based on ray tracing analysis and, as a result, the information that can be obtained are somehow restricted. In this paper, we propose a geometric field tracing technique for the simulation of light propagation through Wollaston prisms. In geometric field tracing we seek for the solutions to Maxwell's equations under the geometrical optics Approximation. The solutions are given in terms of electromagnetic fields, and in this way all the properties of light as electromagnetic fields are retained. These properties includes optical path length (OPL), partial coherence, interference, polarization and so on. Using the proposed simulation technique, we present the simulation of a differential interference contrast (DIC) microscopy, in which the modified Wollaston prism is used as the key component for polarization manipulation.
Bessel beam scanning-laser optical projection tomography for 3D extended-depth cellular imaging

Dongli Xu, Leilei Peng, The Univ. of Arizona (United States)

Optical projection tomography (OPT) creates isotropic 3D imaging of tissue. Two approaches exist today: Wide-field OPT illuminates the entire sample and acquires projection images with a camera; Scanning-laser optical tomography (SLOT) generates the projection with a moving laser beam and point detector. SLOT has superior light collecting efficiency than wide-field optical tomography, making it ideal for tissue fluorescence imaging.

Regardless the approach, traditional OPT has to compromise between the resolution and the depth of view. In traditional SLOT, the focused Gaussian beam diverges quickly from the focused plane, making it impossible to achieve high resolution imaging through a large volume specimen. We report using Bessel beam instead of Gaussian beam to perform SLOT. By illuminating samples with a narrow Bessel beam throughout an extended depth, high-resolution projection images can be measured in large volume.

Under Bessel illumination, the projection image contains signal from annular-rings of the Bessel beam. Traditional inverse Radon transform of these projections will result in ringing artifacts in reconstructed imaging. Thus a modified 3D filtered back projection algorithm is developed to perform tomography reconstructing of Bessel-illuminated projection images. The resulting 3D imaging is free of artifact and achieved cellular resolution in extended sample volume.

The system is applied to in-vivo imaging of transgenic Zebrafish embryos. Results prove Bessel SLOT a promising imaging method in development biology research.

Handheld reflectance confocal endomicroscope for imaging of the oral cavity (Invited Paper)

Kristen Carlson Maitland, Texas A&M University (United States)

No Abstract Available

LED induced autofluorescence (LIAF) imager with eight multi-filters for oral cancer diagnosis

Ting-Wei Huang, Nai-Lun Cheng, National Chiao Tung Univ. (Taiwan); Ming-Hsui Tsai, China Medical Univ. (Taiwan); Jinchern Chiou, China Medical Univ. (Taiwan) and National Chiao Tung Univ. (Taiwan); Mang Ou-Yang, National Chiao Tung Univ. (Taiwan)

Oral cancer has become a serious health problem in many developing and developed countries. The simple oral visual screening by health workers can reduce 37,000 oral cancer deaths annually worldwide. However, the conventional oral examination with the visual inspection and the palpation of oral lesions is not an objective and reliable approach for oral cancer diagnosis, and it may cause the delayed hospital treatment for the patients of oral cancer or leads to the oral cancer out of control in the late stage. Therefore, a device with a highly sensitive and specific manner are developed for early diagnosis and treatment.

A portable LED Induced autofluorescence (LIAF) imager is developed by our group. It contained the multiple wavelength of LED excitation light and eight filters to capture ex-vivo oral tissue autofluorescence images. The advantages of LIAF imager compared to other devices for oral cancer diagnosis are that LIAF imager has a probe of L shape for fixing the object distance, protecting the effect of ambient light, and observing the blind spot in the deep port between the gums-gingiva and the lining of the mouth. Besides, the multiple excitation of LED light source can induced multiple autofluorescence, LIAF imager with eight filters and the higher magnification solution can detect the spectral images of multiple narrow bands. The prototype of a portable LIAF imager is applied in the clinical trials in Taiwan, and the analysis of the clinical trial has the significant differences between normal tissue and oral tissue under some cases.

Excitation-resolved wide-field fluorescence imaging of indocyanine green visualizes the microenvironment properties in vivo via solvatochromic shift

Jaedu Cho, Univ. of California, Irvine (United States); Chang-Seok Kim, Pusan National Univ. (Korea, Republic of); Gultekin Gulsen, Univ. of California, Irvine (United States)

Near-infrared fluorescence imaging (NIRF) is a powerful wide-field optical imaging tool that has a potential to visualize molecular-specific exogenous fluorescence agents, such as FDA approved Indocyanine Green (ICG), in thick tissue. Indeed, ICG is sensitive to biochemical environment such that it can be used to detect micro- or macroscopic environmental changes in tissue by solvatochromic shift that is defined by the dependence of absorption and emission spectra with the solvent polarity. For example, dimethyl sulfoxide (DMSO) is a very powerful drug carrier that can penetrate biological barriers such as the skin, the membranes, and the blood-brain-barrier. In presence of DMSO, ICG in tissue shows the excitation blue shift. However, NIRF imaging of microenvironment dependent changes of ICG has been challenging for the following reasons. First, the Stoke’s shift of ICG is too small to separate the excitation and emission spectra easily. Second, the solvatochromic shift of ICG is too small to be detected by conventional NIRF techniques. Last but not least, the multiple scattering in tissue degrades not only the spatial information but also the spectral contents by the red-shift. We developed a wavelength-swept laser-based NIRF system that can resolve the excitation shift of ICG in tissue such that DMSO can be indirectly visualized. We plan to conduct an in-vivo lymph-node drug-delivery study in a mouse model to show feasibility of the indirect imaging of the drug-carrier with the wavelength-swept-laser based NIRF system.

LED induced autofluorescence (LIAF) imager with eight multi-filters for oral cancer diagnosis, Amaan Mazhar, Pierre Khoury, Chris Campbell, David J. Cuccia, Modulated Imaging, Inc. (United States)

Quantitative assessment of tissue structure and function is one of the most challenging problems in biomedical imaging. A major component for successful commercialization of any technology is a set of design requirements that achieve a product that outputs reliable results. Our team has developed a robust and user-friendly optical imaging platform system based on Spatial Frequency Domain Imaging (SFDI) with the intent of wide-field mapping of optical properties and chromophores in turbid systems. In this presentation, we present: 1) Design and fabrication of a user-friendly hardware platform with large field-of-view (20cm x 15cm), spectral flexibility (10 wavelengths), desired stability (<0.5%/hr) and enhanced ease of use; 2) Development of software with automated acquisition.
Spatial Frequency Domain Imaging (SFDI) is a non-contact, wide-field optical technique that produces quantitative spatial maps of tissue optical properties and chromophores. It has been incorporated into many preclinical and clinical applications including brain function, wound healing, and skin cancer. SFDI technique consists of two main stages: 1) image acquisition and 2) processing to obtain tissue properties that we will refer to as the analysis stage. Acquisition consists of projecting spatially modulated illumination onto the sample and capturing remitted reflectance with a camera. In the analysis stage, a light transport model is applied to the demodulated reflectance images, to extract absorption and reduced scattering maps. Known chromophore extinction coefficients can then be fit, via a least-squares approach, to the absorption coefficient at multiple wavelengths. From this we obtain chromophore concentrations maps such as oxy-deoxyhemoglobin. One continuing challenge associated with sinusoidal pattern projection in conventional SFDI is the inability to acquire images that capture high temporal hemodynamics (e.g., cerebral measurements) and avoid motion artifacts during in-vivo measurements. Our group has recently addressed acquisition speed limits using a demodulation technique called multi spatial frequency synthesis and extraction (MSE). MSE, however, imposes an order of magnitude more computations on the analysis stage. Here, we present improvements to the analysis component of SFDI. This is done using multi-CPU/GPU friendly algorithms, contributing to a real-time in-vivo monitoring strategy for computing tissue properties of up to 33 FPS with 512?64 pixel resolution. We present results in the context of an in-vivo arm pressure cuff occlusion.

Programs and funding opportunities at the National Institute of Biomedical Imaging and Bioengineering

Behrouz Shabestari, National Institutes of Health (United States)

The mission of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) is to improve health by leading the development and accelerating the application of biomedical technologies. This overview described the Institute's commitment to integrating the physical and engineering sciences with the life sciences to advance basic research and medical care. This presentation will provide an overview of the scientific programs and funding opportunities supported by NIBIB, highlighting those that are of particular important to the field of optical imaging and spectroscopy. The presentation will also explore the peer review process and how to structure, write, and fine-tune a competitive application for funding consideration.

Single-channel stereoscopic video imaging ophthalmology surgical microscopes based on TRD

Edalat Radfar, Byungeo Jung, Jihoon Park, Sangyeob Lee, Myungjin Ha, Sungkon Yu, Seulgi Jang, Yonsei Univ. (Korea, Republic of)

A stereoscopic imaging modality was developed in order to apply in ophthalmology surgical microscopes. We have already introduced a single-channel stereoscopic video imaging modality based on a transparent rotating deflector (SSVIM-TRD). In that system, two different viewing angles,
image disparity, are generated via imaging through a rotational optical
deflector which is connected to a motor and is placed inside a lens system.
The image disparity is a function of the refractive index and the rotation
angle of TRD.

A NI data acquisition (DAQ) board was used as a central control unit to
manage signaling and to synchronize a CMOS camera and a 2-phase hybrid
step motor. DAQ system was programmed in NI Labview to have a real-time
control on all components.

The degree of depth perception of human vision in terms of viewing angle
and viewing distance was estimated. For this purpose, angular difference
between left and right views was measured interactively to simulate and
predict the depth perception of human vision.

Image quality assessments were performed to check images quality
and stability during the rotation of TRD. The results show no significant
difference in image quality in terms of stability of structural similarity (SSIM)
during the rotation of TRD.

A subjective analysis was performed to evaluate the depth perception
improvement in 15 blinded observers. The results showed significant
improvement in the depth perception capability of respondents.

The primary results of rabbit eye imaging showed that the single-channel
stereoscopic video imaging modality could be integrated in ophthalmic
operating microscopes to overcome some limitations of conventional ones.

9700-39, Session 8

Fast full 4x4 Mueller polarimeter for
endoscopic applications

Sylvain Rivet, Univ. de Bretagne Occidentale (France) and
Univ. of Kent (United Kingdom); Adrian Bradu, Adrian G. H.
Podoleanu, Univ. of Kent (United Kingdom)

Mueller polarimetry can measure all the polarimetric properties of
biological samples – diattenuation, retardance and depolarization related
respectively to muscles, collagen and tumoral tissues for example. However
Mueller polarimeters are still limited in scope, at least regarding in vivo
investigations because accessing deeper organs requires to incorporate a
fiber lead which compromises or complicates the polarization state of the
light used. Indeed fiber-based systems using single-mode fibers induce
disturbances in the measured polarization due to external factors such as
mechanical stress.

This communication deals with the theoretical frame and calibration steps
for a procedure to deliver full Mueller matrices through a single mode
fiber. This polarimeter has two interesting properties for endoscopy: 1) a
high speed of Mueller matrix acquisitions compatible with the demands
of modern fast laser-scanning imaging systems. The polarimeter is based on
spectral coding of polarization (or channeled spectrum polarimetry)
performed by passive birefringent plates. The speed of a Mueller matrix
acquisition is then related to the speed of running the spectrometer camera,
70 kHz here. 2) simultaneous measurement of the Mueller matrix of both the
fiber and sample through the fiber, to compensate the disturbances due to
fiber handling during measurement. This is achieved by separating the two
signals thanks to coherence gating principle in an interferometric device
illuminated by a broadband source.

Polarimetric measurements of different non-scattering media, such as
retarder and diattenuator, while deliberately introducing fiber disturbances
are presented in order to demonstrate the insensitivity of the polarimetric
measurement to fiber disturbances.

9700-40, Session PSun

The role of cerebral spinal fluid in light propagation through the mouse head:
improving fluorescence tomography with Monte Carlo modeling

Daniele Ancora, Athanasios Zacharopoulos, Foundation
for Research and Technology-Hellas (Greece); Jorge Ripoll,
Univ. Carlos III de Madrid (Spain); Giannis Zacharakis,
Foundation for Research and Technology-Hellas (Greece)

Optical Neuroimaging is a highly dynamical field of research owing to the
combination of many advanced imaging techniques and computational tools
that uncovered unexplored paths through the functioning of the brain. Light
propagation modelling through such complicated structures has always
played a crucial role as the basis for a high resolution and quantitative
imaging where even the slightest improvement could lead to significant
results. Fluorescence Diffuse Optical Tomography (fDOT), a widely used
technique for three dimensional imaging of small animals and tissues, has
been proved to be inaccurate for neuroimaging the mouse head without
the knowledge of a-priori [Neuroimage 44, 1304-1311(2009)] anatomical
information of the subject. Commonly a normalized Born approximation
model is used in fDOT reconstruction based on forward photon propagation
using Diffusive Equation (DE) which has strong limitations in the optically
clear regime. The presence of the Cerebral Spinal Fluid (CSF) instead, a
thin optically clear layer surrounding the brain, can be more accurately
taken into account using Monte Carlo approaches which nowadays is
becoming more useful thanks to parallelized GPU algorithms [Opt. Exp.
17, 20178-20190 (2009)]. In this work we discuss the results of a synthetic
experimental comparison, resulting to the increase of the accuracy for the
Born approximation by introducing the CSF layer in a realistic mouse head
structure with respect to the current model. We point out the importance
of such clear layer for complex geometrical models, while for simple slab
phantoms neglecting it does not introduce a significant error.

9700-41, Session PSun

Towards improved image reconstruction in breast diffuse optical tomography using
compressed sensing: a comparative study among lp (0<sp;2) sparsity regularizations

Bingyuan Wang, Yihan Wang, Yanqi Zhang, Huijuan Zhao,
Feng Gao, Tianjin Univ. (China)

Considering the inverse problem in diffuse optical tomography, far less
measurements than the optical property parameters to be reconstructed
makes the inverse problem underdetermined. The under-determinedness
becomes especially severe when detecting breast cancer because of the
big-size of breasts. With the addition of ill-condition due to the diffusive
nature of light propagation, the ill-posed inverse problem makes the
reconstruction spatial resolution difficult to improve. Fortunately, from
anatomy viewpoint, we know that the cancer is distributed locally and
amounts to a small percentage of the whole breast, which enables
the compressive sensing method. In this paper, we deploy the sparse
regularization method in DOT to mitigate the underdetermined property
and improve the spatial resolution under the premise without increasing
the number of measurements required. Here, we focus on the comparison of
different kinds of Lp (0<sp;2) regularization methods in theory and
real effect respectively. When p takes different values, we compare the
corresponding performances?such as speed of reconstruction process,
robustness and spatial resolution. Just for the sake of comparison, the
performances of several other methods such as Algebraic Reconstruction
Technique (ART), truncated singular value decomposition (TSVD) are also
included in this paper. The comparison implemented using both numerical
and phantom cases show that these methods dramatically improve the
reconstruction quality than the conventional methods.
Validation of MTF measurement for CBCT system using Monte Carlo simulations

Ting Hao, Tianjin Univ. (China); Feng Gao, Huijuan Zhao, Tianjin University (China) and School of Precision Instrument and Optoelectronics Engineering, Tianjin University (China) and Tianjin Key Laboratory of Biomedical Detecting Techniques and Instruments (China); Zhongxing Zhou, Tianjin Univ. (China)

To evaluate the spatial resolution performance of cone beam computed tomography (CBCT) system, accurate measurement of the modulation transfer function (MTF) is required. This accuracy depends on the MTF measurement method and CBCT reconstruction algorithms. In this work, the accuracy of MTF measurement of CBCT system using wire phantom is validated by Monte Carlo simulation. A Monte Carlo simulation software tool BEAMnrc/EGSnrc was employed to model X-ray radiation beams and transport. Tungsten wires were simulated with different diameters and radial distances from the axis of rotation. We adopted filtered back projection technique was adopted to reconstruct images from 360° acquisition. The MTFs for four reconstruction kernels were measured from the corresponding reconstructed wire images, while the hanging kernel increased the MTF slightly relative to the cosine, hann and ram-lak kernel. The results demonstrated that the MTF degraded with the radial distance from the rotation center. This study suggested that an increase in the MTF for the CBCT system is possible by optimizing scanning settings and reconstruction parameters.

Non-contact ECG monitoring

Aleksei Smirnov, Vadim Erlikh, Vladimir Kodkin, Andrei Keller, Vitaly Epishhev, South Ural State Univ. (Russian Federation)

Abstract. Electrocardiography is the most general diagnostic method that allows the assessment both of the human health and the human functional status. All the modern electrocardiographs pick up the signal from the skin, and the major attention is paid to a good contact to skin. Due to these reasons, the scope of electrocardiography application is limited to healthcare facilities.

In recent years it has been reported that several leading foreign companies are working on the driver functional status monitor that may be used in the new generation cars and is based on the non-contact ECG measurement, but the recorded ECG signals are still unstable and provide heart rate monitoring at best.

The specialists of South Ural State University are conducting the study of hardware-software system of non-contact ECG-monitoring. Based on the high-accuracy ECG recorder developed within the framework of ‘START’ programme, one of the University’s small innovative enterprises managed to obtain ECG signals very similar to ECG recorded by SCHILLER device (Switzerland).

Quality of the obtained ECG suggests that the developed system has a vast scope of application – from the up-to-date clinical research to ECG diagnostics and systems of continuous monitoring of functional status in people engaged in labour-intensive and critical activities – motor vehicle operators, aircraft pilots, electric locomotive operators etc. Unfortunately, too many tragic incidents have been reported recently which may have been avoided thanks to ECG monitoring system.

The results of conducted research show that it is possible to perform ECG record without any contact to the human body. The development of a fully functional system will take much effort of various specialists – engineers, chemists, programmers. But in the end a modern technology based on the in-house study will be obtained and further used in widely differing areas of science and engineering.

Embedded infrared imaging system for detection of vein pattern

Mustafa Z. Yildiz, Hyun Soo Lim, Özdemir Cetin, Ömer F. Boyraz, Volkan Seymen, Sakarya Univ. (Turkey)

The purpose of this study is to develop a novel near real-time vein imaging system. This system was realized by a micro-computer (Raspberry-Pi) which was based embedded operating system (OS).

Venipuncture is one of the most used invasive medical procedure but it becomes very hard to conduct. The degree of difficulty of cannulation process depends on factors like vein depth, the amount of fatty tissues, skin pigmentation and blood volume. Finding the vein can be very hard during treatment or examination in children, old persons, obese, severe burn cases and persons with dark skin. To cope with the disadvantages mentioned above, many vein imaging methods have been developed.

The vein images were taken by infrared camera and they were improved by various image processing algorithms in the Python language by using Open CV, which is computer vision library developed by Intel, in the microcomputer system. Thus, the blood veins are detected from the raw images. For detection; grayscaling, histogram equalization, Gaussian blur filter, median filter, bilateral filter, adaptive thresholding and thinning processes are applied respectively.

A comparative study of all these processes is carried out to find the best technique to extract vein pattern. Three filters technique are applied in order to remove noises which are hairs, usual creases on the skin. The result shows the median filter technique provides better to remove noises on the skin thereby being best candidate for Open CV Applications.

Multi-wavelength fluorescence tomography

Tiffany C. Kwong, Jaedu Cho, Farouk Nouizi, Ctr. for Functional Onco-Imaging (United States); Chang-Seok Kim, Pusan National Univ. (Korea, Republic of); Gultekin Gulsen, Ctr. for Functional Onco-Imaging (United States)

The strong scattering and absorption of light in biological tissue makes it challenging to model the propagation of light, especially in deep tissue. This is especially true in fluorescence tomography which aims to recover the internal fluorescence source distribution from the measured light intensities on the surface of the tissue. The inherently ill-posed and underdetermined nature of the inverse problem along with strong tissue scattering makes FT extremely challenging. Previously, it has been demonstrated that the image quality of diffuse optical tomography can be improved with multi-frequency and multispectral information to overcome the inherent absorption and scattering limitations. In this work, we have taken this approach and applied it to FT utilizing a unique rf modulated NIR swept laser to provide multi-wavelength, multi-frequency fluorescent tomography with the aim of improving the overall FT image resolution and quantitative accuracy. The performance of this method is currently investigated with phantom and simulation studies. A finite element method (FEM)-based reconstruction algorithm based on a diffusion equation was utilized to incorporate the multi-wavelength and multi-frequency information to provide superior image quality compared to conventional FT alone.

A modified laminar optical tomography system and initial validation

Huijuan Zhao, Shuang Wang, Mengyu Jia, Feng Gao,
Tianjin Univ. (China)

In a typical laminar optical tomography (LOT) system, the dip-angle between the incident light (or the emitting light) and the normal of the detection plane randomly changes during light scanning. The inconstant dip-angle causes consistency between the measurement and the light transportation model where a fixed dip-angle of the incident light is required. To eliminate the effect from this dip angle, methods such as keeping the angle unchangeable by moving the phantom instead of scanning the light were investigated. In this paper, a LOT system with small dip-angle over the whole detection range is developed and the following investigation for improving LOT system are performed: (1) to obtain the accurate relationship between the situation of the scanning galvanometer and the position of light spot on the detection plane, a method is proposed to correct the pillow distortion of the lens; (2) by measuring the system transfer function, a method for determining the spatial resolution of a mesoscopic system, e.g. a LOT system, is proposed; (3) to eliminate the effect from the indeterminate dip-angle, a bi-telecentric lens were introduced into the system which ensures that both the incident light and the main emitting light are nearly perpendicular to the detection plane. Simulation and experimental evaluation show that the dip-angle of the modified system is much smaller than that of the traditional system. For example, the relative angle between the two incident light on (0mm, 0mm) and (0mm, 2.5mm) is about 0.7° for the traditional system while that is only about 0.02° for the modified system. The main parameters of the system are also evaluated and an image reconstruction algorithm is developed based on Monte Carlo simulation. The reconstructed images show that the spatial resolution and quantitative ratio is improved by the modified system without loss of the scanning speed.
9701-1, Session 1

Quantum yield imaging: a new method for imaging quantum yield in diffusive media
Yanyu Zhao, Darren M. Roblyer, Boston Univ. (United States)

The detected surface emission from a fluorophore embedded in turbid media is a function of the fluorophore concentration and location, the quantum yield (QY) of the fluorophore, and the optical properties of the surrounding media. The QY, which quantifies the efficiency of the imaging fluorescence process, is often sensitive to local tissue environments, such as pH, temperature, and oxygen concentration, suggesting QY could be used as a novel indicator of these parameters. Unfortunately, QY is difficult to measure in turbid media and is most commonly measured by comparison to a well-known standard in non-scattering media. We introduce a new imaging method, called Quantum Yield Imaging (QYI), which uses a combination of quantitative reflectance and fluorescence based methods for spatial mapping of QY in turbid media. First, a wide-field imaging technique called Spatial Frequency Domain Imaging (SFDI) is used to measure the optical properties of the background media and to determine the fluorophore concentration. Then, a planar fluorescence image is modified using the measured background optical properties to produce a quantitative fluorescence image. These items, along with the system instrument response, are used to calculate the pixel-by-pixel QY. QYI was tested with two fluorophores, rhodamine B, which is sensitive to solvent composition, and SNARF-5, which is pH sensitive. Both were tested in phantoms with a range of background optical properties and with different solvent compositions to produce a range of QY values. QYI results showed an agreement of 0.021 for rhodamine B and 0.012 for SNARF-5 compared to gold standard QY measurements.

9701-2, Session 1

A quad-modality molecular small animal imaging system for both optical and radioactive molecular probes
Changhui Li, Yanye Lu, Qiushi Ren, Peking Univ. (China)

In this study, we developed first quad-modality molecular imaging system for small animal study, which includes 3 molecular imaging methods (PET, SPECT, and fluorescence molecular imaging [FMI]) and 1 anatomic imaging modality (CT). This system could study various biologic processes in the same animal using multiple molecular tracers. In addition to the technology development, we also discussed the optimization strategy of the imaging protocols. The performance of this system was tested, and the in vivo animal experiment showed its power to trace three more different molecular probes in living tissues of the same animal during one imaging procedure. Our results demonstrated that this system has a great potential for the preclinical study of diseases.

9701-3, Session 1

Common reduced spaces of representation applied to multispectral texture analysis in cosmetology
Joris Corvo, MINES ParisTech (France) and SILAB (France); Jesus Lopez-Angulo, MINES ParisTech (France); Josselin Breugnot, SILAB (France); Sylvie Borbes, SILAB (France)

Our dimensionality reduction algorithms produce eigenimages that can separate enhance skin component (pores, radiance, vessels...). Accuracy scores associated with the obtained common reduced spaces outperform scores from the PCA. The effect of foundation make-up on skin using multispectral imaging system is more efficiently characterized.

9701-4, Session 1

A combined diffuse correlation spectroscopy and time-resolved near-infrared spectroscopy instrument for calculating absolute cerebral blood flow and cerebral metabolic rate for oxygen
Venkaiah C. Kavuri, Wesley B. Baker, Ashwin B. Parthasarathy, Univ. of Pennsylvania (United States); Ramani Balu, W. Andrew Kofke, Univ. of Pennsylvania School of Medicine (United States); Arjun G. Yodh, Univ. of Pennsylvania (United States)

We investigate the feasibility of constructing an instrument, which can help critical care physicians by real-time monitoring/measuring absolute cerebral blood flow (CBF) and cerebral metabolic rate for oxygen (CMRO2) to facilitate fast diagnosis of Ischemia. Currently there are no straightforward bedside instrument to monitor/measure absolute CBF and CMRO2. Excess of decrements in CMRO2 leads to brain infarction, can be treated if detected early. To address the challenges above we constructed an instrument which utilizes Diffuse Correlation Spectroscopy (DCS) and time domain Diffuse Optical Spectroscopy (DOS). Absolute CBF can be measured by using Indocyanine green (ICG) as a vascular contrast agent. It is calculated by relating the change in absorption due to ICG bolus through the brain to CBF and the arterial concentration of the dye. Change in absorption due to ICG bolus can be estimated by time domain measurements. On the other hand, DCS provides changes in relative blood flow. If calibrated with the flow calculated from time domain data, DCS data can be used for continuous monitoring of the flow and time domain data for monitoring oxygenation. Our instrument is capable of taking simultaneous measurements on both sides of the brain (i.e. stroke side and healthy side). It employs super
continuum source and hybrid detectors for multi-spectral time-domain data collection. For collecting DCS data, the device employs a long-coherence laser and 16 avalanche photodiodes. Other features include real-time data normalization, accelerometers to monitor motion artifacts and application of the glue for stable probe placement. By utilizing intralipid flow phantoms we will optimize and test the instrument in terms of instrument response function and recovered optical properties. We will also present the results from tissue phantom measurements and first patient measurement.

9701-5, Session 1

Deformable medical image registration of pleural cavity for photodynamic therapy by using finite-element based method

Rozhin Penjweini, The Univ. of Pennsylvania Health System (United States); Michele M. Kim, Univ. of Pennsylvania School of Medicine (United States); Andrea Dimofte, Jarod C. Finlay, Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

When the pleural cavity is opened during the surgery portion of pleural photodynamic therapy (PDT) of malignant mesothelioma, the pleural volume will deform. This impacts the delivered dose when using highly conformal treatment techniques. To track the anatomical changes and contour the lung and chest cavity, an infrared camera-based navigation system (Polaris Spectra, NDI) is used during PDT. In the same patient, a series of computed tomography (CT) scans of the lungs are also acquired before the surgery. The reconstructed three-dimensional contours from both NDI and CTs are imported into COMSOL Multiphysics software, where a finite element-based (FEM) deformable image registration is obtained. The CT contour is registered to the corresponding NDI contour by overlapping the center of masses and aligning their orientations. The NDI contour is considered as the reference contour, and the CT contour is used as the target one, which will be deformed. Using COMSOL solid mechanics and deformed- or moving mesh models, the dynamic boundary matching is applied to obtain a deformed target contour. The displacement vectors are mapped to illustrate the transformation of the target contour. The initial assessment shows that FEM-based image deformable registration can fuse images acquired by different modalities. It provides insights into the deformation of anatomical structures along the x, y and z-axes. The deformed contour has good matches to the reference contour after the dynamic matching process. The resulting three-dimensional deformation map can be used to obtain the locations of other critical anatomic structures, e.g., heart, during surgery.

9701-6, Session 1

Correction of reference image distortion in adaptive optics scanning light ophthalmoscopy

Qiang Yang, Ethan Rossi, Univ. of Rochester Medical Ctr. (United States); David R. Williams, Univ. of Rochester (United States)

Registration of images from adaptive optics scanning light ophthalmoscopy (AOSLO) with a single reference image has the disadvantage that the registered and averaged high signal to noise ratio (SNR) images carry random unknown internal distortion from the reference image. To minimize this random unknown distortion, a statistical approach is implemented with data from the existing image sequence or the video itself, without increasing computational cost. The result shows the performance of distortion correction from three typical cases before and after this approach. 1) The internal distortion of registered and averaged images is reduced to a factor of -11 (from -0.398 arcminutes to -0.037 arcminutes) from the same video regardless of the selection of the reference image. 2) It is reduced to a factor of -5.3 from the same retinal location from two videos recorded at 45 minutes apart, from a healthy eye with good fixation. 3) It is reduced to a factor of -4.6 from the same retinal location from two videos recorded on two different days, from a diseased eye with poor fixation.

9701-7, Session 2

Acute and long-term effects of neural implants examined with two-photon and optical coherence microscopy (Invited Paper)

Daniel X. Hammer, U.S. Food and Drug Administration (United States)

No Abstract Available

9701-8, Session 2

Combining large area fluorescence with multiphoton microscopy for improved detection of oral epithelial neoplasia

Rahul Pal, Jinping Yang, Suimin Qiu, Susan McCammon, Vicente Resto, Gracie Vargas, The Univ. of Texas Medical Branch (United States)

Volumetric Multiphoton Autofluorescence Microscopy (MPAM) and Second Harmonic Generation Microscopy (SHGM) show promise for revealing indicators of neoplasia representing the complex microstructural organization of mucosa, potentially providing high specificity for detection of neoplasia, but is limited by small imaging area. Large area fluorescence methods on the other hand show high sensitivity appropriate for screening but are hampered by low specificity. In this study, we apply MPAM-SHGM following guidance from large area fluorescence, by either autofluorescence or a targeted metabolic fluorophore, as a potentially clinically viable approach for detection of oral neoplasia.

Sites of high neoplastic potentially were identified by large area red/green autofluorescence or by a fluorescently labelled deoxy-glucose analog, 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]D-glucose (2-NBDG) to highlight areas of high glucose uptake across the buccal pouch of a hamster model for OSCC. Follow-up MPAM-SHGM was conducted on regions of interests (ROIs) to assess whether microscopy would reveal microscopic features associated with neoplasia to confirm or exclude large area fluorescence findings. Parameters for analysis included cytologic metrics, 3D epithelial connective tissue interface metrics (MPAM-SHGM) and intensity of fluorescence (widelield). Imagined sites were biopsied and processed for histology and graded by a pathologist. A small sample of human ex vivo tissues were also imaged.

A generalized linear model combining image metrics from large area fluorescence and volumetric MPAM-SHGM indicated the ability to delineate normal and inflammation from neoplasia.

9701-9, Session 2

Dual Raman-Brillouin microscope for chemical and mechanical characterization and imaging

Vladislav V. Yakovlev, Texas A&M Univ. (United States)

We present a unique confocal microscope capable of measuring the Raman and Brillouin spectra simultaneously from a single spatial location. Raman and Brillouin scattering offer complimentary information about a material’s chemical and mechanical structure, respectively, and concurrent
Towards in vivo laser coagulation and concurrent optical coherence tomography through double-clad fiber devices

Kathy Beaudette, Ecole Polytechnique de Montréal (Canada) and Massachusetts General Hospital (United States); William Lo, Martin Villiger, Milen Shishkov, 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)

There is a strong clinical need for an optical coherence tomography (OCT) system capable of delivering concurrent coagulation light enabling image-guided dynamic laser marking for targeted collection of biopsies, as opposed to a random sampling, to reduce false-negative findings. Here, we present a system based on double-clad fiber (DCF) capable of delivering pulsed laser light through the inner cladding while performing OCT through the core. A previously clinically validated commercial OCT system (NVisionVLE, Ninepoint Medical) was adapted to enable in vivo esophageal image-guided dynamic laser marking. An optimized DCF coupler was implemented into the system to couple both modalities into the DCF (core signal transmission: -95%; inner cladding coupling: -75%). A DCF-based rotary joint (Princetel Inc.) was used to couple light to the spinning DCF-based catheter for helical scanning. The DCF rotary joint has low insertion loss into the core producing minimal crosstalk-induced artifacts, providing an effective dynamic range of up to 92dB. DCF-based OCT catheters were designed to provide a beam waist diameter of 62±4 μm at a working distance of 9.3±0.4mm, for use with a 17-mm diameter balloon sheath. Insertion loss of the high power laser light (λ = 1436nm, 1-ms pulses at 10 Hz) for the DCF rotary joint and a DCF catheter were -1.0dB in each case at an average input power of 200mW. Our previous ex vivo experiments demonstrate that these settings are suitable for single-pulse laser marking, thereby enabling further in vivo image-guided dynamic laser marking experiments.

Multimodal colposcopy for in vivo detection of cervical intraepithelial neoplasia

Wenqi Ren, YingJie Qu, Univ. of Science and Technology of China (China); JiaoJiao Pei, LinLin Xiao, Chongqing Medical Univ. (China); Shiwu Zhang, Univ. of Science and Technology of China (China); Shufang Chang, Chongqing Medical Univ. (China); Ronald X. Xu, The Ohio State Univ. (United States)

Cervical cancer is the leading cause of cancer death for women in developing countries. Colposcopy plays an important role in early screening and detection of cervical intraepithelial neoplasia (CIN). In this paper, we first studied the optical properties of cervical tissue for instrument design and then developed a multimodal colposcopic system for in vivo detection of CIN. Cervical reflectance and autofluorescence characteristics were simulated by a two-layered Monte Carlo model and analytical
Conference 9701: Multimodal Biomedical Imaging XI

9701-14, Session 3
Towards multimodal detection of melanoma thickness based on optical coherence tomography and optoacoustics
Maik Rahlves, Arthur Varkentin, Maya Otte, Jenny Stritzel, Elias Blumenroether, Mikhail Mazurenka, Merve Meinhardt-Wollweber, Bernhard Roth, Leibniz Univ. Hannover

Melanoma skin cancer is one of the most dangerous types of cancer if not diagnosed at an early stage and treated by surgical excision. It also has the highest rates of incidence compared to all types of cancer and accounts for approximately three percent of new cancer indispositions. The five year survival rate is highly dependent on the penetration depth of the tumor, which determines the probability for metastasis development. Up till now, histopathology is the gold standard when measuring the penetration depth of melanoma. In this work, we aim at the development of an all-optical hand held device to determine the melanoma penetration depth in a non-contact manner. Our method utilizes a multimodal approach based on optical coherence tomography (OCT) and optoacoustics (OA). Combining both methods allows to measure refractive index changes and absorption in skin, which helps not only to identify skin features such as the basal membrane but also pathogenic objects such as cancer cell clusters. We present our recent results on the combination of both modalities in a single hand held device and provide first in vivo OCT data obtained on healthy and cancerous skin lesions in a clinical environment, which are compared to measurement results obtained by high-frequency ultrasound, confocal microscopy and histopathology. As future perspective in clinical applications, our multimodal device aims to help dermatologists to determine the penetration depth of melanoma. Application of such a system could decrease the number of surgical interventions as well as the skin area, which is unnecessarily excised for safety reasons.

9701-15, Session 3
Identification of early cancerous lesion of esophagus with endoscopic images by hyperspectral image technique
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This study presents a method to identify early esophageal cancer within endoscopy using hyperspectral imaging technology. The research samples are three kinds of endoscopic images including white light endoscopic, chromoendoscopic, and narrow-band endoscopic images with different stages of pathological changes (normal, dysplasia, dysplasia - esophageal cancer, and esophageal cancer). Research is divided into two parts: first, we analysis the reflectance spectra of endoscopic images with different stages to know the spectral responses by pathological changes. Second, we identified early cancerous lesion of esophagus by principal component analysis (PCA) of the reflectance spectra of endoscopic images. The results of this study show that the identification of early cancerous lesion is possible achieve from three kinds of images. In which the spectral characteristics of NBI endoscopy images of a gray area than those without the existence of the problem the first two, and the trend is very clear. Therefore, if simply to reflect differences in the degree of spectral identification, chromoendoscopic images are suitable samples. The best identification of early esophageal cancer is using the NBI endoscopic images. Based on the results, the use of hyperspectral imaging technology in the early endoscopic esophageal cancer lesion image recognition helps clinicians quickly diagnose. We hope for the future to have a relatively large amount of endoscopic image by establishing a hyperspectral imaging database system developed in this study, so the clinician can take this repository more efficiently preliminary diagnosis.

9701-16, Session 3
Multimodal imaging of ocular surface of dry eye subjects
Aizhong Zhang, Univ. of Rochester (United States)

We design and build a tearscope in house, which measures the lipids layer thickness of the tear film. Meanwhile, we use a long wave infrared camera to measure the dynamic thermal properties of the ocular surface. We conducted clinical trials for 20 subjects, 15 Meibomian gland dysfunction (MGD) and 5 aqueous-deficient dry eye (ADDE) subjects based on their clinical screening results. And the results of these two methods are analyzed and compared. According to the principal component analysis of the lipid layer thickness, we find that the 20 subjects could be categorized into five statistically significant groups, independent of their original clinical classification: thin (6 subjects), medium (5 subjects), medium and homogenous (3 subjects), thick (4 subjects), and very thick (2 subjects) lipid, respectively. From thin to thick, the average lipid thickness for each group was 31.91 (± 3.57) nm, 48.83 (± 12.91) nm, 53.17 (± 16.07) nm, 71.86 (± 21.91) nm, and 98.59 (± 14.32) nm, respectively. Moreover, using the thermal impulse perturbation (TIP) model proposed by our research group, we also analyzed the thermal performance of these 20 subjects, and we find that the MGD subjects have both a higher initial temperature (p < 0.022) and a higher asymptotic temperature (p < 0.007) than the ADDE subjects. We compared the results of principal component analysis of these two methods after the multimodal measurements, and we didn’t find statistically significant correlation of the lipid thickness and the thermal dynamic performance of the 20 subjects.

9701-17, Session 4
Depth-resolved imaging of colon tumor using optical coherence tomography and fluorescence laminar optical tomography
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Early detection of neoplastic changes remains a critical challenge in clinical cancer diagnosis and treatment. Many cancers arise from epithelial layers such as those of the gastrointestinal (GI) tract. Current standard endoscopic technology is unable to detect those subsurface lesions. Since cancer development is associated with both morphological and molecular alterations, imaging technologies that can quantitative image tissue's morphological and molecular biomarkers and assess the depth extent of a lesion in real time, without the need for tissue excision would be a major advance in GI cancer diagnostics and therapy. In this research, we investigated the feasibility of multi-modal optical imaging including high-resolution optical coherence tomography (OCT) and
depth-resolved high-sensitivity fluorescence laminar optical tomography (FLOT) for structural and molecular imaging. APC (adenomatous polyposis coli) mice model were imaged using OCT and FLOT and the correlated histopathological diagnosis was obtained. Quantitative structural (the scattering coefficient) and molecular imaging parameters (fluorescence intensity) from OCT and FLOT images were developed for multi-parametric analysis. This multi-modal imaging method has demonstrated the feasibility for more accurate diagnosis with 87.4% (87.3%) for sensitivity (specificity) which gives the most optimal diagnosis (the largest area under receiver operating characteristic (ROC) curve). This project results in a new non-invasive multi-modal imaging platform for improved GI cancer detection, which is expected to have a major impact on detection, diagnosis, and characterization of GI cancers, as well as a wide range of epithelial cancers.

9701-18, Session 4
Bioluminescence tomography-guided system for preclinical radiation research
Ken Kang-Hsien Wang, Bin Zhang, Phuoc T. Tran, Julian I. Iordachita, Johns Hopkins Univ. (United States); Michael S. Patterson, McMaster Univ. (Canada); John W. Wong, Johns Hopkins Univ. (United States)
Cone beam computed tomography (CBCT) is limited at guiding irradiation of soft tissue targets. As a complementary imaging modality, bioluminescence tomography (BLT) provides strong tissue contrast. We developed a dual-use configuration for a CBCT/BLT imaging system with the SARRP that can function as a standalone system for longitudinal research and on-board the SARRP to guide irradiation.
The optical assembly includes a CCD camera, lens, filter wheel, 3-way mirrors, and a light-tight enclosure. The rotating mirror system directs the optical signal from the animal surface to the camera at multiple projections. Multiple filters are used for multispectral BLT. SARRP CBCT provides anatomical information and geometric mesh for BLT reconstruction. To facilitate dual use, the 3-way mirror system is cantilevered in front of the camera. The optical assembly is driven by a linear stage to dock onto a mouse bed for standalone application or on-board SARRP. After completion of on-board imaging, the system is retracted from the SARRP to allow irradiation of the mouse. A phantom and mice with luminescence light sources will be used to demonstrate the function of the system. Feasibility data have been obtained based on a manual-docking prototype. The center of mass of light source determined with on-board BLT is within 1±0.2 mm of that with CBCT. The performance of the motorized system is expected to be the same. By supporting off-line longitudinal studies and on-board SARRP for radiation guidance, the dual-use system is a highly efficient and cost-effective platform to facilitate optical imaging for preclinical radiation research.

9701-19, Session 4
Intravascular diagnosis by a dual modality imaging system combining optical frequency domain imaging (OFDI) and intravascular ultrasound imaging (IVUS)
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Intravascular ultrasound imaging (IVUS) has been used worldwide to deepen our understanding of atherosclerosis and to promote accurate diagnosis of intravascular disease. Indeed, IVUS readily images the entire vessel cross-section enabling plaque burden assessment, an important parameter to estimate the vulnerability of a plaque. Its resolution, however, is currently limited to several hundreds microns making it insufficient to disclose lesion microstructures. With a ~10x resolution improvement (<20 microns), intravascular optical frequency domain imaging (OFDI) has been implemented to successfully resolve various intravascular microstructures. Yet, due to tissue scattering, the penetration depth of OFDI is shallower than IVUS. Therefore, the complementary pros and cons of the two approaches suggest that their synergistic combination would offer a comprehensive characterization of intravascular morphology. Such dual modality systems have been previously implemented to demonstrate their unique benefits. Aiming at a direct translation into the clinics, here we present our second-generation integrated IVUS/OFDI system. It includes an imaging console capable of simultaneously performing OFDI and IVUS with over 40KHz axial scan rate and a custom-designed clinical grade catheter (2.6Fr) containing both optical and ultrasonic transducers. Drawing from the experience of our previous studies, we have optimized the optical and the electrical coupling and have developed a new rotary joint and pullback unit allowing for reliable operation in the cardiac catheterization lab and leading to rapid clinical translation. In vivo imaging studies are expected to demonstrate the benefits of co-registered imaging of lesion microstructure and plaque burden.

9701-20, Session 4
Thermal outlining using focused ultrasound (TOFU) with reversible temperature sensitive fluorescent probes
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Optical imaging has long been hindered by the high absorption and scattering of light in biological tissue. This makes it difficult to probe beyond a few millimeters beneath the surface without sacrificing image resolution and quantitative accuracy. Strong scattering and the inherent nature of the inverse problem makes fluorescence diffuse optical tomography (FT) extremely challenging. To this end, multi-modality techniques that combine anatomical imaging with the functional optical information have been used to improve the resolution and accuracy of FT. Previously, we have reported on the feasibility of a new imaging method, “Thermal Outlining using Focused Ultrasound” (TOFU), that combines the sensitivity of FT with the resolution of focused ultrasound using temperature reversible fluorescent probes. In this method, the position of the temperature reversible fluorescent probes are localized by an increase in fluorescent signal when the hot spot of the focused ultrasound is scanned over the medium. This a priori information is then utilized to guide and constrain conventional reconstruction algorithm to recover the position and concentration of the probes more accurately. The small size of the focal spot (~1.4 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. In this work, the performance of the system will be evaluated using simulation, phantom, and animal tissue to investigate the size, concentration, and depth limitations.
Characterization of early-stage ionizing radiation induced skin injury in a mouse model by two-photon microscopy and optical coherence tomography

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Ionizing radiation (IR) induced injuries are tissue destruction or changes by electromagnetic waves of high frequency or subatomic particles that form positively and negatively charged particles. Diagnosis and treatment of IR-induced injuries are difficult due to their clinically latent post-irradiation periods and following successive and unpredictable inflammatory bursts. Skin is the organ exposed to local IR and sensitive IR injury. Early-stage diagnosis of IR-induced skin injury, prior to the appearance of visible clinical symptoms, is essential to maximize treatment efficiency and to prevent aggravation of IR injury. In this study, early stage changes of IR-induced skin injuries at the cellular and physiological levels were studied by using both two-photon microscopy (TPM) and optical coherence tomography (OCT) in an in vivo mouse model. Various doses of localized IR were irradiated on mouse hind limbs. Cells in the epidermis and sebaceous gland, and tissue structure and vasculature were imaged daily for 6 days after IR irradiation. Noticeable changes were detected by both modalities prior to visible clinical symptoms. These results showed that TPM and OCT are sensitive to early-stage changes of IR skin injury and may be useful for its diagnosis.

Multimodal OCT for visualization of animal model tumor response during PDT

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We performed fluorescent and cross-polarization OCT imaging of the animal
Frequency domain diffuse optical tomography using wavelength-swept laser

Hansol Jang, Chang-Seok Kim, Gukbin Lim, Pusan National Univ. (Korea, Republic of); Jaedu Cho, Univ. of California Irvine (United States)

Diffuse optical tomography is an imaging technique using interaction between light and media, such as absorption and scattering. Because of the principle of diffuse optical tomography, diffuse optical tomography has great potential in functional imaging, such as lipid, water and hemoglobin concentration changes in tissue. Conventional diffuse optical tomography instruments are consist of at least two different wavelength laser diodes (LD) in order to obtain chromophore concentration change data from wavelength dependent optical properties. However, for accurate data analysis, absorption variables originated in minor chromophores should be considered with more spectral information. For this reason, we developed a multi-wavelength system for alternative approach to optimize functional information.

In this paper, we propose frequency domain diffuse optical tomography system based on wavelength-swept laser. To acquire biological data effectively, wavelength-swept laser is consisted of semiconductor optical amplifiers around near infrared range. To alter output optical spectra, acousto-optic tunable filter (AOTF) was proposed in the fiber ring cavity configuration. Output of the wavelength-swept laser is intensity-modulated around 100 MHz to acquire phase delay data in frequency domain diffuse optical tomography and also get a sufficiently high signal-to-noise ratio.

Multi-spectral, heterodyne frequency-domain diffuse optical tomography with surface profilometry for breast cancer imaging

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A diffuse optical tomography (DOT) standalone breast imaging device has been developed for use in the clinic. The imager employs a large number of source-detectors along with multi-spectral, frequency-domain data for 3D image reconstructions of oxy-, de-oxo hemoglobin, and scattering in the parallel plate geometry. This imager is combined with custom surface profilometry devices to help segment the breast region from the surrounding optical matching fluid to improve image reconstructions. The frequency-domain detection is facilitated by highly-parallel CCD-based imaging based on gain-modulation heterodyne detection. Other features of the device include both frontal and sagittal breast imaging capabilities and improvements to patient comfort for measurement stability. First imaging experiments with tissue phantoms demonstrating the reduction of absorption and scattering cross-talk using multi-spectral frequency domain data acquisition. Initial breast cancer results from two patients with different breast densities are also shown. Image reconstructions in this work has been done using the TOAST++ package.
Photoplethysmographic imaging via spectrally demultiplexed erythema fluctuation analysis for remote heart rate monitoring

Jason Deglint, Audrey G. Chung, Brendan Chwyl, Robert Amelard, Farnoud Kazemzadeh, Xiao Yu Wang, David A. Clausi, Alexander Wong, Univ. of Waterloo (Canada)

Traditional photoplethysmographic imaging (PPGI) systems use the red, green, and blue (RGB) broadband measurements of a consumer digital camera to remotely estimate a patient’s heart rate; however, these broadband RGB signals are often corrupted by ambient noise, making the extraction of subtle fluctuations indicative of heart rate difficult. Therefore, the use of narrow-band spectral measurements can significantly improve the accuracy. We propose a novel digital spectral demultiplexing (DSM) method to infer narrow-band spectral information from acquired broadband RGB measurements in order to estimate heart rate via the computation of motion-compensated skin erythema fluctuation. Using high-resolution video recordings of human participants, multiple measurement locations are automatically identified on the cheeks of an individual, and motion-compensated broadband reflectance measurements are acquired at each measurement location over time via measurement location tracking. The motion-compensated broadband reflectance measurements are spectrally demultiplexed using a non-linear inverse model based on the spectral sensitivity of the camera’s detector. A PPG signal is then computed from the demultiplexed narrow-band spectral information via skin erythema fluctuation analysis, with improved signal-to-noise ratio allowing for reliable remote heart rate measurements. To assess the effectiveness of the proposed system, a set of experiments involving human motion in a front-facing position were performed under ambient lighting conditions. Experimental results indicate that the proposed system achieves robust and accurate heart rate measurements and can provide additional information about the participant beyond the capabilities of traditional PPGI methods.

Non-contact hematoma damage and healing assessment using reflectance photoplethysmographic imaging

Robert Amelard, Kaylen J. Pfisterer, David A. Clausi, Alexander Wong, Univ. of Waterloo (Canada)

Impact trauma may cause a hematoma, which is the leakage of venous blood into surrounding tissues. Large hematomas can be dangerous as they may inhibit local blood flow. Hematomas are often diagnosed visually, which may be problematic if the hematoma leaks deeper than the visible penetration depth. Furthermore, vascular wound healing is often monitored at home without the aid of a clinician. We therefore investigated the use of near infrared (NIR) reflectance PPGI to assess vascular damage resulting from a hematoma and monitor the healing process. In this case study, the participant experienced internal vascular damage in the form of a hematoma. Using a PPGI system with dual-mode temporally coded illumination for ambient-agnostic data acquisition and mounted optical elements, the tissue was illuminated with a spatially uniform irradiance measurement location over time via measurement location tracking. The motion-compensated broadband reflectance measurements are spectrally demultiplexed using a non-linear inverse model based on the spectral sensitivity of the camera’s detector. A PPG signal is then computed from the demultiplexed narrow-band spectral information via skin erythema fluctuation analysis, with improved signal-to-noise ratio allowing for reliable remote heart rate measurements. To assess the effectiveness of the proposed system, a set of experiments involving human motion in a front-facing position were performed under ambient lighting conditions. Experimental results indicate that the proposed system achieves robust and accurate heart rate measurements and can provide additional information about the participant beyond the capabilities of traditional PPGI methods.

Spectral photoplethysmographic imaging sensor fusion for enhanced heart rate detection

Robert Amelard, David A. Clausi, Alexander Wong, Univ. of Waterloo (Canada)

Continuous heart rate monitoring can provide important context for quantitative clinical assessment in scenarios such as long-term health monitoring and disability prevention. Photoplethysmographic imaging (PPGI) systems are particularly useful for such monitoring scenarios as contact-based devices pose problems related to comfort and mobility. Each pixel can be regarded as a virtual PPG sensor, thus enabling simultaneous measurements of multiple skin sites. Existing PPGI systems analyze temporal PPGI sensor fluctuations related to hemodynamic pulsations across a region of interest to extract the blood pulse signal. However, due to spatially varying optical properties of the skin, the blood pulse signal may not be consistent across all PPGI sensors, leading to inaccurate heart rate monitoring. To increase the hemodynamic signal-to-noise ratio (SNR), we propose a novel spectral PPGI sensor fusion method for enhanced estimation of the true blood pulse signal. Motivated by the observation that PPGI sensors with high hemodynamic SNR exhibit a spectral energy peak at the heart rate frequency, an entropy-based fusion model was formulated to combine PPGI sensors based on the sensors' spectral energy distribution. The optical PPGI device comprised a near infrared (NIR) sensitive camera and an 850 nm LED. Spatially uniform irradiance was achieved by placing optical elements along the LED beam, providing consistent illumination across the skin area. Dual-mode temporally coded illumination was used to negate the temporal effect of ambient illumination. Experimental results show that the spectrally weighted PPGI method can accurately and consistently extract heart rate information where traditional region-based averaging fails.

Integrated polarization-sensitive optical coherence tomography and Stokes imaging polarimeter for birefringent tissues

Yuqiang Bai, Joseph Chue-Sang, Jessica C. Ramella-Roman, Florida International Univ. (United States)

Polarized light-based techniques such as polarized light microscopy, optical polarimetric imaging techniques have shown good sensitivity to the orientation and quantification of birefringent tissue, Polarization-sensitive optical coherence tomography (PS-OCT) with advantages of high resolution, also enables measuring polarization properties of light collected from birefringent samples. Combination of these different techniques, which possess different aspects of view, penetration depth and field of view etc, is extremely useful for optimizing experiments and validating the results. In this study, an integrated polarization-sensitive Fourier domain optical coherence tomography (PS-FDOCT) system and out-of-plane Stokes imaging polarimeter (SIP) was set-up. The two imaging techniques were spatially co-registered by acquiring signal from the same objective. A dichroic mirror is positioned between galvo mirror and objective to separate wavelength of different imaging techniques, 840nm of PS-OCT, and 532nm of SIP. The combined system was tested with tendon, a highly packed structure of birefringent collagen fibers. Maps of birefringence and optic axis orientation from two separate systems were calibrated and analyzed, moving forward the utilization of polarized light-based techniques in birefringent tissues.
Keywords: birefringence, polarization sensitive optical coherence tomography, Stokes imaging polarimeter

9701-40, Session PSun

**Time-resolved hyperspectral single-pixel camera implementation for compressive wide-field fluorescence lifetime imaging**

Qi Pian, Ruoyang Yao, Xavier Intes, Rensselaer Polytechnic Institute (United States)

Single-pixel imaging based on compressive sensing theory has been a highlighted technique in biomedical imaging field for years. Many novel progresses in realms from microscopic to macroscopic imaging are encouraged by the enticing advantages of the technique like avoiding the need of high performance array detectors which is not always available due to financial or technical constraints, increasing the SNR (signal-to-noise ratio) of data sets through light signals integration and enabling high quality image reconstruction with compressed data sets by exploiting the signal sparsity.

In this work, we present our recent work for time domain fluorescence label investigation in thin tissue using a time-resolved hyperspectral single-pixel camera for fast, wide-field mapping of molecular labels in medium with minimum light scattering. The hyperspectral single-pixel camera implements a DMD (digital micro-mirror device) to generate optical masks for modulating the illumination field before it is delivered onto the sample and focuses the emission light signals into a multi-anode hyperspectral time-resolved PMT (photomultiplier tube) to acquire spatial, temporal and spectral information enriched 4-D data sets. Fluorescence dyes with lifetime and spectral contrast are embedded in well plate and thin tissue phantom. L-1 norm based regularization is applied to solve the underdetermined inverse problem during image reconstruction. The result images prove the possibility of fast, wide-field mapping of fluorescent labels with lifetime and spectral contrast in thin medium.

9701-41, Session PSun

**Sparse temporal sampling for fast time-domain wide-field fluorescence molecular tomography**

Ruoyang Yao, Lingling Zhao, Xavier Intes, Rensselaer Polytechnic Institute (United States)

Fluorescence Molecular Tomography is gaining more popularity nowadays as a powerful optical imaging tool for preclinical research. Among all its measurement types, time-domain (TD) provides the most comprehensive information compared to continuous wave (CW) and frequency-domain (FD), allowing lifetime multiplexing and enhancing spatial resolution.

At present, most TD reconstructions require collecting measurements of the whole temporal profile and selecting a subset of floating gates (gates of different source-detector pairs have the same ratio to their own peaks but different absolute numbers). To reduce detection time and avoid collecting more measurements than needed, we come up with an idea of using fixed gates (gates of all source-detector pairs have the same absolute numbers) instead.

We propose a strategy to determine the optimal fixed gate spacing and corresponding gate numbers. Briefly, it measures the TPSF curves of a full illumination pattern as a representation of all illumination patterns, takes the derivative and sets up an early range and a late range. The optimal spacing ensures there's about 1 gate in early range and 2 gates in late range on average.

Several numerical simulations are made using a digimouse model, with one/multiple kinds of fluorophores on one or more organs. We compare reconstructions under different spacing, as well as that of fixed gates and corresponding floating gates. Results show: 1) the proposed method is a valid way to determine optimal spacing for fixed gates; 2) fixed gates provide equivalent or even better reconstruction results than floating gates.

9701-42, Session PSun

**Gate-width impact on NIR FRET lifetime fitting using gated ICCD**

Sez-Jade Chen, Xavier Intes, Rensselaer Polytechnic Institute (United States)

Fürster Resonance Energy Transfer (FRET) is widely used to sense molecular interactions occurring at the nanoscale. In vitro and ex vivo protocols for visualizing FRET are already well-established, but in vivo studies have proven to be more challenging. One issue that hinders in vivo visualization of FRET is the higher absorption and scattering of visible light within tissues. In this case, light in the near-infrared (NIR) spectral window is required for increased depth sensing. Moreover, due to spectral variation in optical properties as well as heterogeneous spatial distribution, lifetime-based FRET imaging is preferred. Herein, we investigate the effect of temporal acquisition settings on the lifetime-based estimation of the fraction of quenched donor molecules (A1) as well as the quenched donor lifetime (?1). We performed in silico, in vitro, and in vivo experiments under gate widths of 300ps to 1,000ps in 100ps intervals to determine the effect on quantification of A1 and ?1. Even though the NIR fluorescent dyes have shorter lifetimes than visible fluorophores, we were still able to accurately quantify FRET under all tested system gate widths and experimental conditions.
The study revealed two salient spectral peak shifts (in the regions 2925 to 2890 cm\(^{-1}\) and 1125 to 1100 cm\(^{-1}\)), which are associated with endotoxin in OVDs. In addition, FO-FTIR experimental results processed using a multivariate analysis confirmed the observed specific peak shifts associated with endotoxin contamination in OVDs. Thus, employing the FO-FTIR sensing methodology integrated with a multivariate analysis could potentially be used as an alternative endotoxin detection technique in OVD.

Novel chalcogenide glass based fiber opens up the mid-infrared (MIR) range for remote, real time monitoring and control in medical diagnostics and chemical processing. Fibers with long wavelength cut-off are of interest here. Sulfide, selenide and telluride based chalcogenide glass are of interest but there are differences in their thermal stability, cut off positions of short and long wavelengths, glass forming region and ability. Sulphide and selenide glasses generally have good glass stability compared to telluride glasses; selenide-telluride glasses are a good compromise.

The Ge-As-Se-Te glass system is a good potential candidate, which has intrinsic transparency from 1.5 to 20 \(\mu\)m. Te could be substituted to the Ge-As-Se system while adequate thermal stability is retained. However, low optical loss MIR fibres fabricated by the Ge-Se-Te glasses have to been investigated in detail. Addition of Te contributes to low phonon energy of the glass system and large refractive indices, this linked with a glass composition which is thermally stable and transparent, gives rise to a good candidate for rare earth doped fibre lasers.

This paper investigates the Ge-As-Se-Te glass system and active systems, in developing fibers with low optical loss and fibre lasing for real time monitoring of medical diagnosis and chemical processing. Several interim objectives needed to be addressed in order for the success of the project: suitable methods of producing the glass material, working towards control of fiber geometry and design of the fiberoptic sensor head.

Fiber-optic Fourier transform infrared (FO-FTIR) spectroscopy for detecting endotoxin contamination in ophthalmic viscosurgical devices (OVDS)

Moinuddin Hassan, Ilko K. Ilev, U.S. Food and Drug Administration (United States)

Ophthalmic Viscosurgical Devices (OVDS) in clinical setting are a major health risk factor for potential endotoxin contamination in the eye, due to their extensive applications in cataract surgery for space creation, stabilization and protection of intraocular tissue and intraocular lens (IOL) during implantation. Endotoxin contamination of OVDS is implicated in toxic anterior syndrome (TASS), a severe complication of cataract surgery that leads to intraocular damage and even blindness. Current standard methods for endotoxin contamination detection utilize rabbit assay or Limulus amoebocyte lysate (LAL) assays. These endotoxin detection strategies are extremely difficult for gel-like type devices such as OVDS. To overcome the endotoxin detection limitations in OVDS, we have developed an alternative optical detection methodology for label-free and real-time sensing of bacterial endotoxin in OVDS, based on fiber-optic Fourier transform infrared (FO-FTIR) transmission spectrometry in the mid-IR spectral range from 2.5 micron to 12 micron. Endotoxin contaminated OVD test samples were prepared by serial dilutions of endotoxins on OVDS. The major results of this study revealed two salient spectral peak shifts (in the regions 2925 to 2890 cm\(^{-1}\) and 1125 to 1100 cm\(^{-1}\)), which are associated with endotoxin in OVDs. In addition, FO-FTIR experimental results processed using a multivariate analysis confirmed the observed specific peak shifts associated with endotoxin contamination in OVDs. Thus, employing the FO-FTIR sensing methodology integrated with a multivariate analysis could potentially be used as an alternative endotoxin detection technique in OVD.

Photoacoustic imaging by using a bundle of thin hollow-optical fibers

Atsushi Seki, Tohoku Univ. (Japan); Katsumasa Iwai, Sendai National College of Technology (Japan); Takashi Katagiri, Yuji Matsuura, Tohoku Univ. (Japan)

We propose a photoacoustic imaging system composed of a flexible bundle of thin hollow-optical fibers that enables endoscopic diagnosis. In this system, we fabricated a hollow-fiber bundle with 37 fibers with an inner diameter of 100 \(\mu\)m and the total diameter of the bundle is 1.2 mm. The bundle is highly flexible and the minimum bending radius is around 10 mm. Owing to the very small numerical aperture of hollow optical fibers, high resolution image is obtained without using a micro-lens array at the distal end.

In the imaging system, the hollow fibers in the bundle is arranged in a line at the input end and therefore, one can excite the hollow fibers sequentially by linearly scanning the laser beam at the input end. A microchip Nd:YAG laser with a 532 nm wavelength was focused on the input end of fiber by using a plano-convex lens with a focal length of 76 mm.

In imaging tests using a 50-\(\mu\)m thick copper-wire sample, imaging resolutions of 65 \(\mu\)m in lateral direction and 355 \(\mu\)m in depth direction were obtained. To test the feasibility of the system, photoacoustic images of a blood vessel phantom made of silicone tube with an inner diameter of 100 \(\mu\)m containing a black ink in the bore were taken. As a result, 3-D photoacoustic images of the phantom were successfully obtained with a laser pulse energy of around 20 \(\mu\)J.

Fiber-optic Fourier transform infrared (FO-FTIR) spectroscopy for detecting endotoxin contamination in ophthalmic viscosurgical devices (OVDS)

Moinuddin Hassan, Ilko K. Ilev, U.S. Food and Drug Administration (United States)

Ophthalmic Viscosurgical Devices (OVDS) in clinical setting are a major health risk factor for potential endotoxin contamination in the eye, due to their extensive applications in cataract surgery for space creation, stabilization and protection of intraocular tissue and intraocular lens (IOL) during implantation. Endotoxin contamination of OVDS is implicated in toxic anterior syndrome (TASS), a severe complication of cataract surgery that leads to intraocular damage and even blindness. Current standard methods for endotoxin contamination detection utilize rabbit assay or Limulus amoebocyte lysate (LAL) assays. These endotoxin detection strategies are extremely difficult for gel-like type devices such as OVDS. To overcome the endotoxin detection limitations in OVDS, we have developed an alternative optical detection methodology for label-free and real-time sensing of bacterial endotoxin in OVDS, based on fiber-optic Fourier transform infrared (FO-FTIR) transmission spectrometry in the mid-IR spectral range from 2.5 micron to 12 micron. Endotoxin contaminated OVD test samples were prepared by serial dilutions of endotoxins on OVDS. The major results of this study revealed two salient spectral peak shifts (in the regions 2925 to 2890 cm\(^{-1}\) and 1125 to 1100 cm\(^{-1}\)), which are associated with endotoxin in OVDs. In addition, FO-FTIR experimental results processed using a multivariate analysis confirmed the observed specific peak shifts associated with endotoxin contamination in OVDs. Thus, employing the FO-FTIR sensing methodology integrated with a multivariate analysis could potentially be used as an alternative endotoxin detection technique in OVD.

Manufacure of micro-fluidic devices by laser welding using thermal transfer printing techniques

Karl-Friedrich Klein, Rolf Klein, Daniel Thölen, Tim Tobisch, Technische Hochschule Mittelhessen (Germany); Mathias Belz, World Precision Instruments (Germany)

Microfluidic devices are widely used today in the areas of medical diagnostics and drug research as well as for applications within the process, electronics and chemical industry. Microliters of fluids or single cell to cell interactions can be conveniently analyzed with such devices using fluorescence imaging, phase contrast microscopy or spectroscopic techniques.

Typical microfluidic devices consist of a thermoplastic base component with chambers and channels covered by a hermetic fluid and gas tight sealed lid from the same or similar material than the base. Different mechanical, adhesive or thermal joining processes can be used to assemble base component and lid.

Today, laser beam welding shows potential to become a novel
manufacturing opportunity for midsize and large scale production of microfluidic devices resulting in excellent processing quality by localized heat input and low thermal stress to the device during processing.

For laser welding, optical absorption of the resin and laser wavelength has to be matched for proper joining. This paper will focus on a new approach to prepare microfluidic channels in such devices using a thermal transfer printing process, where an optical absorbing layer absorbs the laser energy. Advantages of this process will be discussed in combination with laser welding of optical transparent microfluidic devices.

9702-5, Session Key1
Exploring the nanoscale dynamics of biomolecules with optical microcavities (Keynote Presentation)
Frank Vollmer, Max-Planck-Institut für die Physik des Lichts (Germany)

Medicine as well as biology increasingly rely on the use of cutting-edge physics and engineering, in order to pursue the next generation nanomedical applications and to address fundamental questions in the life sciences. Central to this task is the study of micro- and nano systems, focusing on how engineered systems combined with natural ones can advance sensing, medicine, and our understanding of how biological systems work. My research addresses these important questions with state-of-the-art biosensor technologies, capable of detecting single biomolecules and their dynamics; and resolving the kinetics of biomolecular systems on timescales ranging from few nanoseconds to several hours.

9702-6, Session 2
Ultra-sensitive near-infrared fiber-optic gas sensors enhanced by metal-organic frameworks
Xinyuan Chong, Ki-Joong Kim, Erwen Li, Oregon State Univ. (United States); Paul R. Ohodniczki, National Energy Technology Lab. (United States); Chih-Hung Chang, Alan X. Wang, Oregon State Univ. (United States)

Both near-infrared (NIR, 780-2500 nm) and mid-infrared (MIR, 2.5-25 μm) spectroscopy have been developed for gas sensing. MIR spectroscopy offers remarkably high sensitivity by probing the fundamental vibrational and rotational transitions as the fingerprint of various gas molecules. However, most commercial mid-IR spectroscopy systems are large, expensive, heavy tabletop instruments. On the other hand, NIR gas sensing is miniaturized, low cost, and highly reliable; however, it has relatively low sensitivity due to the weak overtone absorption from the fundamental vibrational bond absorption at the mid-IR frequency. In this paper, we demonstrate ultra-sensitive NIR gas sensing for carbon dioxide (CO2) at 1.57 μm wavelength through metal-organic framework (MOF) Cu-BTC (BTC=benzene-1,3,5-tricarboxylate) coated on a single-mode optical fiber. For the first time, we obtained high-resolution NIR spectroscopy of CO2 adsorbed in MOF without seeing any rotational side band, indicating that the bound or tightly confined gas molecules in the pores of MOF do not have any freedom of rotation. Real-time measurement of the mixed gas flow of CO2 and Ar showed different response time depending on the concentration of CO2, which is attributed to the complex adsorption and desorption mechanism of CO2 in Cu-BTC. Most importantly, we obtained ultra-low detection limit of CO2 down to 20 ppm with only 5-cm long Cu-BTC film. Such ultra-sensitive NIR fiber-optic gas sensors can play pivotal roles in medical applications including breath diagnosis for various gas biomarkers such as CO, NO, acetone, and Carbonyl sulfide.

9702-7, Session 2
Label-free tracking of single extracellular vesicles in a nano-fluidic optical fiber
Edwin van der Pol, Univ. van Amsterdam (Netherlands); Stefan Weidlich, Heraeus Quarzglas GmbH & Co. KG (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany); Yoav Lahini, Massachusetts Institute of Technology (United States) and Harvard Univ. (United States); Frank A. W. Coumans, Auguste Sturck, Rienk Nieuwland, Univ. van Amsterdam (Netherlands); Markus A. Schmidt, Leibniz-Institut für Photonische Technologien e.V. (Germany) and Otto Schott Institute of Material Research, Friedrich-Schiller-Univ. Jena (Germany); Sanli Faez, Leiden Univ. (Netherlands) and Utrecht Univ. (Netherlands); Ton G. van Leeuwen, Univ. van Amsterdam (Netherlands)

Background: Extracellular vesicles, such as exosomes, are abundantly present in human body fluids. Since the size, concentration and composition of these vesicles change during disease, vesicles have promising clinical applications, including cancer diagnosis. However, since ~70% of the vesicles have a diameter <70 nm, detection of single vesicles remains challenging. Thus far, vesicles <70 nm have only been studied by techniques that require the vesicles to be adhered to a surface. Consequently, the majority of vesicles have never been studied in their physiological environment. We present a novel label-free optical technique to track single vesicles <70 nm in suspension.

Method: Urinary vesicles were contained within a single-mode light-guiding silica fiber containing a 600 nm nano-fluidic channel. Light from a diode laser (660 nm wavelength) was coupled to the fiber, resulting in a strongly confined optical mode in the nano-fluidic channel, which continuously illuminated the freely diffusing vesicles inside the channel. The elastic light scattering from the vesicles, in the direction orthogonal to the fiber axis, was collected using a microscope objective (NA=0.95) and imaged with a home-built microscope.

Results: We have tracked single urinary vesicles as small as 35 nm by elastic light scattering. Please note that vesicles are low-refractive index (n=1.4) particles, which we confirmed by combining data on thermal diffusion and light scattering cross section.

Conclusions: For the first time, we have studied vesicles <70 nm freely diffusing in suspension. The ease-of-use and performance of this technique support its potential for vesicle-based clinical applications.

9702-8, Session 2
Blood glucose measurement in vivo using hollow-fiber based, mid-infrared ATR probe with multi-reflection prism
Saiko Kino, Suguru Omori, Yuji Matsuura, Tohoku Univ. (Japan)

A hollow-optical fiber probe with a trapezoidal multi-reflection prism is proposed for measurement of blood glucose measurement. By combining the fiber probe with a conventional FT-IR system, ATR spectroscopy of any sites of human body is performed. In this presentation, absorption spectra of human inner lips are measured in vivo and it is shown that area of absorption peaks of glucose highly depends on blood glucose levels.
9702-9, Session 3

**Current progress and perspectives in laser-assisted tissue repair of vascular tissue (Invited Paper)**

Paolo Matteini, Francesca P. Rossi, Martina Banchelli, Istituto di Fisica Applicata “Nello Carrara” (Italy); Stefano Frisoni, Luca Giannoni, El.En. S.p.A. (Italy); Giancarlo Lupi, Univ. di Pisa (Italy); Guido Giachi, Ecopol S.p.A. (Italy); Federica Chiellini, Univ. di Pisa (Italy); Dario Puppi, Ecopol S.p.A. (Italy); Janis Spigulis, Ilze Lihacova, Univ. of Latvia (Latvia); Israel Gannot, Opticul Diagnostics (United States); Roberto Pini, Istituto di Fisica Applicata “Nello Carrara” (Italy)

The development of minimally invasive techniques holding the promise of reduced tissue pain and fast recovery is one of the leading trends in surgical medicine. In particular, the possibility to perform surgeries without sutures or staples is a valuable objective in view of minimally invasive clinical interventions. Laser-assisted sutureless repair consists of the interaction of a laser source with endogenous or exogenous optical absorbers at the wound site that convert the laser light into heat. The light emitted by a laser device is delivered through an optical fiber to the wound site inducing a localized photothermal effect suitable to join the wound walls. Specifically, the thermal modifications induced to biological components may generate strong bonds and in turn the repair of the wound.

Starting from the existing know-how, we developed a rapid, dependable, minimally invasive procedure for laser-mediated repair of arterial wounds and end-to-end anastomosis. The technique rests on the photothermal adhesion of a biocompatible and biodegradable patch that is wrapped over the artery to be repaired along with the use of a biodegradable polymeric stent as intraluminal structural support. The use of millisecond-long-pulsed NIR light generates a strong weld between the patch and the artery wall and under the threshold of irreversible thermal damage. This technique was shown to be effective in vivo and provides several advantages over conventional suturing methods, such as easy and fast surgical operations, as well as minimal surgical trauma to vessels.

9702-10, Session 3

**Closure of incisions in cataract surgery, using a temperature controlled system based on a 1.9µm semiconductor laser and on AgCl/Br fibers**

Svetlana Basov, Ilan Gabay, Tel Aviv Univ. (Israel); David Varsanso M.D., Tel-Aviv Sourasky Medical Ctr. (Israel); Irina S. Barequet, Mordechai Rosner M.D., Sheba Medical Ctr. (Israel); Marcel Rattunde, Joachim Wagner, Fraunhofer-Institut für Angewandte Festkörperphysik (Germany); Abraham Katzir, Tel Aviv Univ. (Israel)

In phacoemulcification cataract surgery a 2.0-3.0 mm long incision is typically made in the cornea and it is closed at the end of the procedure by hydration of the wound lips, or by suturing in complicated cases. We propose to seal the incision by laser soldering, which will provide stronger and more watertight adhesion. In our system the beam of a semiconductor laser based on a 1.9\(\mu\)m semiconductor laser is sent through a glass fiber and used to heat a spot on the approximated edges of the incision, which are covered with albumin solder. IR radiation emitted from the heated spot is transmitted through a small bundle of AgClBr fibers to an IR detector and used to monitor the temperature of the spot. The temperature is controlled at 60-65°C for ~10 sec. The laser beam is then moved to a neighboring spot and the process is repeated, spot by spot, along the incision. Laser radiation at ~1.9\(\mu\)m penetrates ~0.2mm into the cornea and is expected to provide strong bonding, yet it cannot reach the inner parts of the eye. The system was successfully tested in cataract incisions in 30 eyes of pigs, ex vivo. Histopathologic examination showed little thermal damage and good wound apposition. The average burst pressure was 1000±50mmHg, which is higher than the normal pressure in the eye. We will carry now experiments on live pigs. If the results will be good, this method might in the future replace suturing of various corneal wounds, including in traumatic corneal laceration and corneal transplantation.

9702-11, Session 3

**Active depth-guiding handheld micro-forceps for membranectomy based on CP-SSOCT**

Gyeong Woo Cheon, Phillip Lee, Berk Gonenc, Johns Hopkins Univ. (United States); Peter L. Gehlbach, Wilmer Eye Institute (United States); Jin U. Kang, Johns Hopkins Univ. (United States)

In this study, we validate a handheld motion-compensated micro-forceps and intuitive control system that allows the tip of the forceps to lock onto a desired depth during grasping. A thin layer of retina was removed with several tens of micron order accuracy for Epiretinal Membrane Peeling (Membranectomy), which is one of the most common vitrectomy procedures that are required to remove abnormally growing epiretinal membrane, also known as macular pucker and cellophane retinopathy. A surgeon is required to maintain the tool-tip position during the peeling procedure. In practice, these maneuvers are challenging as there is limited depth information available from the microscope and micron order accuracy is not humanly possible due to physiological hand tremor. Normal tremor exists mainly in the 6-12Hz frequency domain with several hundred micrometers of amplitude. There is prior work attempts to compensate for tremor. Our research group has been pursuing an axial motion compensation approach utilizing a swept source optical coherence tomography (SSOCT) distal sensor. The advantages of common path SSOCT (CP-SSOCT) as a distal sensor include that –it’s a simple structure, dispersion free, and polarization free. Here we utilize the ex vivo bovine eye to validate this novel handheld micro-forceps consisting of two piezo-linear motors, a touch sensor and a single mode fiber for motion compensation, grasping motion, and distal sensing.

9702-12, Session 3

**A simply way to establish a dual-core hollow fiber for medical laser surgery applications**

Chengbin Jing, East China Normal Univ (China); Wesley Kendall, James A Harrington, Rutgers University (United States)

A dual-core hollow fiber has a double-core structure. Each core is particularly designed to be capable of delivering a sort of light. Such a fiber is much welcome in hollow waveguide applications for laser surgery. In this case, an infrared laser beam is transmitted for burning tissues while a visible laser beam is simultaneously transmitted as a pilot light. In the past, to construct a dual-core hollow fiber, the inner and outer surfaces of a silica glass tube were coated with some low refractive index materials (i.e. fluorine doped silica glass). Subsequently, Ag and AgI layers were deposited inside the glass tube in sequence. In this work, a simply way was used to establish a dual-core hollow fiber. An AgI/AgI hollow glass fiber was fabricated for transmission of CO2 laser. Another silica glass capillary tube was chosen carefully. Its inner diameter is just slightly higher than the outer diameter of the AgI/AgI hollow fiber. The outer as well as inner surface of the as-selected glass tube was coated with some low refractive index materials (i.e. fluorine doped silica glass). Subsequently, Ag and AgI layers were deposited inside the glass tube in sequence. In this work, a simply way was used to establish a dual-core hollow fiber. An AgI/AgI hollow glass fiber was fabricated for transmission of CO2 laser. Another silica glass capillary tube was chosen carefully. Its inner diameter is just slightly higher than the outer diameter of the AgI/AgI hollow fiber. The outer as well as inner surface of the as-selected glass tube was coated with some low refractive index materials (i.e. fluorine doped silica glass). Subsequently, Ag and AgI layers were deposited inside the glass tube in sequence. In this work, a simply way was used to establish a dual-core hollow fiber. An AgI/AgI hollow glass fiber was fabricated for transmission of CO2 laser. Another silica glass capillary tube was chosen carefully. 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construct a dual-core hollow fiber. A visible laser light can be transmitted through the polymer-coated silica glass fiber according to an attenuated total reflection (ATR) mechanism. The CO2 laser beam can be delivered through the leaky AgI/Ag hollow glass fiber. This method does not involve chemical vapor deposition of a F doped silica glass layer inside a glass tube, which was needed for preparing Ag and AgI layers in the traditional fabrication procedure of a dual-core hollow fiber.

9702-13, Session 3
Endoluminal non-contact soft tissue ablation using fiber-based Er:YAG laser delivery
Dennis Kundrat, Alexander Fuchs, Andreas Schoob, Lueder A. Kahrs, Tobias Ortmair, Leibniz Univ. Hannover (Germany)

The introduction of Er:YAG lasers for soft and hard tissue ablation has proven promising results within the last decade due to strong absorption at 2.94 µm by water molecules. An extension to endoluminal applications demands laser delivery without mirror arms due to dimensional constraints. Therefore, fiber-based solutions are advanced to provide flexible access in combination with reduced space requirements. Conventional fiber-based treatments aim at free beam laser-tissue interactions in (quasi) contact mode. However, this procedure is associated to disadvantages such as advancing decrease in power delivery due to particle coverage of the fiber tip, carbonization, and obstructed observation of the ablation progress. The objective of this work is to overcome aforementioned limitations with a customized fiber-based module for non-contact robot-assisted endoluminal ablation and its associated experimental evaluation. Up to the authors’ knowledge, this approach has not been presented in the context of laser surgery at 2.94 µm. The preliminary system design is composed of an Er:YAG laser, 3D scanning unit enabling automatic laser to fiber coupling, a GeO2 solid core fiber, and a customized module combining collimation and focusing unit (focal length 20 mm, outer diameter 8 mm). The performance is evaluated with in vitro studies on tissue substitutes (agar agar) as well as porcine samples and consecutive optical coherence tomography measurements. Cuts (depths of approx. 3 mm) with strongly reduced carbonization have been achieved with optimized focal distance, adequate moistening and sample movement (~1.5mm/s). Future work aims at module miniaturization and integration into a flexible, endoluminal robot or endoscope.

9702-14, Session 4
Improvement of bimanual SMART micro-surgical system
Hyun-cheol Park, Cheol Song, Daegu Gyeongbuk Institute of Science & Technology (Korea, Republic of)

In microsurgery, precise dissection requires delicate and simultaneous manipulation by both a forceps and a scissors on every hand. Microsurgeons should be able to manipulate two micro-surgical tools freely at different heights. Involuntary hand tremor from each hand has different characteristics and a difficulty to compensate it by an actuator. Previous dual SMART hand-held probes had a difficulty in differentiating two optical coherence tomography (OCT) distance sensor signals from one-channel data acquisition. Two OCT signals can be ambiguous either after exchanging relative height of each probe or when two probes locate in same height. Here, we present bimanual SMART micro-surgical system to accomplish ambidextrous dissection precisely and freely. The system has two handheld probes which could function at any heights independently: a micro-forceps and a micro-scissors. Each probe consists of an actuator and a fiber-optic common-path swept source optical coherence tomography (CP SS-OCT) distance sensor. The bimanual SMART micro-surgical system is improved by implementing two-channel data acquisition scheme. The OCT signal to activate each actuator is obtained from each detection channel independently and simultaneously. This OCT signal could be an efficient guidance for each end-effector’s height without any interference between two hand-held probes. The system performance of both probes was evaluated by analyzing two OCT signals at various heights. Two independent detection channels guided bimanual micro-surgical system could demonstrate accurate dissection on dry phantom efficiently and safely.

9702-15, Session 4
Improved fiber probe for laser tissue ablation with integrated distributed temperature sensor
Yu Liu, Riccardo Gassino, Hao Yu, Politecnico di Torino (Italy); Andrea Braglia, Politecnico di Torino (Italy) and OPI Photonics s.r.l. (Italy); Alberto Vallan, Guido Perrone, Politecnico di Torino (Italy); Daniele Tosi, Nazarbayev Univ. (Kazakhstan)

Laser ablation (LA) is emerging as a more effective alternative to radio-frequency and microwave ablations in the therapy of certain types of solid tumors. According to medical literature, optimal cell necrosis requires an accurate balancing between temperature and exposure time; nevertheless, the temperature during laser irradiation is not easy to predict a priori because it is strongly dependent on the tissue composition, the possible presence of blood vessels and the laser beam characteristics. For this reason in last year BIOS/Photonics West conferences we introduced a probe for LA that is composed of a fiber with micro-structured surface to shape the laser beam irradiation pattern and that integrates for the first time the delivery of a high power beam with a fiber Bragg grating to sense the temperature. Successive experiments demonstrated the usefulness of this probe, despite the limitation of being able to measure the average temperature in a single location only due to the non-negligible length of the grating and the large temperature gradients typical of LA. This paper describes an evolution of such a probe using a chirped fiber grating to allow measuring in real-time the temperature distribution profile along the probe axis. First a model of the grating based on the theory of equivalent transmission lines has been developed to optimize the algorithm for the recovery of the temperature profile from the grating spectral response; then both phantoms and ex-vivo porcine livers have been used to experimentally validate the model and to practically demonstrate the measurement capabilities.

9702-16, Session 4
A sphere-taper cascaded microfiber for temperature sensing
Pei Xian, Guoying Feng, Hong Zhang, Shouhuan Zhou, Sichuan Univ. (China)

We propose a unique sphere-taper cascaded microfiber (STCM) on a standard single-mode fiber for temperature sensing. This device is fabricated by a fusion splicer and electric-arc discharge to form this special microsphere and taper connected in series structure. The whole STCM with a rather smooth surface brings low loss and measurement noise. Benefiting from the large difference of thermal coefficients of the core and cladding modes, the proposed STCM is applied to measure temperature by monitoring the wavelength shift of the interference spectrum and a sensitivity of 18 pm/°C is obtained in the temperature range of 30°-210°C. The sensor is featured with compact size, low cost, stability and easy fabrication, resulting in a very promising device for many other applications.
Comparison of surface micro-structured and plasmonic all-fiber delivery probes for laser-induced thermotherapy of tumor cells

Riccardo Gassino, Politecnico di Torino (Italy); Papiya Dhara, Politecnico di Torino (Italy) and Indian School of Mines (India); Yu Liu, Hao Yu, Politecnico di Torino (Italy); Andrea Braglia, Politecnico di Torino (Italy) and OPI Photonics s.r.l. (Italy); Massimo Oliviero, Alberto Vallan, Guido Perrone, Politecnico di Torino (Italy)

Laser induced thermotherapy or laser ablation (LA) is a very promising alternative to the more common radio-frequency and microwave ablations in the treatment of solid tumors not suitable for surgical resection. LA is performed by inserting a laser delivery probe through a catheter inside the tumor mass; hence, ideally, the irradiation pattern of the probe should cover an area of the right size to affect all the malignant cells without requiring repositioning of the probe. However, solid tumors have shapes and sizes largely variable; therefore a standard laser delivery fiber used in surgery, which irradiates from the tip end-face only, is not suitable for this application since optimal LA requires a more symmetrical heat zone extending all around the probe. This can be obtained by properly treating the delivery tip surface. The paper compares, both with simulations and experiments, two different approaches to design such a probe: i) micro-patterning of the fiber delivery tip surface to introduce controlled side irradiation so to properly shape the heat affected area; ii) deposition of a gold film on the fiber tip surface to realize a heated side area by exploiting the dissipation of the plasmonic waves excited at such gold/silica interface. In addition, for both probes we discuss the integration of a fiber Bragg grating to form an all-optical laser delivery probe with optimized heated area and moreover real-time monitoring of the temperature increase capability. Tests carried out using liver phantoms and ex-vivo porcine livers will be reported.

High-index-contrast multilayer hollow waveguides for mid-IR laser delivery

Jeffrey E. Melzer, Rutgers, The State Univ. of New Jersey (United States) and The Univ. of Arizona, College of Optical Sciences (United States); Wesley Y. Kendall, James A. Harrington, Rutgers, The State Univ. of New Jersey (United States)

Hollow glass waveguides (HGWs) have been researched extensively for the efficient transmission of radiation over a broad spectral range spanning from the visible region to the far-IR. One such HGW film structure consists of a metallic substrate with overlaying multilayer dielectric thin film stack of alternating high and low refractive index films. The optical properties of such multilayer thin film stacks are well established and provide a method for developing photonic bandgap fibers with exceptionally low attenuation losses at a desired wavelength. Transmission losses can be minimized in multilayer waveguides through two main approaches; either maximizing the number of alternating layer pairs or maximizing the index contrast between adjacent films. In practice, it has been shown that for liquid-phase deposition-based procedures, the former approach leads to compounding surface and interface roughness, negating the low-loss advantage of a multilayer waveguide. Thus, this research focuses on maximizing index contrast between adjacent dielectrics in an attempt to minimize the number of films required to achieve acceptable transmission characteristics both in theory and in practice. In this study, multilayer waveguides are fabricated using three dielectric materials: silver iodide, lead sulfide, and cyclic olefin copolymer. Through exploitation of their high index contrast, these materials are used to develop low-film-count multilayer waveguides designed for enhanced transmission at both Er:YAG and CO2 laser wavelengths.

Improved uniformity of meter long continuous sensor gratings in offset core fibers through correction of fiber lensing aberrations

Paul S. Westbrook, Kenneth S. Feder, Tristan Kremp, Thierry F. Taunay, Eric M. Monberg, Gabe S. Puc, OFS Fitel LLC (United States)

As fiber sensing technologies are being considered for a wider range of applications in medical devices, motion control, distributed sensing, and structural health monitoring, there has been renewed interest in the use of multicore fibers to increase sensing capabilities to include shape, force, position and other measurands in a single compact fiber. The use of discrete or continuous gratings in such fibers greatly increases the precision and signal to noise ratio available in various interrogation schemes, and it has been shown that such gratings can be fabricated continuously over long lengths of multicore fiber. A significant problem with gratings, though, is that conventional side writing techniques are impaired by lensing at the fiber surface. Such lensing can result in variations of the grating strength in cores that are offset from the fiber center. Strength variations can degrade the effectiveness of interrogation schemes that require high signal to noise and dynamic range.

In this work, we report the fabrication of grating arrays in multicore fibers with centered and offset cores where grating reflectivity variations from lensing have been greatly mitigated. Our system uses UV transparent coating, thus allowing for flexible and rapid processing without the requirement of stripping the coating. We exploit this flexibility to apply various materials at the surface of the fiber to reduce the effect of lensing at the fiber surface. While our system is effective for point gratings of cm length, it can also be used to mitigate lensing aberration in continuously written gratings over many meters of fiber.

Novel localized surface plasmon resonance based optical fiber sensor

Harald Ian Damm I. Muri, Dag R. Hjelme, Hgskolen i Sor-Trondelag (Norway) and Norwegian Univ. of Science and Technology (Norway)

Over the last decade various optical fiber sensing schemes based on local surface plasmon resonance (LSPR) have been proposed. LSPR sensing is achieved by either by interacting with the evanescent field from light propagating in the fiber core or by interacting with the light at the fiber endface. Sensor designs utilizing the fiber endface is strongly prefered from a manufacturing point of view. However, the different techniques available to immobilize metallic nanostructures on the fiber endface for LSPR sensing is limited to essentially a monolayer, either by photolithographic structuring of metal film, thermal nucleation of metal film, or by random immobilization of nanoparticles (NP). In this paper we report on a novel LSPR based optical fiber sensor architecture. The sensor is prepared by immobilizing gold NP in a hydrogel droplet polymerized on the fiber endface. This design has several advantages over earlier designs. It dramatically increase the number of NPs available for sensing, it offers precise control over the NP density, and the NPs are position in a true 3D aqueous environment. The sensor design is also compatible with low cost manufacturing. The sensor can be configured to measure volumetric changes in a stimuli-responsive hydrogel or to measure binding to receptors on the NP surface. It can also be uses as a two-parameter sensor by utilizing both effects. We present results from proof-of-concept experiments demonstrating a pH sensor based on LSPR sensing in a pAAm hydrogel.
Wavelength and intensity dispersion analysis of Si Av LEDs for futuristic biosensor applications

Timothy A. Okhai, Tshwane Univ. of Technology (South Africa) and Univ. Paris-Est Marne-la-Vallée (France); Lukas W. Snyman, Univ. of South Africa (South Africa); Jean-Luc Polleux, ESIEE Paris (France)

1. Introduction:
Research work around the field of Silicon bio-photonics and lab-on-chip biosensors have become a very significant area of interest in the internationally scientific community [1-10]. The last 10 years particularly has seen the emergence of the field of “Silicon Photonics” and “Biomedical photonics” [11-14]. So also has been the increased utilization of complementary metal oxide semiconductor (CMOS) fabrication processes, which are among the most robust and widely used fabrication processes in the semiconductor industry, for biosensor applications. The advantage of this technology includes advanced processing of data at ultra-high speeds, advanced optical signal processing, and the ability to analyse diverse optical data directly on chip. This paper presents the analysis of a dispersion phenomenon (as a function of exit angle) observed with Si Av LED that emits in the 650 – 850nm range when tilted at various angles.

2. Technical Details
Light emitting devices based on Si Avalanche technology which emit in the 450-650nm regime has been known since quite early [15-19]. Viable CMOS compatible and avalanche based Si LEDs (Si CMOS AvLEDs) have however, only emerged since the 1990’s [20-23]. These devices can emit up to 10nW / lm2 at 450 -650nm regime at compatible CMOS operating voltages and currents, and can be realized using standard CMOS design and processing procedures at great ease [24]. A series of practical first iteration and utilisable LEDs in standard CMOS technology have been realized by Kramer et al [20] and Snyman et al [25-26] using the avalanche light emitting phenomenon. The developed devices showed about three orders of increase in optical output as compared with previous similar work. Substantial development work has subsequently been performed in this field [27-32]. Particularly promising results have then been obtained regarding further increasing the efficiency, as well as the emitted intensity with injection-enhanced and avalanche based technology [27]. This technology utilizes the hypothesis that the light emission in these structures can be increased due to interaction of high energy (hot) electrons, as excited in the avalanching junction.

3. Experimental results
We have performed a dispersion analysis of a Si Av LED test structure to analyze the dispersion of red, green and blue pixels at tilt angles of 10°, 15°, 30°, 45° and 60°. We took a pixel at lateral tilts to the right and to the left at tilt angles of 10°, 15°, 30°, 45° and 60°. We did the same for horizontal tilt forwards and backwards at same tilt angles of the Silicon Avalanche LED test structure. For each tilt position, a dark field CCD photomicrograph was captured at x1000 magnifications showing the dispersion of red, green and blue pixels. The number of RBG pixels per exit angle at each tilt position were analysed and plotted on RGB curves.

4. Conclusion
The dispersion characteristics is very important as we find that CCD mapping of wavelength (?) and intensity (I) would provide future potential to enable complete identification of bio species through absorption and receptor analysis. We also conclude that surface morphology has an effect on the light dispersion due to tilt.

Electric field Monte Carlo simulation for studying the backscattering coherence phenomenon with diverging beam illumination from fiber

Wenli Wu, Andrew J. Radosевич, Adam Eshein, The-Quyen Nguyen, Vadim Backman, Northwestern Univ. (United States)

Diverging beam illumination is widely used in many optical techniques especially in fiber optic applications and coherence phenomenon is one of the most important properties to consider for these applications. Until now, people have used Monte Carlo simulations to study the backscattering coherence phenomenon in collimated beam illumination only. We are the first one to study the coherence phenomenon under the exact diverging beam geometry by taking into account the impossibility of the existence for the exact time-reversed pair paths of photons, which is the main contribution to the backscattering coherence pattern in collimated beam. In this work, we present a Monte Carlo simulation that considers the influence of the illumination numerical aperture. The simulation tracks the electric field for the unique paths of forward path and reverse path in time-reversed pairs of photons as well as the same path shared by them. With this approach, we can model the coherence pattern formed between the pairs by considering their phase difference at the collection plane directly. To validate this model, we use the Low-coherence Enhanced Backscattering Spectroscopy, one of the instruments looking at the coherence pattern using diverging beam illumination, as the benchmark to compare with. In the end, we show how this diverging configuration would significantly change the coherent pattern under coherent light source and incoherent light source. This Monte Carlo model we developed can be used to study the backscattering phenomenon in both coherence and non-coherence situation with both collimated beam and diverging beam setups.

Advanced biosensing methodologies developed for evaluating performance quality and safety of emerging biophotonics technologies and medical devices (Keynote Presentation)

Ilko K. Ilev, Bennett Walker, William Calhoun, Moinuddin Hassan, U.S. Food and Drug Administration (United States)

Biophotonics is an emerging field in modern biomedical technology that has opened up new horizons for transfer of state-of-the-art techniques from the areas of lasers, fiber optics and biomedical optics to the life sciences and medicine. This field continues to vastly expand with advanced developments across the entire spectrum of biomedical applications ranging from fundamental “bench” laboratory studies to clinical patient “bedside” diagnostics and therapeutics. However, in order to translate these technologies to clinical device applications, the scientific and industrial community, and FDA are facing the requirement for a thorough evaluation and review of laser radiation safety and efficacy concerns. In many cases, however, the review process is complicated due the lack of effective means and standard test methods to precisely analyze safety and effectiveness of some of the newly developed biophotonics techniques and devices. There is, therefore, an immediate public health need for new test protocols, guidance documents and standard test methods to precisely evaluate fundamental characteristics, performance quality and safety of these technologies and devices. Here, we will overview our recent developments of novel test methodologies for safety and efficacy evaluation of some emerging biophotonics technologies and medical devices. These methodologies are based on integrating the advanced features of state-of-the-art optical sensor technologies and approaches such as high-resolution fiber-optic
Fiber optic biofluorometer for physiological research on muscle slices

Mathias Belz, World Precision Instruments (Germany); Andreas Dendorfer, Walter Brendel Zentrum für experimentelle Medizin, Ludwig-Maximilians-Univ. München (Germany); Jan Werner, Karl-Friedrich Klein, Technische Hochschule Mittelhessen (Germany)

A focus of physiological research is the detection of Ca²⁺, NADH, FAD, ATPase activity or Membrane Potential, only to name a few, in muscle slices. In this work, we report on a biofluorometer using UV-LEDs, optical fibers, and two PMTs using synchronized fluorescence detection with integrated background correction in a horizontal tissue bath.

A fiber optic probe with imaging optics has been designed to transport excitation light from the biofluorometer’s light output to a horizontal tissue bath and to collect emission light from a tissue sample of interest to two PMTs allowing either single excitation / single emission or ratiometric, dual excitation / single emission or single excitation / dual emission fluorescence detection of indicator dyes or natural fluorophores. We discuss the efficient transport of light from the excitation LEDs to the tissue sample, bleaching effects of the excitation light in both, polymer and fused silica-based fibers. Further, we show an approach to maximize light collection of the emission light using high NA fibers and a high NA coupling optics. Finally, we show first results on Ca²⁺ measurements in muscle slices in a horizontal tissue bath.

Biophotonic low-coherence sensors with boron-doped diamond thin layer

Daria Milewska, Katarzyna Karpienko, Michal Sobaszek, Małgorzata Jóźdrzewska-Szczerska, Gdansk Univ. of Technology (Poland)

Low-coherence sensors using Fabry-Perot interferometers are finding new applications in biophotonic sensing, especially due to the rapid technological advances in the production of new materials. In this paper we discuss the possibility of using boron-doped nanodiamond layers to protect mirror in a Fabry-Perot interferometer. A low-coherence sensor using Fabry Perot interferometer with a boron-doped nanodiamond (B-NCD) thin protective layer has been developed. B-NCD layers with different boron doping level were investigated. The boron level, expressed as the [B]/[C] ratio in the gas phase, was 0, 2000, 5000 or 10000 ppm B-NCD layers were processed with the use of chemical vapor deposition (CVD).

The sensing Fabry Perot interferometer, working in the reflective mode, was connected to the source and to the optical processor by single-mode fibers. Supravisionaceous diodes with Gaussian spectral density were used as sources, while an optical spectrum analyser was used as an optical processor. The design of the sensing interferometer was optimized to attain the maximum interference contrast. Performance of the sensor was consistent with design predictions, and have shown that B-NCD B-NCD thin layers can be successfully used in biophotonic measurements.

Simultaneous monitoring the real and imaginary parts of the analyte refractive index using liquid-core photonic bandgap Bragg fibers

Jingwen Li, Ecole Polytechnique de Montréal (Canada)

We demonstrate simultaneous monitoring of the real and imaginary parts of the liquid analyte refractive index by using a hollow-core Bragg fiber. We apply this two-channel fiber sensor to monitor concentrations of various commercial cooling oils including heat transfer fluid and sawing fluid. The sensor operates using spectral monitoring of the fiber bandgap center wavelength, as well as monitoring of the fiber transmission amplitude at mid-bandgap position. Both measurements are highly sensitive to the complex value of the fiber core refractive index, thus allowing its efficient determination and cross-correlation with the concentrations of the cooling oils. The fiber sensor is first calibrated using NaCl solutions of different concentrations. By measuring spectral shift of the fiber bandgap, the sensitivity of changes in the real part of the core refractive index is found to be 1460nm/RIU, which translates into 2.6nm/w% sensitivity to changes in the NaCl concentration. Additionally, using changes in the mid-bandgap transmission amplitude of a Bragg fiber, sensitivities of 3.55dB/W% for NaCl solution are demonstrated.

In this work, we demonstrate the simplification and development of label-free fiber optic biosensors based on immobilization of oligonucleotides on dual-peak long period fiber gratings

Xianfeng Chen, Chen Liu, Bangor Univ. (United Kingdom); Marcos R. Cardoso, Cleber R. Mendonça, Univ. de São Paulo (Brazil); David A. Nagel, Anna V. Hine, Lin Zhang, Aston Univ. (United Kingdom)

In this work, we demonstrate the simplification and development of label-free fiber optic biosensors based on immobilization of oligonucleotides on dual-peak long period gratings (dLPGs). A one-step 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-mediated reaction has been developed for the straightforward immobilization of unmodified oligonucleotides on the glass fiber surface along the grating region, leading to covalent attachment of a 5′-phosphorylated probe oligonucleotide to the amino-derivatized fiber grating surface. The simplification of biomolecule immobilization procedure is feasible by using only EDC as the heterobifunctional cross-linker for 5′-phosphorylated oligonucleotides. The dual long period grating has been measured in different external media to demonstrate its inherent ultrahigh sensitivity to the surrounding-medium refractive index (RI) achieving 50-fold improvement in RI sensitivity over the previously-published LPG sensor in media with RI’s relevant to biological assays. The probe oligonucleotide- functionalized dLPG has been used for experimentally monitoring the hybridization of probe and complementary oligonucleotides in real-time, showing a detectable oligonucleotide concentration of 4 nM. The proposed one-step EDC reaction approach can be further extended to develop fiber optic biosensors for disease analysis and medical diagnosis with the advances of label-free, real-time, multiplex, high sensitivity and specificity.
Estimating needle-tissue interaction forces for hollow needles using fiber Bragg grating sensors

Saurabh Kumar, Indian Institute of Science (India) and Bosch Research and Technology Ctr. (India); Sundarrajan Asokan, Bharadwaj Amrutur, V. Shrikanth, M. S. Bobji, Indian Institute of Science (India)

Needles form a critical component of several simple and complex procedures like drug delivery, anesthetic delivery, biopsy, radiological cancer therapy, neurological procedures etc. Complex procedures like Radiological Cancer Therapy (e.g. Brachytherapy) and Neurological Procedures can benefit from real-time estimation of needle-tissue interaction forces specifically for robotic or robot-assisted procedures.

Steering of solid flexible needles has been extensively studied including determination of bending using Fiber Bragg Grating (FBG) sensors. However, hollow needles with bevel have differences in properties. Though some work has been published in this area, details of needle-tissue interactions for hollow needles and the effective mechanisms to steer needles accurately to the correct location are still being studied. Methods involving force measurements at the needle hold require modelling of tissue properties including friction which have several challenges. The work presented in this paper shows the use of FBG sensors bonded at the tip inside the needle lumen to detect the needle-tissue interaction forces and identify events of interest. Phantoms with multiple layers of varying stiffness have been created using Polydimethylsiloxane along with small nodules with high stiffness to induce bending. The identification of events of interest is highlighted.

Both simulations and measurements show that the tip forces are small and hence it is required to accurately perform strain-temperature discrimination. Another important aspect of using FBG sensors is the interrogation method and this work shows the applicability of relatively lower cost interrogation systems based on linear CCD array.

Sensorization of a surgical robotic instrument for force sensing

Kaspar S. Shahzada, Aaron Yurkewich, Ran Xu, Rajni V. Patel, Western Univ. (Canada) and Canadian Surgical Technologies and Advanced Robotics (Canada)

This paper presents the development and application of an approach for sensorizing a surgical robotic instrument for two degree-of-freedom lateral force sensing. The sensorized instrument is compatible with the daVinci® Surgical System and can be used for skills assessment and force control in specific surgical tasks. The sensing technology utilizes a novel layout of four fiber Bragg grating (FBG) sensors attached to the shaft of a daVinci® surgical instrument. The two cross-section layout is insensitive to error caused by combined force and torque loads, and the orientation of the sensors minimizes the condition number of the instrument’s compliance matrix. To evaluate the instrument’s sensing capabilities, its performance was tested using a commercially available force-torque sensor, and showed a resolution of 0.05N at a 1 kHz sampling rate. The performance of the sensorized instrument was evaluated by performing three surgical tasks on phantom tissue using the daVinci® system with the daVinci® Research Kit (dVRK): tissue palpation, knot tightening during suturing and Hem-O-Lok® tightening during knotless suturing. The tasks were designed to demonstrate the robustness of the sensorized force measurement approach. The full paper will report the results of further evaluation by a group of expert and novice surgeons performing the three tasks mentioned above.

Curvature and torsion sensing for pre-curved continuum robots

Ran Xu, Aaron Yurkewich, Rajni V. Patel, Western Univ. (Canada) and Canadian Surgical Technologies and Advanced Robotics, London Health Sciences Ctr. (Canada)

Concentric-tube robots (CTR) are a recent development in surgical robotics for demanding operations such as neurosurgery and beating-heart surgery. The series of pre-curved flexible tubes that make up the robot structure provide the high dexterity required for performing surgical tasks in constrained environments. However, this special design introduces new challenges in shape sensing as large twisting is experienced by the robot structure. In the literature, fiber Bragg grating (FBG) sensors are attached to needle-sized continuum robots for curvature sensing, from which the shape and pose of the robot are estimated. These sensing designs are limited to obtaining bending curvatures since a straight sensor layout is utilized. For a CTR, in addition to bending curvatures, the torsion along the robot’s shaft is required to calculate the shape and pose of the robot accurately. As a solution for this requirement, our novel design involves engraving helical slots into the body of a continuum robot using a custom-designed micromachining system, and embedding FBG sensors into these grooves. A strain-curvature model is proposed to convert the strain values from helically-oriented FBG sensors to torsion and bending curvatures. To evaluate this sensing technology for CTRs, helical grooves were engraved into a Nitinol tube. The tube was then heated treated into a curved shape and three FBG sensors with 1 mm sensing segments were bonded into the grooves. Experimental results gave accurate measurements, and using a 100 Hz sampling rate, resolutions of 0.02 rad/m for torsion and bending curvatures were achieved.

Quantitative optical coherence elastography based on fiber-optic probe with integrated Febry-Perot force sensor

Yi Giu, Yahui Wang, New Jersey Institute of Technology (United States); Yiqing Xu, The Univ. of Hong Kong (Hong Kong, China); Namas Chandra, James Haorah, New Jersey Institute of Technology (China); Basil Hubbi, Bryan J. Pfister, Xuan Liu, New Jersey Institute of Technology (United States)

Optical coherence tomography (OCT) is a versatile imaging technique and has great potential in tissue characterization for breast cancer diagnosis and surgical guidance. In addition to structural difference, cancerous breast tissue is usually stiffer compared to normal adipose breast tissue. However, previous studies on optical coherence elastography (OCE) are qualitative rather than quantitative. It is challenging to identify the cancerous status of tissue based on qualitative OCE results obtained from different measurement sessions or from different patients. Therefore, it is critical to develop a technique that integrates structural imaging and force sensing, for quantitative elasticity characterization of breast tissue.

In this work, we demonstrate a fiber-optic quantitative OCE (qOCE) microsurgery device which simultaneously quantifies force exerted to tissue and measures the resultant tissue deformation. The qOCE system is based on a spectral domain OCT engine operated at 1300 nm and a fiber-optic probe with an integrated Febry-Perot (FP) interferometric cavity at its distal end. The FP cavity is formed by the cleaved end of the lead-in fiber and the end surface of a GRIN lens which allows light to incident into tissue for structural imaging. The force exerted to tissue is quantified by the change of FP cavity length which is interrogated by a fiber-optic common-paths phase resolved OCT system with sub-nanometer sensitivity. Simultaneously, image of the tissue structure is reconstructed from tissue through the GRIN lens. Tissue deformation is obtained through Doppler
9702-33, Session 9  
**Development of nanomaterials-based strain sensor for OCT sensor-guided SMART surgical tool**  
Phillip Lee, Gyeong Woo Cheon, Peter L. Gehlbach, Jin U. Kang, Johns Hopkins Univ. (United States)

Strain sensors with nanomaterials were designed and their properties were measured for surgical tool application. Nanomaterials-based strain sensor is useful in various medical applications because most surgical tools are composed of small components. This technology is expected to have a great potential especially in handheld surgical tools that require compact and light parts with good performance. We also developed OCT-guided SMART surgical tool which the strain sensor can be mounted on. OCT sensor enables the surgical tool to accurately measure the distance from target surface, allowing the operating tip to be precisely positioned. The positional information from the OCT sensor is converted to data for motor control in real time. The motor is connected to the operating tip of surgical tool. This system allows the operating tip to maintain the same distance from the target surface, thereby compensating errors from human tremor. In addition to minimizing human tremor using the OCT sensor’s distance control ability, strain sensor can also benefit surgical procedures by detecting force applied on the operating tip. Characteristics of the suggested strain sensor can be modulated depending on the types of nanomaterials, the amount of nanomaterials, the structure of nanomaterials and etc. In this research, various nanomaterials such as metallic nanowires, carbon nanotubes and graphene flakes were applied in strain measurement. Silver nanowires, carbon nanotubes and graphene flakes were filtered sequentially to make a thin composite film and this film was transferred to a stretchable polymer. As a result, the film showed strain-sensitive conductance. This fabricated thin film can be used as a strain sensor and can be mounted on handheld surgical tools. This research shows that OCT-guided surgical tool with nanomaterials-based strain sensor has ability to not only promote efficient surgery but also provide beneficial information about the surgery.

9702-34, Session 9  
**Mechanical properties of polyimide coated optical fiber at elevated temperatures**  
Lei Huang, OFS Fitel LLC (United States)

As applications for and adoption of fiber optics continue to expand beyond telecommunications, optical fiber may need to survive much harsher environments, such as at elevated temperatures in a sterilization oven or in the extreme conditions found in an oil and gas well. The majority of the knowledge of optical fiber’s mechanical properties and lifetime predictions exist around traditional uses in optical telecommunication. In order to answer the questions regarding the performance and reliability of optical fiber for harsh environment applications, new knowledge is necessary. In this paper, we will report our results on tensile strength and fatigue resistance factor, or n value, of polyimide coated optical fiber that is tested while at elevated temperatures in the vicinity of 300 °C. We will compare the result with the weight loss behavior of the coating in a similar environment to understand the relationship between mechanical strength and coating integrity. This will, for the first time, provide a direct observation that will allow us to gain important insight into the mechanism of fiber’s mechanical failure at elevated temperatures. We will also describe the test apparatus developed and test methodology employed in addition to the analysis and discussion on the results.

9702-35, Session 9  
**Silver hollow optical fibers with acrylic silicone resin coating as buffer layer for sturdy structure**  
Katsumasa Iwai, Hiroyuki Takaku, Sendai National College of Technology (Japan); Mitsunobu Miyagi, Tohoku Institute of Technology (Japan); Yi-Wei Shi, Xiao-Song Zhu, Fudan Univ. (China); Yuji Matsuura, Tohoku Univ. (Japan)

For sturdy silver hollow optical fibers, acrylic silicone resin is newly used as a buffer layer between an inner silver layer and a silica capillary. This acrylic silicone resin film prevents the glass surface from chemical and mechanical micro damages during silver plating process, which deteriorate mechanical strength of the hollow fibers. In addition, it keeps high adhesion of the silver layer with the glass surface. We discuss improvement of mechanical strength of the hollow glass fibers without deterioration of optical properties.

9702-36, Session 9  
**Fiber optic probes based on silver-only coated hollow glass waveguides for ionizing beam radiation dosimetry**  
Arash Daraafsheh, Haoyang Liu, Univ. of Pennsylvania (United States); Jeffrey E. Melzer, James A. Harrington, Rutgers, The State Univ. of New Jersey (United States); Timothy C. Zhu, Jarod C. Finlay, Univ. of Pennsylvania (United States)

Fiber optic probes with scintillating materials are promising candidates for radiation therapy dose assessment and quality assurance. However, in therapeutic radiation fields, the total optical signal carried by the fiber has unwanted components in addition to the useful scintillator’s signal. The main problem with fiber optic dosimeters is these unwanted signals that are primarily the Čerenkov radiation generated in the irradiated portion of the fiber. In this work, in order to enhance the scintillation signal transmission while minimizing the effect of Čerenkov contamination, we design, fabricate, and characterize a fiber probe based on using a silver-only coated hollow waveguide (HWG). The probe is composed of scintillating material inserted inside a silver-only coated HWG. The probe, embedded in tissue-mimicking phantoms, was irradiated with electron and photon beams produced by a medical linear accelerator. Optical spectra of irradiated tips were taken using a fiber spectrometer coupled to the distal end of the HWG by a solid-core glass fiber. By using a singular value decomposition method with basis spectra for the scintillator and Čerenkov, the luminescence signal from the scintillator is spectrally separated from the total recorded signal. The resultant decomposed spectra corresponding to the emission from the scintillator obtained with and without explicitly accounting for Čerenkov signal were in good agreement with measurements performed by an electron diode and ion chamber, indicating the minimal effect of Čerenkov contamination. Compared with a silver/dielectric coated HWG fiber dosimeter design we observed higher signal transmission in our design based on the silver-only HWG.

9702-37, Session PMon  
**Time resolved based fiber optic FRET sensor: a potential lead in medical diagnostic and remote sensing applications**  
Nabarun Polley, Samir K. Pal, S.N. Bose National Ctr. for
Bowel perforation detection using metabolic fluorescent chlorophylls

Jung Hyun Han, Gwangju Institute of Science and Technology (Korea, Republic of); Young Goun Jo, Chonnam National Univ. Hospital (Korea, Republic of); Yong-Chul Kim, In-Wook Hwang, Gwangju Institute of Science and Technology (Korea, Republic of)

Chlorophyll derivatives that abundantly exist in food plants show brighter and longer-wavelength-regime emissions (at >650 nm) than those of auto-fluorescence of bio-tissues or -organs. In bowel perforation, intestinal fluids, which can leak from perforation sites, may show brighter and longer-wavelength regime emissions of chlorophyll derivatives than those of pure peritoneal fluid or bio-organs. In order to examine a feasibility of fluorescence spectroscopy to be utilized as a real-time bio-sensor without using contrast agents for monitoring bowel perforation, we comparatively analyses fluorescence obtained from peritoneal and intestinal fluids of mouse and rat models administered with alfalfa free feed known to minimize auto-fluorescence and improve imaging clarity. Fluorescence measurements are conducted with a portable optical-fiber coupled-fluorescence spectrometer equipped with a continuous-wave diode laser operating at 532 nm and a spectral analyzer coupled with a photodiode-array (detecting wavelengths of 187-1045 nm with a spectral resolution of 0.1 nm). Compared to pure peritoneal fluid and bio-tissues or -organs, perforated sites contaminated with intestinal contents show much brighter emissions i.e., increased in intensity by a factor of >8 and longer wavelength regime fluorescence bands at ~680 and ~710 nm, which are assigned to vibronic bands of fluorescence of pheophytin (a chlorophyll derivative). We also performed fluorescence imaging measurements for bowel perforation, which are enabled by selective monitoring of long wavelength regime emissions.

Black-glass optical-fiber preform preparation for a high resolution endoscope

Soichi Kobayashi, Kaoru Fukuda, Chitose Institute of Science and Technology (Japan); Yusuke Fuji, Photonic Science and Technology, Inc. (Japan)

A higher resolution endoscope for photodynamic therapy (PDT), diagnosis and detection is required for irradiation with excitation light, improvement of color reproducibility and detection of fluorescence in ultraviolet or far-infrared light than a CCD camera. An ideal optical fiber bundle can be
expected to have smaller pixels and clear images compared with the CCD. In this report the new black-glass fiber-preform fabricated by the vapor-phase axial deposition (VAD) method to realize high-resolution optical bundle fibers is discussed with the Energy Dispersive X-ray (EDX) analysis and the transmittance spectrum measurement. The black glass consists of SiO2, GeO2, Bi2O3 and Al2O3. Firstly, the rod-shaped soot of SiO2 and GeO2 is prepared by blowing SiCl4 and GeCl4 into the oxyhydrogen burner. Then the soot is soaked into the solution of the Bi and Al compounds. After drying the soot with Bi and Al penetrated, the soot is consolidated into the glass preform by heating with the carbon heater at 1650 degree. The diameter of the obtained preform is 12 mm and the black glass layer thickness is 3 mm located at the periphery. The Bi concentration distribution shows the content of 5.4 ~ 13 wt% in the black glass layer. By using the tungsten lamp and the spectrometer the very low transmittance of the black glass was measured as the same value over the visible and infrared wavelength ranges. The black glass preform is drawn into the black optical fiber being expected to make a clear image because of no light leaking from the neighboring optical fibers as compared to the conventional fiber endoscope.

9702-42, Session PMon

Optical coherence tomography application by using optical phase shift based on fiber optic sensor

Seung Suk Lee, Joo Ha Kim, Chosun Univ. (Korea, Republic of); Tae Joong Eom, Gwangju Institute of Science and Technology (Korea, Republic of) and Advanced Photonics Research Institute (Korea, Republic of); Eun-Seo Choi, Chosun Univ. (Korea, Republic of)

We present the performance of fiber optics sensor based on in-fiber interferometer for the application of full range complex optical coherence tomography(OCT). To extend imaging range in OCT, real value measured from interferometer is converted to complex value by using various treatments such as mechanical phase stepping, offset shifting of scanner pivot, optical phase shift using fiber coupler and so on. As an alternative for optical phase shifter, we suggested optical phase shift method based in-fiber Mach-Zehnder interferometer. Higher mode excitation in the optical fiber could be utilized for in-fiber optical interferometer and the external perturbation such as pressure, vibration and temperature is detected by using this kind of fiber sensors. Reverse, intentionally induced external vibration could be operated for the optical phase shifter in the optical fiber depending on the amount of phase shift. By controlling operating frequency and amplitude of the external vibration, we could exactly adjust the phase shift of fiber-based optical coherence tomography system. With presenting real-time full range complex OCT imaging, the feasibility of the proposed method on the extending of optical imaging range would be proven. This method could be optical delay line with increasing the amount of phase shift and all-optical solution for optical treatment in OCT.

9702-43, Session PMon

Fiber optic pH-sensor with fast response time, broad operating range and simple readout system

Krister Hammarling, Magnus Engholm, Mid Sweden Univ. (Sweden); Beatrice Fiorini, Università degli studi di Modena e Reggio Emilia (Italy)

In this work we demonstrate a compact, fast and cost effective pH-sensor system for use in e.g. medical applications. The sensor is based on a fiber-optic interferometer technology. The fiber optic sensor is coated with a bio-compatible pH-sensitive polymer and can be customized for a large operating range (pH2-15) or for high sensitivity within a specific range, for ex. in the blood. The sensor has a very short response time, less than 15s. The readout system for optical sensors have a tendency to be expensive and complicated to manufacture, thereby limit any potential markets. By using standard optical telecom components, together with a smart system setup and readout, one can greatly reduce the total cost for manufacturing and components, thereby making a more cost effective sensor system. Our readout system is based on an Erbium doped fiber amplifier (EDFA) used as an amplified spontaneous emission (ASE) light source, together with a few optical filters and photo diodes. A smart algorithm ensure that the system has a high accuracy, without the need for expensive optical components. The sensor works as a Mach-Zehnder interferometer capable of sensing the surrounding effective refractive index. Together with a pH stimuli-sensitive hydrogel made of 1,4-Butanediol Diacrylate and Piperazine one get a fast and accurate pH sensor.

This sensor system can easily be integrated in a multiple sensor systems for simultaneous measurement of e.g. glucose, temperatures, blood gases etc. The sensor can be built either as a single strand sensor or as an inline sensor.

9702-44, Session PMon

Novel light diffusing fiber for use in medical applications

W. Spencer S. Klubben III, Stephan L. Logunov, Edward J. Fewkes, Jeff Mooney, Paul M. Then, Horst Schreiber, Cynthia J. Wilson, Kaitlyn Matias, Manuela Ocampo, Corning Incorporated (United States)

Fiber-based cylindrical light diffusers are often used in photodynamic therapy to illuminate a luminal organ, such as the esophagus. The diffusers are often made of plastic and suffer from short diffusion lengths, on the order of centimeters. We have developed Fibrance (TM), a glass-based fiber optic cylindrical diffuser which can illuminate from 0.5 cm to 10 meters over a broad wavelength range. With these longer illumination lengths, a variety of other medical applications are possible beyond photodynamic therapy. We present a number of applications for Fibrance ranging from in situ controllable illumination for Photodynamic Therapy to light guided anatomy highlighting for minimally invasive surgery and more.
Towards the mid-infrared optical biopsy (Invited Paper)

Angela B. Seddon, Trevor M. Benson, Slawomir Sujecki, Nabil S. Abdel-Moneim, Zhuoqi Tang, David Furniss, Lukasz Sojka, The Univ. of Nottingham (United Kingdom); Ian Lindsay, Jon D. Ward, Gooch & Housego PLC (United Kingdom); Mark Farries, Gooch & Housego (Torquay) Ltd. (United Kingdom); Peter M. Moselund, NKT Photonics A/S (Denmark); Bruce Napier, Vivid Components Ltd. (Germany); Samir Lamrini, LISA Laser Products OHG (Germany); Christian R. Petersen, Uffe V. Møller, DTU Fotonik (Denmark); Irnis Kubat, Technical Univ. of Denmark (Denmark); Ole Bang, DTU Fotonik (Denmark)

We are establishing a new paradigm in mid-infrared molecular sensing, mapping and imaging to open up the mid-infrared spectral region for in vivo (i.e. in person) medical diagnostics and surgery (Seddon-Intl-J-Appl-Glass-Sci-2011). Thus, we are working towards the mid-infrared optical biopsy (‘opsy’ look at, bio the biology) in situ in the body for real-time diagnosis. This new paradigm will be enabled through focused development of devices and systems which are robust, functionally designed, safe, compact and cost effective and are based on active and passive mid-infrared optical fibers. In particular, this will enable early diagnosis of external cancers, mid-infrared detection of cancer-margins during external surgery for precise removal of diseased tissue, in one go during the surgery, and mid-infrared endoscopy for early diagnosis of internal cancers and their precision removal.

The mid-infrared spectral region has previously lacked portable, bright sources. We set a record in demonstrating extreme broad-band supercontinuum generated light 1.4 to 13.3 microns in a specially engineered, high numerical aperture mid-infrared optical fiber (Petersen-et-al-Nat-Photon-2014). In addition, record loss of 80 dB/km is achieved in 52 m of mid-infrared transmission fibers (Tang-et-al-Opt-Mat-Exp-2015). The active mid-infrared fiber broadband supercontinuum for the first time offers the possibility of a bright mid-infrared wideband source in a portable package as a first step for medical fibre-based systems operating in the mid-infrared. Moreover, mid-infrared molecular mapping and imaging is potentially a disruptive technology to give improved monitoring of the environment, energy efficiency, security, agriculture and in manufacturing and chemical processing (Seddon-Intl-J-Appl-Glass-Sci-2011).

Identification of GI cancers utilising rapid mid-infrared spectral imaging (Invited Paper)

Nick Stone, Jayakrupakar Nallala, Univ. of Exeter (United Kingdom); Gavin R. Lloyd, Rebecca Griggs, Oliver Old, Neil A. Shepherd, Hugh Barr M.D., Gloucestershire Hospitals NHS Foundation Trust (United Kingdom)

Pathologists find it are notoriously difficult to provide both inter- and intra-observer agreement on a diagnosis of early gastrointestinal cancers. Vibrational spectroscopic approaches have shown their value in providing molecular compositional data from tissue samples and therefore enabling the identification of disease specific changes, when combined with multivariate techniques.

Mid-infrared microscopic imaging is undergoing rapid developments in sources, detectors and spectrometers. Here we explore the use of high magnification FTIR and discuss alternative approaches for spectral image analysis of gastrointestinal tissues. We will outline results of our ongoing study MINERVA (Mid- to NEAr infrared spectroscopy for improvement of medical diagnostics).

A two-step framework for the registration of HE stained and FTIR images

Francisco Peñaranda, Valery Naranjo, Univ. Politècnica de València (Spain); Rafael Verdú, Univ. Politècnica de Cartagena (Spain); Gavin R. Lloyd, Gloucestershire Hospitals NHS Foundation Trust (United Kingdom); Jayakrupakar Nallala, Nick Stone, Univ. of Exeter (United Kingdom)
FTIR spectroscopy is an emerging technology with high potential for cancer diagnosis but with particular physical phenomena that require special processing. Little work has been done in the field with the aim of registering hyperspectral FTIR images and Hematoxinlin and Eosin (HE) stained histological images of contiguous slices of tissue. This registration is necessary to transfer the location of relevant structures that the pathologist may identify in the gold standard HE images. A two-step registration framework is presented where a representative gray image extracted from the FTIR hypercube is used as an input. This representative image, which must have a spatial contrast as similar as possible to a gray image obtained from the HE image, is calculated through the spectrum variation in the fingerprint region. In the first step of the registration algorithm the similarity transformation is estimated from interest points, which are automatically detected by the popular SURF algorithm. In the second stage, a variational registration framework defined in the frequency domain compensates for local anatomical variations between both images. After a proper tuning of some parameters the proposed registration framework works in an automated way. The method was tested on 7 samples of colon tissue in different stages of disease and very promising qualitative and quantitative results were obtained (a mean correlation ratio of 92.16% with a standard deviation of 3.10%).

9703-7, Session 2

Sensing applications of silver halide mid-IR fibers (Invited Paper)
Abraham Katzir, Tel Aviv Univ. (Israel)

We developed crystalline AgClBr fibers of diameters 0.7-0.9mm that are flexible, non-toxic, insoluble in water and highly transparent between 4-15um. We used these fibers for various sensing applications. Highly sensitive absorption measurements in the mid-IR may be carried out by Fiber-optic Evanescent Wave Spectroscopy (FEWS). A typical FEWS system is based on three mid-IR components: a tunable source, a detector and a AgClBr fiber sensor that is brought in contact with the samples. We used FTIR spectrometers or tunable gas lasers or quantum cascade lasers (QCLs) as mid-IR sources. We used this FEWS system for measurements on gases, liquids and solids. In particular we used it for several biomedical applications. Measurements in vivo: (1) Early detection of skin diseases (e.g. melanoma). (2) Measurements on cells and bacteria. (3) Measurements on cornea. Measurements in vitro: (4) Characterization of urinary and biliary stones. (5) Blood measurements. The FEWS method is simple, inexpensive and does not require sample processing. It would be useful for diagnostic measurements on the outer part of the body of a patient, as well as for endoscopic measurements. It would also useful for measurements on tissue samples removed from the body. In addition we develop Scanning Near-field Infrared Microscope that will be used for spectral imaging with sub-wavelength resolution in the mid-IR. The various AgClBr fiber-optic sensors are expected to be important diagnostic tools at the hand of physicians in the future.

9703-8, Session 2

Ge-Sb-Se glass fiber-optics for the mid-infrared optical biopsy
Harriet A. Parnell, David Furniss, Trevor M. Benson, Colin Scotchford, Hesham Sakr, Zhuoqi Tang, Jessica H. Butterworth, Angela B. Seddon, The Univ. of Nottingham (United Kingdom)

In the UK, it is now recognised that 1 in 2 people born after 1960 will develop some form of cancer during their lifetime [1]. Diagnosing patients whilst in the early stages drastically improves their chances of survival but up until now the most common form of detection is via a lengthy excision biopsy procedure, which relies on the skill of a histopathologist. Evidently, the need for a faster solution is paramount.
The mid-infrared (MIR) spectral region covers the wavelengths 3-25 μm and characteristic vibrational spectra unique to each molecular type. Subtle changes within this region are indicative of changes within the cells relative to normal cells, signifying the presence or absence of a disease. Our goal is to carry out disease diagnosis in vivo. Reaching these wavelengths has previously presented difficulties as conventional MIR blackbody light sources are weak and optical fibers for transmitting MIR light to/from tissue in vivo fibers can be limited by strong material absorption such as silica glass >2.4 μm, and tellurite and fluoride >4.75 μm. However, chalcogenide glasses have been shown to transmit MIR light out to 25 μm and, moreover, have recently been demonstrated capable of bright, wideband supercontinuum (SC) emission across the MIR region. This paper will report glass fibers in the Ge-Sb-Se system. Successful fibers are considered for broadband, MIR fiber, SC generation and transmission to enable in-vivo mapping for an immediate response to the crisis termed ‘optical biopsy’. Basic biophysics of MIR light-tissue interactions are also reviewed.


9703-9, Session 2

Mid-IR supercontinuum generation beyond 7 μm using a silica-fluoride-chalcogenide fiber cascade

Christian R. Petersen, DTU Fotonik (Denmark); Peter M. Moselund, Christian Pedersen, NKT Photonics A/S (Denmark); Uffe V. Møller, DTU Fotonik (Denmark) and NKT Photonics A/S (Denmark); Ole Bang, DTU Fotonik (Denmark)

Broadband mid-infrared (MIR) light sources based on supercontinuum generation (SCG) in optical fibers have great potential for applications in vibrational spectroscopy, optical coherence tomography, and hyperspectral imaging microscopy. For example probing of the Amide I stretch (~5.30-6.11 μm) may be used for determining structural changes of proteins for non-invasive food analysis and medical diagnostics1,2. Recent experimental demonstrations of SCG in chalcogenide fibers have shown promising results for spectral coverage beyond 7 μm-3. Unfortunately, these demonstrations required expensive MIR pump systems based on high-power pulsed lasers and free-space wavelength conversion. Here, we demonstrate experimentally a cascaded SCG approach in which a MIR continuum from 3.5-4.4 μm is used to pump a chalcogenide fiber. The initial pump continuum is generated from a standard 1.55 μm laser diode and silica fiber, which is then amplified in a thulium-doped silica fiber amplifier, and finally red-shifted in a commercial fluoride fiber. By pumping a commercial Ge10As22Se68 single-material photonic crystal fiber with 135.7 mW, we obtained a continuum from 3.1-7.2 μm with a total output power after the collimating lens of 54.5 mW and 3.7 mW above 4.5 μm. The low power conversion above 4.5 μm was primarily due to the large core diameter of 14 μm, resulting in a fiber zero-dispersion wavelength at around 4.5 μm. These results may be improved by further optimization of fiber and pump parameters, making the reported cascaded silica-fluoride-chalcogenide SCG technique an attractive alternative to direct pumping schemes in terms of cost, flexibility and all-fiber integration.


9703-10, Session 2

All-fiber mid-IR supercontinuum: a powerful new tool for IR-spectroscopy

Peter M. Moselund, NKT Photonics A/S (Denmark); Laurent Huot, NKT Photonics A/S (Denmark) and DTU Fotonik (Denmark); Christopher D. Brooks, NKT Photonics A/S (Denmark)

Mid-IR spectroscopy is a powerful tool to detect and analyze a wide range of samples, however, its application has until now been greatly limited by its availability of light sources. The choice has generally stood between a laser whose narrow spectrum limits flexibility or a globar, whose low brightness limits signal to noise ratio.

Mid-IR supercontinuum sources, which can deliver an ultra-broad spectrum with a million times higher brightness than a globar, are now appearing to fill the performance gap between the traditional light sources. The generation of a supercontinuum is a highly nonlinear process produced by high peak power pulses propagating through a nonlinear medium. Since the underlying processes are fundamentally random there will normally be some pulse to pulse fluctuation in the output light which can cause problems in spectroscopy. Most of the mid-IR supercontinuum sources shown to date have also been limited to pulse repetition rates of only a few tens of kilohertz which makes it difficult to apply them to the popular FTIR spectroscopy techniques.

Here we will demonstrate a fully packaged, all-fiber, turn-key, low noise, >2W, 1.8-4.5 μm supercontinuum source, which can operate with variable repetition rates of up to 10 MHz. In addition we will discuss ways to counter the effect of pulse fluctuations and we will show the optimization of the output spectrum of the source for various applications. Such a source can give any mid-IR optics lab access to a performance which has previously only been available from dedicated beamlines at huge synchrotron facilities.

9703-11, Session 3

Spectrally encoded confocal microscopy (SECM) for rapid assessment of breast excision specimens (Invited Paper)

Elena F. Brachtel, Massachusetts General Hospital (United States); Nicole B. Johnson, Beth Israel Deaconess Medical Ctr. (United States); Amelia E. Huck, Travis L. Rice-Stitt, Mark G. Vangel, Barbara L. Smith, Guillermo J. Tearney M.D., DongKyun Kang, Massachusetts General Hospital (United States)

Unacceptably large percentage (20-40%) of breast cancer lumpectomy patients are required to undergo multiple surgeries when positive margins are found upon post-operative histologic assessment. If the margin status can be determined during surgery, surgeons can resect additional tissues to achieve tumor-free margin, which will reduce the need for additional surgeries. Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance confocal microscopy technology that has the potential to image the entire surgical margin within a short procedural time. Previously, SECM was shown to rapidly image a large area (10 mm by 10 mm) of human esophageal tissue within a short procedural time (15 seconds). When used in lumpectomy, SECM will be able to image the entire margin surface of ~30 cm2 in around 7.5 minutes. SECM images will then be used to determine margin status intra-operatively. In this paper, we present results from a study of testing accuracy of SECM for diagnosing malignant breast tissues.
We have imaged freshly-excised breast specimens (N=46) with SECM. SECM images clearly visualized histomorphologic features associated with normal, benign and malignant breast tissues in a similar manner to histologic images. Diagnostic accuracy was tested by comparing SECM diagnoses made by three junior pathologists with corresponding histologic diagnoses made by a senior pathologist. SECM sensitivity and specificity were high, 0.91 and 0.93, respectively. Intra-observer agreement and inter-observer agreement were also high, 0.87 and 0.84, respectively. Results from this study showed that SECM has a potential to accurately determine margin status during breast cancer lumpectomy.

9703-12, Session 3

Rapid full-field OCT assessment of clinical tissue specimens

Eugénie Dalimier, LLTech SAS (France); Fabrice Harms, Institut Langevin (France) and LLTech SAS (France) and Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France); Charles Brossollet, Emilie Benoît, Franck Martins, LLTech SAS (France); Claude A. Boccara, Institut Langevin (France) and LLTech SAS (France) and Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France)

FFOCT (Full Field Optical Coherence Tomography) is a novel optical technology that gives access to very high resolution tomography images of biological tissues within minutes, non-invasively. This makes it an attractive tool to bridge the gap between medical imaging modalities (MRI, ultrasound, CT) used for cancer lesion identification or targeting and histological diagnosis. Clinical tissue specimens, such as surgical cancer margins or biopsies, can potentially be assessed rapidly, by the clinician, in the aim to help him decide on the course of action.

A fast FFOCT prototype was built, that provides 1cm² images with 1 µm resolution in 1 minute, and can accommodate samples up to 50mm diameter. Specific work was carried out to implement a large sample holder, high-speed image acquisition system, optimized scanning, and accelerated GPU tiles stitching. Results obtained on breast, urology, and digestive tissues show the efficiency of the technique for the detection of cancer on clinical tissue specimens, and reinforce the clinical relevance of the technique. The technical and clinical results show that the fast FFOCT system can successfully be used for a fast assessment of cancer excision margins or biopsies providing a very valuable tool in the clinical environment.

9703-14, Session 3

Line-scanning, stage scanning confocal microscope

Daniel S. Gareau, The Rockefeller Univ. (United States); John Carucci, New York Univ. (United States)

Confocal microscopes are complex, in part due to optomechanics that enable raster beam scanning of a point of focused laser light within a 2D section of whole tissue. Line-scanning is a potential simple alternative that eliminates the need to scan the beam in the direction of the line. Scanning is still required in the other direction (perpendicular to the line), so to eliminate the need for optomechanics in that direction too, a translating stage can be implemented. We present early developments on a line-scanning, sample scanning microscope aimed at use in the surgical suit for rapidly obtaining images of cancer specimens for the purposes of margin screening, including standard target imaging and the first human tissue images. Our design includes four-mode fluorescence and reflectance “simultaneous” imaging (488nm & 532nm excitation) enabled by an optical chopper, which eliminates the need to scan back across the sample to obtain multiple confocal images. An air objective lens eliminates the need for messy coupling media. Other nice features that are attractive from a clinical and translational perspective include an improved tissue fixture, tele-pathology linking and a touchscreen interactive display that mimics the flow that typically results from the use of a standard light microscope. The diffraction-limited theoretical resolution limit of the system is 1µm laterally and 3.7 µm axially. The system can potentially image larger areas (µm) with high resolution (~µm) in short periods (~60seconds), so it may be an attractive bedside surgical pathology technology.

9703-13, Session 3

Rapid breast cancer assessment using a high resolution microendoscope system with structured illumination

Jessica Dobbs, Rice Univ. (United States); Matthew Kyrish, Fresnel Technologies Inc. (United States) and Rice Univ. (United States); Savitri Krishnamurthy M.D., The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); Noah Bedard, Univ of California, San Francisco (United States); Ben Grant, Rice Univ. (United States); Wei T, Yang M.D., The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); Tomasz Tkaczyk, Rebecca Richards-Kortum, Rice Univ. (United States)

The standard procedure for breast cancer diagnosis is time-intensive and requires extensive resources and trained personnel to perform. High resolution microendoscopy has the potential to address these limitations, by acquiring images at near video rate without the need for extensive tissue preparation. A challenge of using optical imaging for breast lesion diagnosis is the presence of both neoplastic and non-neoplastic tissue with high nuclear density. To determine if reduction of out of focus signal could improve the potential to evaluate neoplastic and non-neoplastic breast tissue we designed a high resolution microendoscopy system that uses structured illumination. Following surgical excision, fresh breast tissue specimens containing neoplastic and non-neoplastic tissue were stained with profafine, a nuclear contrast agent, and imaged with high resolution microendoscopy (HRME) and high resolution microendoscopy with structured illumination (SI-HRME). In addition to profafine, we also evaluated the contrast agent, Lugol’s Iodine, for its potential to improve tissue assessment by reducing out of focus light. Specimens were imaged with confocal fluorescence microscopy (CFM) and standard histologic preparation. Images were qualitatively evaluated based on standard histologic criteria by a dedicated breast pathologist. We used grey-level co-occurrence matrix (GLCM)-based parameters to quantitatively evaluate all optical images (HRME, SI-HRME, and CFM). Our findings indicate that by incorporating structured illumination into the HRME system, there is significant improvement in image quality relative to HRME alone. Lugol’s Iodine application also improved image quality by reducing background light. Larger studies are needed to determine if SI-HRME images have sufficient information to identify neoplastic breast lesions.

9703-15, Session 4

Nonlinear microscopy for rapid assessment of breast surgical specimens

Michael G. Giacomelli, Tadayuki Yoshitake, Lucas C. Cahill, Osman O. Ahsen, Massachusetts Institute of Technology (United States); Yuri Sheykin, Hilde Vardeh, Beth Israel Deaconess Medical Ctr. (United States); Jeffrey Brooker, Thorlabs Imaging Systems (United States); Lennart A. Husvogt, Joachim Hornegger, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); James L. Connolly M.D.,
demonstrated the capability of this technology for accurately differentiating each patient. Thus, representative sampling of the tumor is needed for the optimal cancer therapy. To enable individualized treatment, the genetic analysis, which will become one of the next big advances in our search for reorient the needle during the biopsy and sample the most representative studies of breast pathology in the clinic and comparison to conventional H&E pathology are reported. We will also discuss the clinical study design to assess multiphoton microscopy of surgical breast specimens and the impact on repeat surgeries. We present a novel method, based on encoder mapping OCT imaging, requires no lasers, confocal, multiphoton or optical coherence tomography signals from only the most superficial tissue elements. The method is one such technique that can generate histology-quality images from fresh tissue solely based on their intrinsic autofluorescence emission, without the need for tissue processing or staining. New imaging protocols and a novel image processing algorithm are presented that generate real-time images of fresh, unfixed, rapid stained tissue where the multiphoton microscopy channels are mapped to an H&E like color scale to facilitate interpretation. The combination of optimized scan optics and GPU-accelerated processing enable high resolution, wide field of view video rate, low latency virtual H&E histology of thick, unfixed tissue specimens. The system images a greater than 2 mm x 2 mm field of view, comparable to a 10x objective which is typically used to assess breast pathology, with 2048 x 2048 pixels at <1 um transverse resolution and 8 Hz frame rate. In addition, rapid scanning enables visualization of many-centimeter scale specimens at surgically relevant time scales. Imaging studies of breast pathology in the clinic and comparison to conventional H&E pathology are reported. We will also discuss the clinical study design to assess multiphotonic microscopy of surgical breast specimens and the impact on repeat surgeries. [1] Y.K. Tao, D. Shen, Y. Shekine, O.O. Ahsen, H.H. Wang, D.B. Schmolze, N.B. Johnson, J.S. Brooker, A.E. Cable, J.L. Connolly, and J.G. Fujimoto, “Assessment of breast cancer pathologies using nonlinear microscopy,” Proc. Natl. Acad. Sci U.S.A. 111, 15304-15309, October 2014.
9703-20, Session 5

**Fluorescence lifetime spectroscopy and imaging of tissue specimens: applications in oncology and cardiovascular pathology (Invited Paper)**
Laura Marcu, Univ. of California, Davis (United States)

Fluorescence measurements provide information about biochemical, functional and structural changes in fluorescent bio-molecular complexes in tissues and cells including structural proteins, enzyme metabolic cofactors, lipid components, and porphyrins. This presentation will overview multispectral time-resolved fluorescence spectroscopy (ms-TRFS) and fluorescence lifetime imaging microscopy (FLIM) instrumentation developed in our laboratory. We present studies that demonstrate the potential of these techniques as clinical tool for rapid detection of positive tumor margins in freshly excised tumor tissue specimens and as research tool for comprehensive ex-vivo analysis of coronary atherosclerosis.

9703-21, Session 5

**Development and clinical translation of OTIS: a wide-field OCT imaging device for ex-vivo tissue characterization (Invited Paper)**
Elizabeth A. Munro, David Rempel, Christine Danner, Yaasen Atchua, Michael S. Valic, Andrew Berkeley, Bahar Davoud, Paul A. Magnin, Perimeter Medical Imaging (Canada); Susan J. Done, Supriya Kulkarni, Wey-Liang Leong, Brian C. Wilson, Univ. Health Network (Canada)

We have developed an automated, wide-field optical coherence tomography-based imaging device (OTIS™, Perimeter Medical Imaging) for peri-operative, ex-vivo tissue imaging. This device features automated image acquisition, enabling rapid capture of high-resolution (15-µm) OCT images from samples up to 10 cm in diameter. Further, the device features a software interface for scroll-through review and annotation of OCT image sets from multiple sides of a given sample.

We present the iterative progression of device development from phantom and pre-clinical (tumor xenograft) models through to initial clinical results. We discuss the relative ease of demonstrating device efficacy in phantom and pre-clinical models, and the steps required to translate the technology to clinical applicability.

We report also on initial clinical results from a 60-patient study at Princess Margaret Cancer Center, Toronto, where intact breast lumpectomy specimens were imaged following excision and intra-operative radiology but prior to formalin fixation. All imaging was performed within cold ischemic time guidelines. We discuss the challenges and opportunities associated with proving a novel imaging technology against the clinical “gold standard” of conventional post-operative pathology.

Finally, we present an atlas of correlative images comparing OCT to conventional histopathology, including healthy ducts, fibrous bands, fatty tissue, ductal carcinoma in situ (DCIS) and invasive carcinoma.

9703-22, Session 5

**Parametric approaches to micro-scale characterization of tissue volumes in vivo and ex vivo: Imaging microvasculature, attenuation, birefringence, and stiffness (Invited Paper)**
David D. Sampson, Lixin Chin, Peijun Gong, Philip Wijesinghe, Shaghayehg Es’haghian, Wesley M. Allen, Blake R. Klyn, Rodney W. Kirk, Brendan F. Kennedy, Robert A. McLaughlin, The Univ. of Western Australia (Australia)

Advances in imaging tissue microstructure in living subjects, or in freshly excised tissue with minimal preparation and processing, are important for future diagnosis and surgical guidance in the clinical setting, particularly for application to cancer. Whilst microscopy methods continue to advance on the cellular scale and medical imaging is well established on the scale of the whole tumor or organ, it is attractive to consider imaging the tumor environment on the micro-scale, between that of cells and whole tissues. Such a scenario is ideally suited to optical coherence tomography (OCT), with the twin attractions of requiring little or no tissue preparation, and in vivo capability. OCT’s intrinsic scattering contrast reveals many morphological features of tumors, but is frequently ineffective in revealing other important aspects, such as microvasculature, or in reliably distinguishing tumor from uninvolved stroma. To address these shortcomings, we are developing several advances on the basic OCT approach. We are exploring speckle fluctuations to image tissue microvasculature and we have been developing several parametric approaches to tissue micro-scale characterization. Our approaches extract, from a three-dimensional OCT data set, a two-dimensional image of an optical parameter, such as attenuation or birefringence, or a mechanical parameter, such as stiffness, that aids in characterizing the tissue. This latter method, termed optical coherence elastography, parallels developments in ultrasound and magnetic resonance imaging. Parametric imaging of birefringence and of stiffness both show promise in addressing the important issue of differentiating cancer from uninvolved stroma in breast tissue.

9703-23, Session 5

**Fluorescein as a contrast agent for confocal intra-operative imaging of basal cell carcinomas: a preliminary ex vivo study (Invited Paper)**
Heidy Sierra, Memorial Sloan-Kettering Cancer Ctr. (United States); Qiaochu Qi, Weill Cornell Medical College (United States); Nikash Taskar, Edgemont Jr. / Sr. High School (United States); Anthony Rossi, Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

When used for intra-operative imaging of residual basal cell carcinomas (BCCs), reflectance confocal microscopy (RCM) is limited to detection of relatively large tumors. Small tumors remain hidden in the surrounding bright dermis. Fluorescence confocal microscopy (FCM) may improve the sensitivity for detecting small tumors. Fluorescein enhances cell cytoplasm contrast in fluorescence confocal images and is a potential topical contrast agent in FCM. However, it has had limited clinical impact on imaging BCCs in vivo because there is a lack of a well-defined protocol (concentration and application time) that can be effectively used for intraoperative imaging.

We conducted an ex vivo study, using discarded tissue from Mohs surgery and a benchtop FCM with 488nm wavelength for excitation and 521nm detection for imaging. Concentrations of 6 and 0.6 mM with immersion times of 5, 15, 30, and 60 seconds were repeatedly tested in 41 specimens.
The 0.6 mM and immersion time of 60 seconds showed that cellular cytoplasm could be labeled with controlled saturation and minimum tissue staining. This combination of parameters was further validated in 48 additional samples. Confocal images were compared with histology by experts. Results show that, fluorescein enhances the contrast of cellular structures relative to other normal dermal structures, improving the detection of small BCCs. This study provides an optimized set of parameters for subsequently testing of topical application in vivo for intra-operative imaging of BCCs.

9703-24, Session 5
Real-time digital signal processing in multiphoton and time-resolved microscopy (Invited Paper)
Jesse W. Wilson, Colorado State Univ. (United States); Martin C. Fischer, Warren S. Warren, Duke Univ. (United States)

The use of multiphoton interactions in biological tissue for imaging contrast requires highly sensitive optical measurements. These often involve signal processing and filtering steps between the photodetector and the data acquisition device, such as photon counting and lock-in amplification. These steps can be implemented as real-time digital signal processing (DSP) elements on field-programmable gate array (FPGA) devices, an approach that affords much greater flexibility than commercial photon counting or lock-in devices. We will present progress in developing three new FPGA-based DSP devices for multiphoton and time-resolved microscopy applications. The first is a high-speed multi-harmonic lock-in amplifier for transient absorption microscopy, which is being developed for real-time analysis of the intensity-dependence of melanin, with applications in vivo and ex vivo (noninvasive histopathology of melanoma and pigmented lesions). The second device utilizes spread-spectrum modulation, in which a pseudo-random binary code is imposed on the pump beam, in order to resolve slow (~ ms time scale) optical transients with high repetition-rate (~100 MHz) pulse trains. The third device is a kHz lock-in amplifier running on a low cost (~$30) development platform. It is our hope that these FPGA-based DSP devices will enable new, high-speed, low-cost applications in multiphoton and time-resolved microscopy.

9703-25, Session 6
Implementation of fluorescence confocal mosaicing microscopy by “early adopter” Mohs surgeons: a review of recent progress in five settings (Invited Paper)
Manu Jain M.D., Memorial Sloan-Kettering Cancer Ctr. (United States)

Fluorescent confocal mosaicing microscopy (CMM) has recently advanced from pre-clinical laboratory studies to implementation and testing in Mohs surgery settings. To date, fluorescent CMM has been used to guide Mohs surgery of basal cell carcinomas (BCCs) in approximately 400 procedures. We will provide a critical review of the initial outcomes of these advances. Two pre-clinical laboratory studies reported sensitivity of 97%-94% and specificity of 89-94%. A recent large clinical study, with implementation during Mohs surgery, reported sensitivity of 89%, specificity of 99%, positive predictive value of 98% and negative predictive value of 97%. Another study reported good to excellent agreement (kappa 0.68 to 1.00) between a Mohs surgeon, a dermatologist and a pathologist for reading mosaics and excellent agreement (kappa 0.98) for classifying sub-type of BCCs. Other studies in other settings report the ability of fluorescent CMM to guide Mohs surgery of recurrent BCCs and eccrine carcinomas. Another larger study on about 600 Mohs procedures is currently in progress. As the implementation of this technology advances further, it may serve as an adjunct to standard pathology and enable rapid detection of residual BCC margins in freshly excised Mohs surgical tissue at the bedside.

9703-26, Session 6
Differentiation of cancerous and normal brain tissue using label free fluorescence and Stokes shift spectroscopies
Yan Zhou M.D., The General Hospital of the Air Force, PLA (China); Leana Wang, Columbia Univ. (United States); Cheng-Hui Liu, The City College of New York (United States); Yong He, Beijing Normal Univ. (China); Xinguang Yu M.D., Gangge Cheng M.D., Peng Wang M.D., Cheng Shu M.D., The General Hospital of the Air Force, PLA (China); Robert R. Alfano, The City College of New York (United States)

Optical biopsy techniques are based on optical spectroscopy that includes native fluorescence, Stokes shift, multiphoton fluorescence images, and special frequency analysis. In this presentation, optical biopsy was applied to diagnose human brain cancer in vitro. The concentration of this study was the identification of brain cancer from normal tissues using native fluorescence and Stokes shift spectra (SSS). Five types of brain tissues (normal, primary tumor, and metastatic brain carcinoma) from five grades were studied from twenty five brain specimens. In order to observe the fluorescence spectral changes of fluorophores, a wide excitation wavelength ranging from UV to visible (260 to 530 nm) for the emission spectra was examined. In addition, the Stokes shift spectra with intervals, Δ?, from 10 to 120 nm were measured. The fluorescence spectra and SSS from multiple key native molecular markers, such as tryptophan, collagen, NADH, alanine, ceroid and lipofuscin, were observed in normal and diseased brain tissues. Two diagnostic criteria were established based on the ratios of the peak intensities and peak position in both the fluorescence and SSS spectra. It was observed that the ratio of the spectral peak intensity of tryptophan (340 nm) to NADH (440 nm) was higher in glioma and malignant meningeal tissues when compared with the same ratio present in normal tissues. The SSS peak (Δ? ~40nm) intensity ratio of 292 nm to 366 nm increased by a set amount as higher grades of glioma tumors were measured.

9703-27, Session 6
Deconstructing native fluorescence: non-invasive detection and monitoring of biochemistry in cells and tissues
Ewa M. Goldys, Martin E. Gosnell, Ayad G. Anwer, Macquarie Univ. (Australia); Juan C. Cassano, Carolyn M. Sue, The Univ. of Sydney (Australia); Saabah B. Mahbub, Sandeep M. Perinchery, David W. Inglis, Macquarie Univ. (Australia); Partho P. Adhikary, Jalal A. Jazayeri, Michael A. Cahill, Charles Sturt Univ. (Australia); Sonia Saad, Carol Pollock, The Univ. of Sydney (Australia); Melanie L. Sutton-McDowall, Jeremy G. Thompson, The Univ. of Adelaide (Australia)

Automated and unbiased methods of non-invasive cell monitoring able to deal with complex biological heterogeneity are fundamentally important for biology and medicine. Label-free cell imaging provides information about endogenous fluorescent metabolites, enzymes and cofactors in cells. However extracting high content information from imaging of native fluorescence has been hitherto impossible. Here, we quantitatively characterise cell populations in different tissue types, live or fixed, by using novel image processing and a simple multispectral upgrade of a wide-field fluorescence microscope. Multispectral intrinsic fluorescence...
imaging was applied to patient olfactory neurosphere-derived cells, cell model of a human metabolic disease MELAS (mitochondrial myopathy, encephalomyopathy, lactic acidosis, stroke-like syndrome). By using an endogenous source of contrast, subtle metabolic variations have been detected between living cells in their full morphological context which made it possible to distinguish healthy from diseased cells before and after therapy. Cellular maps of native fluorophores, flavins, bound and free NADH and retinoids unveiled subtle metabolic signatures and helped uncover significant cell subpopulations, in particular a subpopulation with compromised mitochondrial function. The versatility of our method is further illustrated by detecting genetic mutations in cancer, non-invasive monitoring of CD90 expression, label-free tracking of stem cell differentiation, identifying stem cell subpopulations with varying functional characteristics, tissue diagnostics in diabetes, and assessing the condition of preimplantation embryos. Our optimal discrimination approach enables statistical hypothesis testing and intuitive visualisations where previously undetectable differences become clearly apparent.

9703-28, Session 6

Raman spectroscopy of hamster buccal pouch tissues: investigating suitability of ex vivo models to evaluate in vivo spectra

Piyush Kumar, C. Murali Krishna, Advanced Ctr. for Treatment, Research & Education in Cancer (India)

Raman spectroscopy (RS) has been shown to be useful in classification of many cancers using both tissues and in vivo approaches. While cancers of oral and cervix can be directly studied, internal organs like stomach can be carried out using endoscopy mediated spectroscopy. However, spectroscopy of organs like brain is complicated as obtaining tissue samples, especially healthy tissues is difficult. Thus, in this study, we have simulated spectra from hamster buccal pouch tissues to evaluate both ex vivo and in vivo control (216 ex vivo;866 in vivo) and tumor spectra (63 ex vivo;40 in vivo) using HE785 commercial Raman instrument. Principal Component Analysis (PCA) scatter plots showed exclusive clusters for control and tumor spectra. PCA based Linear Discriminant analysis (PC-LDA) findings were cross validated using leave one out algorithm and further evaluated. In case of control spectra, 193 and 805 spectra were predicted as either of the controls, respectively. In case of tumor spectra, 53 and 38 spectra were predicted as tumors. In the next step, in vivo spectra were used a standard models and evaluated against both types of spectra to obtain similar results. Thus, findings suggest that in spite of differences between ex vivo and in vivo spectra, ex vivo models may be used for identification of cancers in inaccessible organs like brain, for applications in diagnostics and surgical demarcation.

9703-29, Session 7

Label-free fluorescence spectroscopy detecting Alzheimer disease in brain tissue of a mouse model

Lingyan Shi, The City College of New York (United States); George Harvey, Thomas Harvey, Riverdale Country School (United States); Paulo Marques, City College of New York (United States); Robert R. Alfano, The City College of New York (United States); Adrián Rodríguez-Contreras, The City Univ. of New York (United States)

Alzheimer’s disease (AD), a degenerative disorder that attacks neurons in the brain and leads to the loss of proper cognition, ravages the lives of millions of people all across the world. A large proportion of people with Alzheimer’s disease remained undiagnosed. Physicians diagnose Alzheimer’s disease with just an examination of the patient’s state, inquiries into the familial history of psychiatric and neurological disorders, and a neurological exam. Other newer methods of diagnosis include Magnetic Resonance Imaging (MRI) to look for Hippocampal atrophy, Positron Emission Tomography (PET) scans, and examining levels of beta-amyloid and tau protein in cerebrospinal fluids taken from the patient. But these methods are relatively low resolution and high cost. Fluorescence of certain amino acids, proteins and molecules within human tissue, has been expanded upon and applied to examine levels of tryptophan, NADH, flavin, and collagen. Optical spectroscopy has not been employed to study the linear fluorescence of these biomarkers excited at various wavelengths in AD and normal (N) brain tissue. This study is to apply optical fluorescence spectroscopy for measuring fluorescence levels of key biomolecules (tryptophan, NADH, collagen, and flavin) in AD and N mouse brain tissues. The results demonstrate significant differences of emission peaks of these molecules in AD and N brain. It showed that fluorescence intensity levels from tryptophan: AD > N; from collagen: AD - N; from NADH: N > AD and from flavin: AD > N. This is a potential method for detection and diagnosis of Alzheimer’s disease in humans.

9703-30, Session 7

Rapid spatial frequency domain inverse problem solutions using look-up tables for real-time processing

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Imaging technologies working in the spatial frequency domain are becoming increasingly popular for generating wide-field optical property maps, enabling further analysis of tissue parameters such as absorption or scattering. While acquisition methods have witnessed a very rapid growth and are now performing in real-time, processing methods are yet slow preventing information to be acquired and displayed in real-time. In this work, we present solutions for rapid inverse problem solving for optical properties by use of advanced look-up tables. In particular, we present methods and results from a dense, linearized look-up table and an analytical representation that currently run 100 times faster than the standard method and within 10% in both absorption and scattering. With the resulting computation time in the tens of milliseconds range, the proposed techniques enable video-rate feedback of real-time techniques such as snapshot of optical properties (SSOP) imaging, making full video-rate guidance in the clinic possible.

9703-31, Session 7

Measuring sampling depth of lens-based single fiber reflectance spectroscopy with optical coherence tomography

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Standard prostate cancer diagnosis involves ultrasound guided biopsies. Unfortunately, this series of 10-24 biopsies have a low sensitivity and the tendency to underage. The result: 30% of the patients undergo a second round of biopsies.

To overcome this problem, we are developing an optical biopsy tool that will provide real-time information of location and tissue characteristics. The aim is to reduce the amount of biopsies while increasing diagnostic accuracy. In our biopsy tool we combine Optical Coherence Tomography (OCT) and Single Fiber Reflectance Spectroscopy (SFR) in a needle. These techniques can offer structural, physiological and biochemical information of tissue in vivo.
Different from previous SFR studies that used a bare fiber forward firing setup in contact with tissue, we are using a lens-based side firing setup. This change in design might influence the SFR sampling depth. We will investigate the sampling depth of our lens-based SFR measurements using a phantom consisting of an obliquely placed absorbing filter in a container filled with Intralipid. By moving our probe along the filter, the absorber will be at increasing distance from the probe. Since the sampling depth also depends on the optical properties of the sample, we will use different concentrations of Intralipid to measure the sampling depth at different scattering coefficients. Simultaneous measurements will be obtained with SFR and OCT, so exact depth location of the filter will be measured with OCT. We will compare the measurements for our probe and a bare fiber to each other and to Monte Carlo simulations.

9703-32, Session 7
Label-free pathological evaluation of grade 3 cancer using Stokes shift spectroscopy
Laura A. Sordillo, Peter P. Sordillo M.D., Robert R. Alfano, The City College of New York (United States)

Evaluation of a cancer’s optical properties may provide useful prognostic information. Tissue samples from 15 patients with breast carcinoma were evaluated using Stokes shift spectroscopy (S3). S3 is an optical tool which utilizes the difference between emission wavelength (?e) and absorption wavelength (?abs) (known as the Stokes’ shift) to give a fixed wavelength shift (?S). Studies by Yang et al highlighted the efficacy of this method over conventional fluorescence, and showed that this technique could be used to detect cancerous versus normal tissue based on the key biomolecules in tissue. ?S=20, 40, 60, 80 and 100 nm were used on the breast cancer samples. We show that tumors from patients with grade 3 (high grade) cancers have increased relative tryptophan content compared to grade 1 or 2 tumors, and that this technique may be useful in determining the aggressiveness of a patient’s cancer.

9703-33, Session 7
Tryptophan as a biomarker for cancer detection in terahertz (THz) sensing
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Tryptophan is an extremely important amino acid for a variety of biological functions in living organisms. Changes in the concentration of this amino acid can point to identification of cancerous tissues or even confirm symptoms of depression in patients. Therefore it is extremely important to identify and quantify tryptophan concentrations in human blood as well as in in vivo diagnostic studies. Pioneering studies done in the THz region have shown that tryptophan has unique resonances which can enable its use as a biomarker. These studies which were done for high concentrations showed that the resonances were a result of torsional modes due to C-C bonds. This development opened up the possibility of using THz systems to directly detect tryptophan in a liquid, biological environment without the need to apply complex approaches in sample preparation such as that which is required with high performance liquid chromatography (HPLC) equipment. Studies have shown that tryptophan concentration is increased in cancer and in the last few years efforts have been made to directly measure the presence of cancerous tissue by targeting these observed resonances. While these studies have shown that THz sensing is a viable option for clinical studies of skin cancers, its impact has yet to be understood for the broad variety of this disease. With the recent development of high resolution THz spectroscopy methods the potential to detect the concentration of this amino acid with micromol/L precision in blood will allow for fast simple diagnosis of various cancers.

9703-34, Session 8
Resonance Raman spectroscopy detection of vulnerable atherosclerotic plaque
Cheng-Hui Liu, The City College of New York (United States); Susie Boydston-White, Borough of Manhattan Community College (United States); Arel Weisberg, Energy Research Co. (United States); Wubao B. Wang, Laura A. Sordillo, Yury Budansky, The City College of New York (United States); Adler Perotte, Vincent P. Tomaselli, Columbia Univ. (United States); Stephanie S. Lubicz M.D., Peter P. Sordillo M.D., Robert R. Alfano, The City College of New York (United States)

The Resonance Raman (RR) spectroscopy is shown provide molecular vibrational fingerprints for identifying and classifying vulnerable plaques (VP) in arteries. The RR spectra of fibro lipid plaque, calcification, and vulnerable atherosclerotic plaques that reside in the aortic/artery intimal wall tissues from human corpses were measured. Three types of calcified atherosclerotic plaque lesions were observed and investigated using their key RR molecular fingerprints. Different RR peaks and peak intensities of proteins and lipids components used as biomarkers for VP detection were revealed. The vibrational modes of carotenoids at 1012 cm\(^{-1}\), 1156 cm\(^{-1}\), and 1517 cm\(^{-1}\), the tryptophan and heme protein at 758 cm\(^{-1}\) and 1596 cm\(^{-1}\), the amide I, II, III at 1658 cm\(^{-1}\), 1554 cm\(^{-1}\) and 1228 cm\(^{-1}\), respectively; and methyl/methylene group at 2935 cm\(^{-1}\), 2854 cm\(^{-1}\) and 2895 cm\(^{-1}\) from the intrinsic atherosclerotic VPVs tissues were studied. A clear correlation between RR spectra and different atherosclerotic plaque type evolutions was observed due to changes of the fibrous cap thickness of VP. The cap thicknesses were examined using standard histopathology methods and compared with the RR results. The critical ruptured cap thickness near 66 µm was using the curve fitting with two exponential decay algorithms for ballistic and diffusion propagation through the cap.

9703-35, Session 8
Shedding light inside middle ear: discerning the differential molecular pathology of proliferative middle ear lesions
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Morphologic recognition based on white light otoscopic examination remains the gold standard for diagnoses of most middle ear pathological conditions. However, stratifications using morphology alone involve significant inter-observer variability and provide limited insight into a disease’s defining biochemistry. The inability to biochemically define pathologies is particularly evident in the management of cholesteatoma and myringosclerosis that on gross inspection exhibit nearly identical features. While cholesteatoma is characterized by keratinization of squamous epithelium and aggressive growth of the tissue in the middle ear and mastoid cavity, myringosclerosis is marked by the calcification and hyalinization in the tympanic membrane.

Here we present a novel in-vivo Raman spectroscopic detection that has potential to non-invasively identify two key lesions, namely cholesteatoma and myringosclerosis, by providing real-time information of differentially expressed molecules. Our spectroscopic measurements reveal that, in
addition to the presence of calcifications, myringosclerosis presents novel spectral markers that collectively can be used to construct an accurate decision algorithm. A model of silicate-substitution in the calcifications is proposed to explain these new spectral markers that represent an unreported biochemical moiety in middle ear pathology. Availability of real-time and molecular fingerprint information would enable more complete removal of cholesteatoma thereby reducing the number of unwanted repeat surgeries and allowing for better hearing preservation.

9703-36, Session 8
Assessing the effects of antihistamines in rodents with Raman and Brillouin spectroscopy

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Histamine-1 receptor blockers are a commonly prescribed medication taken to alleviate allergy symptoms. However, multiple reports have shown that among the possible side effects of its use are an increase in appetite and weight gain. Taking into account the alarming trend of increased obesity rates in the nation, it is important to examine the effect of antihistamines on the body. Raman spectroscopy allows non-invasive chemical assessment, while Brillouin spectroscopy affords non-invasive measurements of local elasticity of tissues, which, as we hypothesize, are being affected by antihistamines. In this study, three groups of rats were fed a diet supplemented with antihistamines for different durations, while the control group was fed a regular diet. Two spectroscopic techniques were used to evaluate the variations of chemical and mechanical properties in the skin, inguinal and interscapular adipose tissues between the four groups. Supplementary measurements were collected from the femoral vein and bone samples.

9703-37, Session 8
The Raman spectrum character of Skin tumor induced by UVB

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Skin cancer is the most common cancer in the world, with over 3.5 million cases diagnosed annually in the United States and incidence increasing at a staggering 3% per year. Basal cell carcinoma and squamous cell carcinoma (SCC) are the main types of skin cancer. The major carcinogenic factor for most skin cancers has been confirmed as solar ultraviolet light, especially the chronic exposure to UVB (280 nm-320nm). Diagnosis of skin cancers mainly relies on visual inspection by doctor. Although skin presents an easy position for inspection, the skin cancers diagnosis is difficult, because of many benign lesions visually resemble. The combination of Raman spectroscopy with optical microscopy developed into a powerful imaging technique with molecular specificity and lateral resolution down to the subcellular level. In our study, the skin canceron processes induced by UVB were analyzed from the perspective of tissue spectrum. A home-made Raman spectral system with a millimeter order excitation laser spot size and molecular specificity was used to generate diagnostic algorithms for the classification of different stages of skin SCC process. The results indicated that Raman spectroscopy combined with PCA-LDA demonstrated good potential for improving the diagnosis of skin cancers.

9703-39, Session PTues
Tissue slides analysis using red, green and blue LEDs as microscope light source

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The optical microscopy is one of the most powerful tools in the analysis of biological systems. The usual optical transmission microscopy uses a white light lamp as source, what sometimes does not bring optimal results, making it necessary to introduce filters to change some illumination properties like the temperature or the color itself. There is, of course, an intrinsic limitation on the use of filters that is the lack of an analogical control on the illumination properties and a practical limitation that depends on the number of available filters. To address this need, we developed an illumination system based on (Red, Green and Blue) RGB LEDs, were the microscope operator can control the intensity of each one independently and manually. This paper details the developed system and describes the methods used to compare quantitatively the images acquired while using the standard white light illumination and the images obtained with the developed system. To quantify the contrast, we calculated the relative population standard deviation for the intensities of each channel of the RGB image. This procedure allowed us to compare and understand the major advantages of the developed illumination system. All analysis methods have shown that an increased contrast can be obtained under the RGB LEDs light. The presented illumination allowed us to visualize the structures in different samples with higher contrast without the need of any additional filters.

9703-52, Session PTues
Assessing the photoaging process at sun exposed and non-exposed skin using fluorescence lifetime spectroscopy

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Photoaging is the skin premature aging due to light exposure, which damage the collagen, elastin and other fibers that give skin a smooth and youth appearance. The chronic exposure of skin to the sunlight can also induce an accumulation of alterations on the cells DNA. Induced tissue changes range from wrinkled and discoloured skin through to cancer lesions. The fluorescence spectra and lifetime analysis in biological tissues has been presented as a technique of great potential for tissue characterization and can be used to evaluate the level of ultraviolet light exposure of the skin. The objective of this study is to evaluate the variation on fluorescence lifetimes of normal skin at sun exposed and non-exposed areas. A portable system using the Time Correlated Single Photon Counting (TCSPC) (HPM-100-50, Becker and Hickl, Berlin, Germany) technique was assembled and used to perform the measurements. Fluorescence lifetime spectra of sun exposed and non-exposed regions of volunteers are measured using two diode pulsed lasers: one emitting at 378 nm and another at 445 nm (BDL-375-SMC and BDL-445-SMC, Becker and Hickl, Berlin, Germany). The data were processed using the SPCM Software (Becker and Hickl, Berlin, Germany) analyzed using a MATLAB R2012a (version R2012a, Mathworks, Natick, MA, USA) home-made script. The collected fluorescence decay curve was fitted by a biexponential function. The sun exposed and non-exposed areas showed statistical differences with p<0.05 at student's t-test in thirteen of a total of sixteen parameters of the biexponential decays fitted to the experimental curve.
Evaluation of actinic cheilitis using fluorescence lifetime spectroscopy

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Actinic cheilitis is a potentially malignant disorder that mostly affects the vermilion border of the lower lip and can lead to squamous cell carcinoma. Because of its heterogeneous clinical aspect, it is difficult to indicate representative biopsy area. Late diagnosis is a limiting factor of therapeutic possibilities available to treat oral cancer. The diagnosis of actinic cheilitis is mainly based on clinical and histopathological analysis and it may take some time due to the need of a biopsy. Information about the organization and chemical composition of the tissues can be obtained using fluorescence lifetime spectroscopy techniques without the need for biopsy. The main targeted fluorophores are NADH (nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide), with free and bound states showing different fluorescence average lifetimes. The average lifetimes for free and bound NADH and FAD change according to tissue metabolic alterations, allow a quick and non-invasive clinical investigation of injuries and may help clinicians with the early diagnosis of actinic cheilitis. This study aims the evaluation of the fluorescence lifetime spectroscopy to distinguish three categories of epithelial dysplasia, the most important predictor of malignant development, described in up to 100% of actinic cheilitis cases: absent, mild and moderate dysplasia. The results show a differentiation between the normal lip (absent dysplasia) and actinic cheilitis cases (mild and moderate dysplasia).

Imaging using a supercontinuum laser to assess tumor margins in patients with breast carcinoma

Laura A. Sordillo, Peter P. Sordillo M.D., Robert R. Alfano, The City College of New York (United States)

Transmission images of breast normal and malignant tissue samples were obtained using the Leukos supercontinuum (SC) laser light source and IR-CCD InGaAs camera detector (Goodrich Sensors Inc. high response camera SUJ20KTSW-1.7RT with spectral response between 900 nm and 1,700 nm highlighting the second and third optical windows). Patient n=1 was a 60 year old female with invasive ductal carcinoma. Patient n=2 was a 63 year old female who also had invasive ductal carcinoma. Normal and paired malignant tissue samples were imaged in the same frame (no visible space between them) to highlight the differences between these tissue samples. Due to the relative lipid, water and protein content, images of the malignant tissue samples appeared darker (transmitted less light) than their paired normal. This suggests that the use of SC laser and InGaAs detector may provide an alternative optical technique for the assessment of tumor in patients with breast carcinoma. Corresponding optical attenuation measurements from patients n=1 and n=2 were obtained from the tissue samples.

Optical pathology: real time evaluation of disease

Laura A. Sordillo, Peter P. Sordillo M.D., Robert R. Alfano, The City College of New York (United States)

Pathologic assessment of tumor samples is a subjective measure of a cancer, and is therefore subject to human error. Optical pathology utilizes non-invasive, label-free techniques to evaluate the cancer based on the emission of key native biomolecules in the tissue. We have shown that the relative tryptophan content in breast cancer tissue samples increases as a function of histologic grade (high grade correlated with increased emission intensity peak at 340 nm from a 280 nm or 300 nm excitation due largely to tryptophan residues). Stokes shift spectroscopy was also used to better highlight the relative content of the biomolecules in the tissue. Patients with grade 3 (high grade) cancers consistently show increased relative tryptophan content. These results can be obtained instantaneously, without waiting for staining of samples or pathologic review. Rapid, label-free evaluation of disease using native fluorescence and Stoke shift spectroscopy can be an important tool which may reduce waiting time for patients with grade 3 (highly malignant) breast tumors.

Evaluation of algorithmic methods for analyzing fluorescence spectra of cancerous and normal human tissues

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The aim of this study is to evaluate the efficacy of different spectra unmixing methods to identify fresh cancerous and normal prostate tissues from the measured fluorescence spectra. Twenty-four pieces of prostate adenocarcinoma as well as thirty pieces of normal tissues confirmed by pathologist were excited by selective wavelength of 340 nm. The emission spectra of resected fresh tissue were used to evaluate the relative changes of collagen and NADH by various spectral unmixing methods. These methods include two categories: forward methods (ratios at key spectral peaks and key biochemical spectral fitting), and Blind Source Separation (such as Principal Component Analysis (PCA), Independent Component Analysis (ICA), and Nonnegative Matrix Factorization (NMF) etc.). The purpose of the spectral analysis is to discard the redundant information which conceals the differences in the two types of tissues, but keep their diagnostically significance. The facts predicted by different methods were compared to the gold standard of histopathology. The results indicate that the key fluorophores in tissue such as tryptophan, collagen, NADH, and flavin show different relative contents among human cancerous and normal prostate tissues. The sensitivity, specificity, and receiver operating characteristic (ROC) are finally employed as criteria to evaluate efficacy of these methods in cancer detection. This ex vivo preliminary trial demonstrates that these different algorithmic methods can be used to distinguish carcinoma from normal prostate tissues with good sensitivity and specificity, while among them; ICA appears to be the superior method in predication accuracy.

Transcutaneous in vivo Raman spectroscopy: discrimination of benign from malignant breast lesions in animal models

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Mortality of breast cancer, the most fatal cancer amongst women worldwide, can be reduced substantially by early detection of the cancer. However currently available screening tools suffer from several disadvantages. One major disadvantage is their inability to accurately distinguish benign from malignant lesions. A lot of time is wasted on unnecessary biopsy procedures and histopathology, not to mention patient anxiety, since majority of lesions detected turn out to be benign. Therefore, the current study aims to test the...
ability of in vivo Raman spectroscopy to distinguish benign fibroadenomas of breast from malignant breast adenocarcinomas in animal models. Transcutaneous in vivo Raman spectra were acquired from control breast (n=8), breast fibroadenoma (n=10) and breast adenocarcinoma (n=7) of Sprague-Dawley rats and analyzed using Principal-Component-Analysis (PCA) and Principal-Component-Linear-Discriminant-Analysis (PC-LDA). PCA shows three clusters of control, adenocarcinoma and fibroadenoma, of which there is a slight overlap between adenocarcinoma and fibroadenoma clusters. PC-LDA after leave-one-out-cross-validation (LOOCV) shows classification efficiency of 88, 67 and 76% for control, fibroadenoma and adenocarcinoma, respectively. Thus, results suggest feasibility of distinguishing benign from malignant lesions using in vivo Raman spectroscopy. Further studies may help establish Raman spectroscopy as an invaluable adjunct to currently available screening tool.

9703-61, Session PTues

Transcutaneous in vivo Raman spectroscopy: study of pre-adenocarcinoma condition for early breast cancer detection in animal models

Tanmoy Bhattacharjee, Mahazabeen Sayyed, Arvind Ingle, Girish B. Maru, C. Murali Krishna, Advanced Ctr. for Treatment, Research & Education in Cancer (India)

Early detection of breast cancer, the most fatal female cancer worldwide, can substantially reduce mortality. However, currently available screening tools have low sensitivity and specificity. Therefore, alternate screening tools are being extensively explored. One such tool under investigation is Raman spectroscopy owing to its rapidity, sensitivity, specificity and amenability to in vivo applications. In this study, we have explored the possibility of detecting adenocarcinoma early using in vivo Raman spectroscopy. We treated Sprague-Dawley rats with carcinogen and acquired spectra from breast before tumor appearance. The spectra were labelled ‘pre-tumor’ (PT). These spectra were analyzed using Principal-Component-Linear-Discriminant-Analysis (PC-LDA). After leave-one-out-cross-validation (LOOCV), the spectra were classified with 90% efficiency. The results clearly indicate that PT spectra can be classified with high efficiency. If we consider misclassifications with FT, 10% FT spectra were misclassified as ‘normal’ (C/NT) while 15% PT and 94% FT were predicted as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency. If we consider misclassifications with FT, 10% C or NT spectra were classified as ‘abnormal’ (PT/FT). The results clearly indicate that C and FT can be classified with high efficiency.

9703-62, Session PTues

Fluorescence anisotropy characterization of urine in the diagnosis of cancer

Ramu Rajasekaran, Brindha Elumalai, Aruna Prakasa Rao, Anna Univ. Chennai (India); Dornadula Koteeswaran, Meenakshi Univ. (India); Singaravelu Ganesan, Anna Univ. Chennai (India)

Cancer is one of the important global health problems. It is well known that most of the cancer patients diagnosed with advanced stages of cancer and require more aggressive treatment. This yields very poor survival rate and higher morbidity, and hence there arises a need for improved detection techniques. There is a pressing need for improved methods to detect cancer and its precursors. Fluorescence spectroscopy has been considered as one of the tools to identify different fluorophores conformational state under different environment and many investigators explored the diagnostic potential of fluorescence spectroscopy. Fluorescence polarization spectroscopy is one such method to analyze and helps us to elucidate protein structure, molecular dynamics, and characteristics of the local environment. It has been reported that the polarized spectroscopic properties of bio-molecule in particular, fluorophores are highly depends on polarity, pH and viscosity. In this context, many reported to use the fluorescence polarization and anisotropy in diagnostic and imaging the cells and tissues. However, only limited data are available on the characterization of urine using the above said technique. Since urine has many metabolites, attempts were made to study fluorescence anisotropic characterization of the human urine of the patients having malignancy as well as normal subjects and to verify whereas there exist any diagnostic potential. Significant differences were observed between the anisotropic values of malignant and normal subjects. The details of the results and statistical analysis will be discussed in detail.

9703-63, Session PTues

The effect of Stokes shift in the discrimination of urine of cervical cancer from normal subjects

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Native fluorescence spectroscopy of tissues and body biofluids with real time evaluation is considered to be one of the potential methods for monitoring the minor changes in the structure and microenvironment of the native fluorophores. Urine is considered as one of the diagnostically important biological fluid as it has complex mixture of different metabolites where number of them are natural fluorophores. Among various fluorescence spectroscopic techniques, the Stokes shift spectroscopy (SSS), also referred to synchronous luminescence spectroscopy (SLS) technique provides highly resolved spectral signatures of the endogenous fluorophores even in complex systems. Although many reported extensively on the use of fluorescence spectroscopy in the discrimination of different pathological conditions of tissue from their normal counterpart, only limited reports are available on the reasons for the altered spectral signature between normal and abnormal cells and tissues. In particular, the application of SSS of biofluids in diagnostic oncology is in the initial stage. However, still the use of SSS in the characterization of urine and other biofluids is under progress as there is a controversy in the fixing of the A2 value. In this regard, an attempt was made to characterize the urine of patients having cervical cancer and normal subjects with different A2 value. The results of the spectral variation with respect to different Stokes shift values and the diagnostic potential of the A2 value based on the statistical analysis will be discussed in detail.

9703-64, Session PTues

Optical pathology of human brain metastasis of lung cancer using combined resonance Raman and spatial frequency spectroscopies

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Raman spectroscopy has become widely used for diagnostic purpose of breast, lung and brain cancers. This report, a new approach was introduced based on Fourier spatial frequency spectrum analysis of the underlying pattern structure of tissue state. Spatial frequency spectroscopy (SFS) is combined with Resonance Raman (RR) spectroscopic method for the first time to discriminate human brain metastasis of lung cancer from normal tissues. A total of thirty-one label-free micrographic images of normal and metastatic brain cancer tissues was obtained from a confocal micro-Raman spectroscopic system with synchronously examined RR spectra of the corresponding samples were collected from the identical site of tissue. The difference of the randomness of tissue structures between the micrograph images of metastatic brain tumor tissues and normal tissues can be recognized by analyzing the spatial frequency.

By fitting the distribution of the spatial frequency spectra of human brain tissues as a Gaussian function, the standard deviation, ?, can be obtained, which was used to generate a criterion to differentiate human brain cancerous tissues from the normal ones using Support Vector Machine (SVM) classifier. This SFS-SVM analysis on micrograph images presents good results with sensitivity (85%), specificity (75%) in comparison with gold standard reports of pathology and immunology. The dual-modal advantages of SFS combined with RR spectroscopy method may open a new way in the neuropathology applications.

9703-65, Session PTues

Optical pathology study of human abdominal aorta tissues using confocal micro resonance Raman spectroscopy: vibrational fingerprints

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Resonance Raman (RR) spectroscopic technique has a high potential for label-free detection of biomedical lesions in vivo. This study evaluates RR spectroscopy method as an optical pathology tool to detect the abdominal aortic atherosclerosis in vitro. This part presents the RR spectral molecular fingerprint features from different types of the atherosclerotic aortic wall tissues. Total 65 sites of five pieces aortic wall (intimal and adventitial wall) tissues from an autopsy specimen were examined using confocal micro Raman system of WITec 300R with excitation wavelength of 532nm. Among of the tested sites, 16 sites were repeated 3 times. The preliminary RR spectral biomarkers (molecular fingerprints) indicated that typical calcified atherosclerotic plaque (RR peak at 964cm-1) tissue; fibrolipid plaque (RR peaks at 1007, 1161, 1517 and 2888cm-1) tissue, lipid pool with the fatty precipitation (cholesterol) with collagen type I increased (RR peaks at 864, 1452, 1658, 2888 and 2948cm-1) in the soft tissue were observed and investigated. We also observed a pair of Raman peaks appears at 2441cm-1 and 487cm-1 that may arise from chemicals used in initial treatment.

9703-66, Session PTues

Raman spectroscopy of bio fluids: an exploratory study for oral cancer detection

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Optical spectroscopic techniques have shown great promise in extracting the biochemical and morphological information for various disease diagnosis including cancers. Among various cancers, oral cancer is the most common cancers in India and it accounts for one third of the global oral cancer burden. Recently, Raman spectroscopy has gained much attention in the diagnostic oncology, as it provides unique spectral signature corresponding to metabolic alterations under different pathological conditions and micro-environment. Based on these, several studies have been reported on the use of Raman spectroscopy in the discrimination of diseased conditions from their normal counterpart at cellular and tissue level, but only limited studies were available on bio-fluids. In this regard, optical characterization of bio-fluids has geared up for biomarker identification in the disease diagnosis, as they have several advantages over conventional biopsy. Based on these, in the present study an attempt has been made to study the metabolic variations in the blood, urine and saliva of oral cancer patients and normal subjects using Raman spectroscopy. For this, blood, urine and saliva from oral cancer patients and normal subjects were collected and they were analyzed under 785nm excitation to probe the molecular/spectral differences between normal and malignant subjects. Principal Component based Linear Discriminant Analysis (PC-LDA) followed by Leave-One-Out Cross-Validation (LOOCV) was employed to find the statistical significance of the present technique in discriminating the malignant conditions from normal subjects, and the results will be discussed in detail.

9703-67, Session PTues

An empirical formula based on Monte Carlo simulation for diffuse reflectance from turbid media

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The surface of diffuse reflectance with respect to μa and μs’ is a continuous monotonous surface. The fitting surface was chosen as a function of μa and μs’. A program in Matlab was written, which estimates variance between Monte Carlo simulation values and diffuse reflectance values obtained from empirical equation. The coefficients of the fitting surface were estimated using optimization algorithm for which the variance reaches a minimum value. The variance between Monte Carlo simulation values and fitting surface was found to be 0.004% error. Both classical Monte Carlo simulation and proposed empirical formula reveal same result.

Further, the result was compared with diffusion approximation theory and other empirical methods. This comparative study shows that the proposed empirical formula has the accuracy of Monte Carlo simulation values and the predictions of the empirical formula is valid for a wide range of scattering values.

9703-38, Session 9

Nanosecond coherent anti-Stokes Raman scattering for particle size characterization

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Conference 9703: Optical Biopsy XIV:
Toward Real-Time Spectroscopic Imaging and Diagnosis

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Laser diffraction particle size analyzers commonly make use of light diffraction and scattering around the particle considered in its medium. For particle size below 50 μm, the Fraunhofer theory is abandoned and an alternative theory is required to know the complex refractive index of both the particle and the medium. Here we demonstrate that particle size characterization can be realized by measuring the macroscopic Raman spectral response of the whole set of particles excited by a laser beam. We use a home-made spectroscope based on coherent anti-Stokes Raman scattering (CARS) and having a 0.36 cm⁻¹ spectral resolution, in which the laser source is a dual-output infrared monochromatic supercontinuum source (1064 nm monochromatic pump wave, 1100-2000 nm broadband Stokes wave). The samples are latex beads in water with different diameters (20 nm, 50 nm, 100 nm, 5 μm). The C-H stretching line around 3050 cm⁻¹ is studied. For this vibration, we show the variation of both the CAR central frequency and linewidth as a function of particle size. A quasi linear increase of the linewidth with the inverse of the diameter is measured; a difference of 15 cm⁻¹ is obtained between beads of 5 μm and 20 nm respectively. The physical phenomena at the origin of this difference are discussed, especially considering the contributions of the center of the object and of the edges of the object to the global Raman response.

9703-39, Session 9
Penetration depth in biological tissues from hyperspectral imaging in SWIR in transmission and reflection geometry
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Large depth penetration in tissue is one of the main objectives of in vivo optical imaging and non invasive diagnosis. The use of visible light in imaging is convenient but only feasible for thin slices of tissue due to the strong scattering and absorption effects of tissue components. To alleviate this effect, near infrared (NIR) light is commonly used in thick tissue samples. While photon penetration in NIR range is well studied and optical imaging in the traditional NIR is established, the field of SWIR imaging is a relatively less explored area. In this work, we have focused on the optical properties of a biological tissue in SWIR. For that we developed a hyperspectral imager with high acquisition speed and spatial resolution and conducted the study on intralipid phantoms and chicken tissues in transmission and reflection geometries.

9703-40, Session 9
Upconversion imaging using an all-fiber supercontinuum source
Laurent Huot, NKT Photonics A/S (Denmark) and DTU Fotonik (Denmark); Christian Pedersen, Peter Tidemand-Lichtenberg, Jeppe S. Dam, DTU Fotonik (Denmark); Peter M. Moselund, Christopher D. Brooks, NKT Photonics A/S (Denmark)

The combination of high brightness mid-IR supercontinuum lasers and upconversion detection systems opens a unique possibility for combining two leading edge technologies pointing towards extremely fast and/or extremely sensitive imaging and spectroscopy in the mid-IR range.

We present a novel mid-IR imaging system born from the combination of an all fiber mid-IR supercontinuum source and an all fiber upconversion detection system. The source delivers 1.2W of average power and its spectrum ranges from 1.8μm to 2.6μm. In this wavelength region traditional IR solid state detectors are known to suffer from significant dark noise compared to silicon based detectors operating in the NIR. In this setup however, the supercontinuum signal is mixed in bulk lithium niobate with a mixing pump at 1550nm, thus resulting in an upconverted signal ranging from 830nm to 980nm. This upconverted signal is acquired on an affordable standard silicon CCD array with much better noise and time performance than direct detection systems in the mid-IR. Additionally, the high brightness of the pump source allows for efficient upconversion and enables the use of a large mixing mode which enhances spatial and spectral resolution.

We investigate the influence of temporal, spectral and polarization noise of the supercontinuum source on the overall performance of the device. We also study the influence of the spatially coherent nature of the source on speckle formation in the upconverted image.

9703-41, Session 9
Measuring the scattering anisotropy by combining SFR spectroscopy and OCT: accounting for the phase function dependence
Anouk L. Post, Xu U. Zhang, Academisch Medisch Centrum (Netherlands); Nienke Bosschaart, Univ. Twente (Netherlands); Ton G. Van Leeuwen, Henricus J. C. M. Sterenborg, Dirk J. Faber, Academisch Medisch Centrum (Netherlands)

Both Optical Coherence Tomography (OCT) and Single Fiber Reflectance Spectroscopy (SFR) are used to determine various optical properties of tissue. We developed a method combining these techniques to measure the scattering anisotropy (g1) and ? (=1-g2/1-g1), related to the 1st and 2nd order moments of the phase function. The phase function is intimately associated with the cellular organization and ultrastructure of tissue, and physical parameters that may change during disease onset and progression. Quantification of these parameters may therefore allow for improved non-invasive, in vivo discrimination between healthy and diseased tissue. With SFR the reduced scattering coefficient and ? can be extracted from the reflectance spectrum (Kanick et al., Biomedical Optics Express 2(6), 2011). With OCT the scattering coefficient can be extracted from the signal as a function of depth (Faber et al., Optics Express 12(19), 2004). Consequently, by combining SFR and OCT measurements at the same wavelengths, the scattering anisotropy (g) can be resolved using μs² = μs²(1-?g). We performed measurements on a suspension of silica spheres as a proof of principle. The SFR model for the reflectance as a function of the reduced scattering coefficient and ? is based on semi-empirical modelling. These models feature Monte-Carlo (MC) based model constants. The validity of these constants - and thus the accuracy of the estimated parameters - depends on the phase function employed in the MC simulations. Since the phase function is not known when measuring in tissue, we will investigate the influence of assuming an incorrect phase function on the accuracy of the derived parameters.

9703-42, Session 9
Label-free vascular imaging in a spontaneous hamster cheek pouch carcinogen model for pre-cancer detection
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Inducing angiogenesis is one hallmark of cancer. Tumor induced neovascularure is often characterized as leaky, tortuous and chaotic, unlike a highly organized normal vasculature. Additionally, in the course of carcinogenesis, angiogenesis precedes a visible lesion. Tumor cannot grow beyond 1-2 mm in diameter without inducing angiogenesis. Therefore,
Hyperspectral imaging fluorescence excitation scanning for detecting colorectal cancer: pilot study

Silas J. Leavesley, Mikayla Wheeler, Carmen Lopez, Thomas Baker, Peter F. Favreau, Thomas C. Rich, Paul F. Rider, Carole W. Boudreaux, Univ. of South Alabama (United States)

Optical spectroscopy and hyperspectral imaging have shown the theoretical potential to discriminate between cancerous and non-cancerous tissue with high sensitivity and specificity. To date, these techniques have not been able to be effectively translated to endoscope platforms. Hyperspectral imaging of the fluorescence excitation spectrum represents a new technology that may be well-suited for endoscopic implementation. However, the feasibility of detecting differences between normal and cancerous mucosa using fluorescence excitation-scanning hyperspectral imaging has not been evaluated. The objective of this pilot study was to evaluate the changes in the fluorescence excitation spectrum of resected specimen pairs of colorectal adenocarcinoma and normal colorectal mucosa. Patients being treated for colorectal adenocarcinoma were enrolled. Representative adenocarcinoma and normal colorectal mucosa specimens were collected from each case. Specimens were flash frozen in liquid nitrogen. Adenocarcinoma was confirmed by histologic evaluation of H&E permanent sections. Hyperspectral image data of the fluorescence excitation of adenocarcinoma and surrounding normal tissue were acquired using a custom microscope configuration previously developed in our lab. Results demonstrated consistent spectral differences between normal and cancerous tissues over the fluorescence excitation spectral range of 390-450 nm. We conclude that fluorescence excitation-scanning hyperspectral imaging may offer an alternative approach for differentiating adenocarcinoma and surrounding normal mucosa of the colon. Future work will focus on expanding the number of specimen pairs analyzed and will utilize fresh tissues where possible, as flash freezing and reconstituting tissues may have altered the autofluorescence properties.

Protocol using elliptically polarized light for enhanced contrast in polarization gating imaging of biological tissues

Susmita Sridhar, Anabela da Silva, Institut Fresnel (France) and Aix-Marseille Univ. (France); Ivo Vanzetta, Institut des Neurosciences de la Timone, Aix-Marseille Univ. (France)

Polarization gating is a popular technique in biomedical optics to classify and select photons based on their state of polarization. Contrast of tissue surface is enhanced by selecting the polarization maintaining photons, usually using a collinear imaging channel, and, in reverse, deeper volumes are probed by selecting the depolarized ones, usually by performing cross-linear measurements. Instead of using the conventional linearly polarized illumination, we propose to take advantage of using elliptically polarized light as it allows for more selective probing in terms of depth. Co-elliptical measurements allow access to deeper subsurface volumes than collinear measurements, the depth of probing being controlled by the ellipticity of polarization. Counter-elliptical measurements attenuate subsurface signal and, hence, enhance the signal coming from deeper volumes, provided that mirror reflections are filtered. We propose a new protocol of polarization gating data-acquisition that combines co-elliptical and counter-elliptical measurements. Validations of the approach include measurements on phantoms and ex vivo tissues. For the purpose of illustrating different modes of application, two series of in vivo measurements were performed: examination of volunteers’ skin abnormalities and visualization of the exposed cortex of an anaesthetized rat. In the first application, one seeks at accessing subsurface information, co-elliptical imaging configuration is
adopted in order to illustrate the selective probing in depth. In the second application, the aim was to selectively probe first and second vascular layers, co-elliptical and counter-elliptical imaging channels are used for subsurface probing or, in reverse, for better probing of deeper volume signals and attenuating subsurface signals.

9703-47, Session 10

Characterizing microstructural changes of skeletal muscle tissues using spectral transformed Mueller matrix polarization parameters

Chao He, Honghui He, Jintao Chang, Hui Ma, Graduate School at Shenzhen, Tsinghua Univ. (China)

Polarization imaging techniques are recognized as potentially powerful tools to detect the structural changes of biological tissues. Meanwhile, spectral features of the scattered light can also provide abundant microstructural information, therefore can be applied in biomedical studies. In this paper, we adopt the polarization reflectance spectral imaging to analyze the microstructural changes of hydrolyzing skeletal muscle tissues. We measure the Mueller matrix, which is a comprehensive description of the polarization properties, of the bovine skeletal muscle samples in different periods of time, and analyze its behavior using the multispectral Mueller matrix transformation (MMT) technique. The experimental results show that for bovine skeletal muscle tissues, the backscattered spectral MMT parameters can be used to distinguish the different stages of hydrolysis. The MMT parameters have different values and variation features at different stages. We also use Monte Carlo simulations based on the sphere-cylinder birefringence model (SCBM) to study the relationships between the backscattered spectral MMT parameters and the microstructural changes of the skeletal muscle tissues during hydrolysis. Both the experimental and simulated results indicate that the stages of hydrolysis for bovine skeletal muscle samples can be judged by the spectral MMT parameters, while the microstructural variations can also be detected. The results presented in this work show that combining with the spectral technique, the MMT parameters have the potential to be used as tools for meat quality detection and monitoring.

9703-48, Session 11

Preliminary investigation of intrinsic UV fluorescence spectroscopic changes associated with proteolytic digestion of bovine articular cartilage

William Lewis, Walfre Franco, Juan Pablo Padilla-Martinez, Antonio Ortega-Martinez, Wellman Ctr. for Photomedicine (United States)

Degradation and destruction of articular cartilage is the etiology of osteoarthritis (OA), an entity second only to cardiovascular disease as a cause of disability in the United States. Joint mechanics and cartilage biochemistry are believed to play a role in OA; an optical tool to detect structural and chemical changes in articular cartilage might offer benefit for its early detection and treatment. The objective of the present study was to identify the spectral changes in intrinsic ultraviolet (UV) fluorescence of cartilage that occur after proteolytic digestion of cartilage. Bovine articular cartilage samples were incubated in varying concentrations of collagenase ranging from 10ug/mL up to 5mg/mL for 18 hours at 37 °C, a model of OA. Pre- and post-incubation measurements were taken of the UV excitation-emission spectrum of each cartilage sample. Mechanical tests were performed to determine the pre- and post-digestion force/displacement ratio associated with indentation of each sample. Spectral changes in intrinsic cartilage fluorescence and stiffness of the cartilage were associated with proteolytic digestion. In particular, changes in the relative intensity of fluorescence peaks associated with pentosidine crosslinks (330nm excitation, 390 nm emission) and tryptophan (290 nm excitation, 340 nm emission) were found to correlate with different degrees of cartilage digestion and cartilage stiffness. In principle, it may be possible to use UV fluorescence spectral data for early detection of damage to articular cartilage, and as a surrogate measure for cartilage stiffness.

9703-49, Session 11

In vivo detection of apoptosis with cellular level resolution using fluorescence lifetime imaging

Andrew J. Bower, Marina Marjanovic, Youbo Zhao, Joanne Li, Eric J. Chaney, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Cell death plays a crucial role in homeostasis as well as in the pathogenesis and treatment of many diseases. Of great importance in the study of cell death is the identification of apoptosis. While this complex process has been extensively characterized in vitro, label-free detection of apoptosis in vivo at the cellular level remains difficult. In this study, for the first time, fluorescence lifetime imaging microscopy (FLIM) of intracellular reduced nicotinamide adenine dinucleotide (NADH) was utilized to assess the metabolic response of in vivo mouse epidermal keratinocytes following induction of apoptosis and necrosis. Results show significantly elevated levels of both the mean lifetime of NADH and the intracellular ratio of protein bound-to-free NADH in apoptotic compared to necrotic and healthy tissue. In addition, single cell analysis of these metabolic profiles is performed to study the cell death process in single cells in vivo. Through identifying these temporal metabolic signatures, apoptosis in single cells can be studied in native tissue environments within the living organism, allowing direct study of pathological alterations to cell death pathways.

9703-50, Session 11

Wound size measurement of diabetic foot ulcers using region growing algorithm

Arash Dadkhah, Elizabeth Solis, Ruogu Fang, Anuradha Godavarty, Florida International Univ. (United States)

Diabetic foot ulcer is one of the most common complications, which occurs in approximately 15 percent of the diabetes patients. It is also one of leading causes of lower extremity amputations in the U.S., and about 14-24% of the severe cases of diabetes mellitus require amputations. Clinical studies have shown that reduction in the wound size by 40% or more within 4 weeks is an acceptable progress in the healing process. Quantification of the wound size plays a crucial role in assessing the extent of healing and determining the treatment process. To date, wound healing is visually inspected and the wound size is measured from surface images. The extent of wound healing internally may vary from the surface. A near-infrared (NIR) optical imaging approach has been developed for non-contact subsurface imaging of wounds and differentiating healing from non-healing wounds. Herein quantitative wound size measurements from NIR and white light images are estimated using a region growing based image segmentation algorithm. The extent of wound healing from weekly NIR imaging of diabetic foot ulcers (IRB approved study) are quantified and compared across NIR and white light images. NIR imaging and wound size measurements can play a significant role in predicting the extent of internal healing on a weekly basis, thus allowing better treatment plans for each subject.
9703-51, Session 11

Monitoring combat wound healing by IR hyperspectral imaging

Christopher R. Howle, Abigail M. Spear, Ehsan Gazi, Defence Science and Technology Lab. (United Kingdom); Nicole J. Crane, Naval Medical Research Ctr. (United States) and Uniformed Services Univ. of the Health Sciences (United States)

In recent conflicts, battlefield injuries consist largely of extensive soft injuries from blasts and high energy projectiles, including gunshot wounds. Repair of these large, traumatic wounds requires aggressive surgical treatment including multiple surgical debridements to remove devitalised tissue and to reduce bacterial load. Identifying those patients with wound complications, such as infection and impaired healing, could greatly assist health care teams in providing the most appropriate and personalised care for combat casualties.

Candidate technologies to satisfy these requirements include the fusion of imaging and optical spectroscopy to enable rapid identification of key markers. Hence, a novel system based on IR negative contrast imaging (NCI) is presented. Two NCI systems have been developed, which are based upon optical parametric oscillator (OPO) sources comprising either a periodically-poled LiNbO3 (PPLN) crystal that operates in the shortwave and midwave IR spectral regions (ca. 1.5 – 1.8 µm and 2.6 – 4 µm, respectively) or a ZGP crystal that operates in the longwave IR region (ca. 6 – 8 µm). Wavelength tuning is achieved by translating the crystal within the pump beam. System size and complexity are minimised by the use of single element detectors and the intracavity OPO design. Images are composed by raster scanning the monochromatic beam over the scene of interest; the reflection and/or absorption of the incident radiation by target materials and their surrounding environment provide a method for spatial location. Results using these NCI systems to characterise wound biopsies are presented here.

9703-55, Session 12

Study on discrimination of oral cancer from normal using blood plasma based on fluorescence steady and excited state at excitation wavelength 280 nm

Rekha Pachaiappan, Aruna Prakasa Rao, Singaravelu Ganesan, Anna Univ. Chennai (India)

Many research works based on fluorescence spectroscopy have proven its potential in the diagnosis of various diseases using the spectral signatures of the native key fluorophores such as tryptophan, tyrosine, collagen, NADH, FAD and porphyrin. These fluorophores distribution, concentration and their conformation may be changed depending upon the pathological and metabolic conditions of cells and tissues. Although many have reported on cells and tissues, not much of work on the characterization of biofluids. In this study, we have made a pilot attempt to characterize the blood plasma of normal subject and oral cancer patients by native fluorescence spectroscopy at 280 nm excitation. Further, the fluorescence data were analyzed by employing the multivariate statistical method - linear discriminant analysis using leave one out cross validation method. The analysis yielded a diagnostic sensitivity of 93.8% and a specificity of 80% in the discrimination between normal and oral cancer blood plasma. An efficiency of 87.1% obtained for both the original and cross validated groups. The results illustrate the potential of fluorescence spectroscopy technique in the diagnosis of oral cancer using blood plasma. The details of results will be discussed.

9703-56, Session 12

Serum-based Raman spectroscopy: discrimination of benign from malignant lesions and identification of pre-tumor condition

Tanmoy Bhattacharjee, Sneha Tawde, Aarif Khan, Piyush Kumar, Arvind Ingle, Girish B. Maru, C. Murali Krishna, Advanced Ctr. for Treatment, Research & Education in Cancer (India)

Although the most fatal cancer among women worldwide, breast cancer mortality can be reduced by early detection of the ailment. The current screening tools have two major disadvantages – they cannot effectively distinguish malignant from benign lesions (90% lesions are found to be benign) and their sensitivity and specificity to early carcinogenic changes is low. To circumvent these disadvantages, we have investigated use of serum-based Raman spectroscopy to distinguish benign from malignant changes and to identify pre-tumor condition in this study. Blood was collected from adenocarcinoma bearing (FT), fibroadenoma bearing (FF) and control (C) rats and carcinoma. The blood samples and analysed using Principal-Component-Linear-Discriminant Analyses (LDA) using leave one out cross validation method. The analysis yielded a diagnostic sensitivity of 93.8% and a specificity of 80% in the discrimination between normal and oral cancer blood plasma. An efficiency of 87.1% obtained for both the original and cross validated groups. The results illustrate the potential of fluorescence spectroscopy technique in the diagnosis of oral cancer using blood plasma. The details of results will be discussed.

9703-53, Session 12

LED-based endoscopic light source for spectral imaging

Craig Browning, Samuel Mayes, Silas J. Leavesley, Univ. of South Alabama (United States)

Colorectal cancer is the 3rd leading cause of cancer deaths in the United States.[1] The standard screening technique is colonoscopy using white light endoscopy (WLE). Several newer methods have been implemented to improve the performance of WLE, including narrow band imaging and autofluorescence imaging.[2] However, these methods have demonstrated negligible increases in sensitivity and specificity in large-scale studies. The goal for the project is to implement a real time spectral imaging endoscope using 16 narrow-band LEDs that increases detection sensitivity and specificity.

An Olympus CLK-4 light source was modified to accommodate a novel spectral LED array and circuitry. Custom electronics were designed to allow high-speed wavelength switching and independent wavelength dimming. Computer control was provided via National Instruments hardware and NIS Elements software.

The retrofit design was successfully fitted with the spectral array and circuitry. LED testing resulted in effective wavelength switching and independent wavelength dimming. Optical output scaled linearly with LED current, which scaled linearly with reference voltage to the current driver. A look-up table was created allowing all LEDs to be set to an equal power output (flat spectral illumination).

The spectral light source was successfully coupled to the endoscope. A high-speed scCMOS camera was also coupled to the endoscope. Ongoing work includes testing the system performance. Future work will focus on acquiring spectral image data from ex vivo pairs of normal, precancerous and cancerous tissues, and assessing the ability to detect early-stage colorectal cancer.
9703-57, Session 12

**Steady state fluorescence spectroscopic characterization of normal and diabetic urine at 280 nm excitation**

Anjana Kesavan, Rekha Pachaiappan, Aruna Prakasa Rao, Singaravelu Ganesan, Anna Univ. Chennai (India)

Urine is considered for its diagnostically important native fluorophores present in it. These fluorophores varies both in their distribution, concentration and physiochemical properties depending upon the metabolic condition of the subject. Many have reported on the cells and tissues, not much work have been reported on the characterization of biofluids. In this study, we have made a pilot attempt to characterize the urine of normal subject and diabetic patients by native fluorescence spectroscopy at 280 nm excitation. Further, the fluorescence data were analyzed employing the multivariant statistical methods such as principal component analysis (PCA) coupled with linear discriminant analysis (LDA) using leave one out cross validation method. The analysis yielded a diagnostic sensitivity of 90% and a specificity of 80% in the classification of normal and diabetic urine. An efficiency of 85% obtained for both the original and cross validated groups. The results were promising in discriminating diabetic urine from that of normal urine. The details of results will be discussed.

9703-68, Session 12

**Time resolved fluorescence spectroscopy with multi-photon or single-photon excitation of label free molecules in brain tissue of an Alzheimer’s mouse model**

Bidyut Das, Lingyan Shi, The City College of New York (United States); Adrian Rodriguez-Contreras, The City Univ. of New York (United States); Robert R. Alfano, The City College of New York (United States)

Multi-photon excitation in the time-resolved domain is explored as a potential non-invasive neuroimaging technique for early detection and monitoring of the neurodegenerative condition of Alzheimer’s disease (AD). The definitive diagnosis of AD is currently possible by detecting the key fingerprints of extracellular aggregates of amyloid-β plaques after brain autopsy. Fluorescence lifetime measurements at various emission bands were performed ex vivo on normal and AD mouse brain tissues using multi-photon or single-photon excitation. Fluorescence life times of emissions from label free molecules, such as Tryptophan ~340nm (excited at 800nm), NADH~440nm (excited at 400nm or 800nm), Flavin~525nm (excited at 400nm or 800nm) were measured using 100 femtosecond pulses from a Ti:Sapphire laser and a streak camera with a time-resolution of 10ps. The emission decay time of these molecules may indicate the environmental changes inside the brain such as the deposition of amyloid-beta or tau plaques and neurofibrillary tangles—the characteristic signature of Alzheimer’s disease. The potential for near infrared brain imaging for AD with multi-two-photon and three-photon time-resolved microscopy will be presented.
9704-2, Session 1

Functionalized plasmonic nanostructure arrays for direct and accurate mapping extracellular pH of living cells using SERS
Fang Sun, Shaoyi Jiang, Qiuming Yu, Univ. of Washington (United States)

The extracellular pH (pHe) of living cells is one of the major factors that influence cell behaviors including cycle progression, migration, and proliferation, as well as metastasis and invasion of tumor cells. Thus, accurate sensing and mapping of the pHe is still a critical yet challenging task in the study of pHe-dependent cell behaviors. In this work, we present a method to map pHe of living cells based on surface-enhanced Raman spectroscopy (SERS). We immobilized a pH probe molecule, 4-mercaptobenzoic acid (4-MBA), on a gold quasi three-dimensional plasmonic nanostructure array (Q3D-PNA) to enable an exceptionally sensitive and reproducible pH measurement. We prudentially investigated the influences of cations and complexity of detecting solutions on the responses of 4-MBA SERS spectra to pH variations to ensure the accuracy. The detection curves of pH were obtained for different buffers and cell culture media. Herein, a normal cell line (NIH/3T3) and a tumor cell line (HepG2) were cultured on the 4-MBA modified SERS substrates. Localized pHe was detected and mapped with good spatial resolution and pH sensitivity showing pHe domains on both cells. Moreover, the averaged pHe of tumor cells was shown to be more acidic compared with that of normal cells.

9704-3, Session 1

Determination of optical properties of porcine skin in the mid-infrared regime
Arthur Schönhals, Niels Kröger-Lui, Annemarie Pucci, Wolfgang Petrich, Ruprecht-Karls-Univ. Heidelberg (Germany)

In the last decades, the development of non-invasive methods for clinical practice has advanced continuously. The scope of using electromagnetic radiation in the ultraviolet, visible or infrared spectral range substantially depends on the optical properties of the sample investigated. Therefore, a detailed knowledge of the respective optical parameters is essential for the successful implementation.

While numerous studies of the optical properties of tissue were carried out in the visible and near-infrared wavelength range, the optical properties of tissue in the mid-infrared (MIR) spectral range are only known to a rather limited extend. The MIR regime provides spectroscopic access to many molecules in blood or the interstitial fluid due to their strong vibrational bands, such as glucose, proteins, etc. However, due to strong absorption by water it has been cumbersome to use conventional MIR methods, e.g. FTIR-spectroscopy, in order to determine optical properties of biological tissue. Here we show that by virtue of using quantum cascade lasers (QCLs) it is possible to fill this gap. Because of their high spectral power density QCLs are subject to particular interest for biomedical applications, such as novel monitoring techniques or MIR-microscopy.

In order to determine the optical properties of biological tissues in the MIR a QCL-based setup has been assembled. First investigations of the optical parameters of porcine skin in the MIR will be presented.

9704-4, Session 1

UV-resonance Raman spectroscopy of amino acids
Martin Höhl, Merve Meinhardt-Wollweber, Uwe Morgner, Leibniz Univ. Hannover (Germany); Heike Schmitt, Thomas Lenarz, Medizinische Hochschule Hannover (Germany)

Resonant enhancement of Raman signals is a useful method to increase sensitivity in samples with low concentration such as biological tissue. The investigation of resonance profiles shows the optimal excitation wavelength and yields valuable information about the molecules themselves. However careful characterization and calibration of all experimental parameters affecting quantum yield is required in order to achieve comparability of the single spectra. We present an experimental technique for measuring the resonance profiles of different amino acids. The absorption lines of these molecules are located in the ultraviolet (UV) wavelength range. One limitation for broadband measurement of resonance profiles is the availability of Raman filters in the UV for blocking the Rayleigh scattered light. Here, a wavelength range from 244.8 nm to 266.0 nm was chosen. The excitation emission maps reveal the optimal wavelength for distinguishing amino acids in aqueous solutions. Challenges occurring and possible solutions are discussed. This study provides the basis for measurements on more complex molecules like proteins in the perilymph. The composition of this liquid in the inner ear is essential for hearing and cannot be analyzed non-invasively so far. The long term aim is to implement this technique as a fiber based endoscope for non-invasive measurements during surgeries (e.g. cochlear implants) making it available as a diagnostic tool for physicians. This project is embedded in the interdisciplinary cluster of excellence “Hearing for all” (H4A) which aims at a major breakthrough in hearing research.

9704-5, Session 1

Raman spectroscopy for highly accurate estimation of the age of refrigerated porcine muscle
Constantinos Timinis, Costas Pitris, Univ. of Cyprus (Cyprus)

The high water content of meat, combined with all the nutrients it contains, make it vulnerable to spoilage at all stages of production and storage even when refrigerated at 5°C. A non-destructive and in situ tool for meat sample testing, which could provide an accurate indication of the storage time of meat, would be very useful for the control of meat quality as well as for consumer safety. The proposed solution is based on Raman spectroscopy which is non-invasive and can be applied in situ. For the purposes of this project, 42 meat samples from 14 animals were obtained and three Raman spectra per sample were collected every two days for two weeks. The spectra were subsequently processed and the sample age was calculated using a set of linear differential equations. In addition, the samples were classified in categories corresponding to the age in 2-day steps (i.e., 0, 2, 4, 6, 8, 10, 12 or 14 days old), using linear discriminant analysis and cross-validation. Contrary to other studies, where the samples were simply grouped into two categories (higher or lower quality, suitable or unsuitable for human consumption, etc.), in this study, the age was predicted with a mean error of ~1 day (20%) or classified, in 2-day steps, with 100% accuracy. Although Raman spectroscopy has been used in the past for the analysis of meat samples, the proposed methodology has resulted in a prediction of the sample age far more accurately than any report in the literature.
9704-37, Session 1

**Discrete frequency infrared imaging using quantum cascade lasers for biological tissue analysis**

Kevin L. Yeh, Univ. of Illinois at Urbana-Champaign (United States); Rohit Bhargava, Beckman Institute for Advanced Science and Technology (United States)

Infrared (IR) spectroscopic imaging is an emerging modality for biological tissue analysis that has traditionally employed an interferometer for spectral discrimination. Recent technology developments have made discrete frequency sources, both lasers and filters, practical for imaging. The use of quantum cascade lasers in particular, presents new opportunities as well as challenges. Here we describe results from a novel optical setup for an IR microscope and characterize the performance in terms of important spectral properties of lung tissue. The relation of spectral quality to histologic analysis is discussed. Results show the possibility of rapid imaging with a smaller number of frequencies and the importance of the data quality in determining the same.

9704-6, Session 2

**Serum Raman spectroscopic classification of buccal mucosa and tongue cancers**

Aditi Sahu, Sharada Sawant, C. Murali Krishna, Advanced Ctr. for Treatment, Research & Education in Cancer (India)

Oral cancers are the most common cancers in Indian men, with many anatomical sites associated with less favorable prognosis. Although tongue and floor-of-mouth are the globally most common sites for oral cancer, buccal mucosa and tongue are the most common oral cancer subsites in the Indian sub-continent. These two cancers demonstrate differing biological characteristics with respect to aggressiveness, metastasis and angiogenesis with proteomic and molecular studies indicating differential expression of several proteins, including cell-cycle regulatory proteins. Previous serum Raman spectroscopic (RS) pilot studies demonstrating potential in classifying normal and oral cancers had indicated a feasibility of classification between buccal and tongue cancers. In this evaluation study, spectra were recorded from serum of buccal mucosa (n=62) and tongue cancer (n=58) subjects using Raman microprobe. Mean spectral comparisons indicate intensity-related variations and minor shifts at 1008 (Phe), 1085-1095 (DNA phosphate backbone), 1270 (amide III), 1320, 1344 (DNA bases and CH2, CH3 twisting in proteins), 1450 (CH2 bending in proteins), and 1660 cm-1 (amide I). Multivariate analysis using principal-component-analysis (PCA) and principal-component-linear-discriminant-analysis (PC-LDA) gave two almost distinct clusters corresponding to buccal-mucosa and tongue cancer. Leave-one-out-cross-validation yields average classification efficiency of ~71%. Thus, buccal mucosa and tongue cancers, sub-entities of oral cancers can be differentiated. The preliminary findings indicate that different cancer sub-sites may have different Raman spectroscopic signatures. Prospectively, site-specific cancer detection may be possible using serum RS.

9704-7, Session 2

**Raman-based identification of circulating tumor cells for cancer diagnostics**

Christoph Krafft, Claudia Beleites, Iwan Schie, Leibniz-Institut für Photonische Technologien e.V. (Germany); Joachim H. Clement, Universitätsklinikum Jena (Germany); Jürgen Popp, Leibniz-Institut für Photonische Technologien e.V. (Germany)

Circulating tumor cells (CTCs) that can be extracted from body fluids offer new prospects in cancer diagnostics. An overview about our achievements is presented to use Raman-based methodologies to distinguish cancer cells from normal blood cells. In a first approach, a microfluidic chip was developed to collect Raman spectra from optically trapped cells. Whereas sensitivities and specificities were promising, the throughput was not compatible with the expected low number of CTCs per million white blood cells. A second approach utilized surface enhanced Raman scattering (SERS) active nanoparticles that were functionalized for CTC detection. Signal enhancement of factor 1000 enabled shorter exposure time to acquire Raman spectra of SERS-labeled CTCs in continuous flow without trapping. Another strategy immobilize up to 300000 cells onto a microhole array made of silicon nitride. Rapid microscopic screening pre-selects a subset of cells from which Raman spectra are collected for specific CTC identification. As this approach is compatible with living cells and Raman spectroscopy with 785 nm excitation is non-destructive, a robotic arm can select positively identified CTCs for in-depth biochemical assessment. Finally, an in vivo approach uses a functionalized needle that was developed by GLUPI (Germany). After injecting the needle in the blood vessel, CTCs are collected from the blood stream by antibodies in an alginate coating, detached from the needle, transferred to a microfluidic cartridge and subjected to Raman spectroscopy for cell typing and enumeration.

9704-8, Session 2

**Preanalytical considerations in detection of colorectal cancer in blood serum using Raman molecular imaging**

Patrick J. Treado, Shona D. Stewart, Aaron Smith, Heather Kirschner, Christopher Post, ChemImage Corp. (United States); Bergein F. Overholt M.D., Gastrointestinal Associates, P.C. (United States)

Colorectal cancer (CRC) is the third most common cancer in men and women in the United States. Raman Molecular Imaging (RMI) is an effective technique to evaluate human tissue, cells and bodily fluids, including blood serum for disease diagnosis. Chemimage Corporation, in collaboration with clinicians, has been engaged in development of an in vitro diagnostic Raman assay focused on CRC detection. The Raman Assay for Colorectal Cancer (RACC) exploits the high specificity of Raman imaging to distinguish diseased from normal dried blood serum droplets without additional reagents. Pilot Study results from testing of hundreds of biobank patient samples have demonstrated that RACC detects CRC with high sensitivity and specificity. However, expanded clinical trials, which are ongoing, are revealing a host of important preanalytical considerations associated with sample collection, sample storage and stability, sample shipping, sample preparation and sample interferents, which impact detection performance. Results from recent clinical studies will be presented.

9704-9, Session 2

**Blood test using surface-enhanced Raman spectroscopy with colloidal silver nanoparticle substrate to detect polyps and colorectal cancer**

Wenbo Wang, BC Cancer Agency Research Ctr. (Canada); Shangyuan Feng, BC Cancer Agency Research Ctr. (Canada) and Fujian Normal Univ. (China); Isabella T. Tai, Canada’s Michael Smith Genome Sciences Ctr. (Canada) and The Univ. of British Columbia (Canada); Guannan Chen, Rong Chen, Fujian Normal Univ. (China); Haishan Zeng, BC Cancer Agency Research Ctr. (Canada)

Colorectal cancer (CRC) is the third most common type of cancer in men and women in the United States. Raman Molecular Imaging (RMI) is an effective technique to evaluate human tissue, cells and bodily fluids, including blood serum for disease diagnosis. Chemimage Corporation, in collaboration with clinicians, has been engaged in development of an in vitro diagnostic Raman assay focused on CRC detection. The Raman Assay for Colorectal Cancer (RACC) exploits the high specificity of Raman imaging to distinguish diseased from normal dried blood serum droplets without additional reagents. Pilot Study results from testing of hundreds of biobank patient samples have demonstrated that RACC detects CRC with high sensitivity and specificity. However, expanded clinical trials, which are ongoing, are revealing a host of important preanalytical considerations associated with sample collection, sample storage and stability, sample shipping, sample preparation and sample interferents, which impact detection performance. Results from recent clinical studies will be presented.
forth leading cause of cancer-related death. Early diagnosis is the key to long-term patient survival. Programmatic screening for the general population has shown to be cost-effective in reducing the incidence and mortality from CRC. Current CRC screening strategy relies on a broad range of test techniques such as fecal based tests and endoscopic exams. Occult blood tests like fecal immunochemical test is a cost effective way to detect CRC but have limited diagnostic values in detecting adenomatous polyph, the most treatable precursor to CRC. In the present work, we proposed the use of surface enhanced Raman spectroscopy (SERS) with silver nanoparticles as substrate to analyze blood plasma for detecting both CRC and adenomatous polyps. Blood plasma samples collected from healthy subjects and patients diagnosed with adenomas and CRC were prepared with nanoparticles and measured using a real-time fiber optic probe based Raman system. The collected SERS spectra are analyzed with partial least squares-discriminant analysis. Classification of normal versus CRC plus adenomatous polyphs achieved diagnostic sensitivity of 86.4% and specificity of 80%. This exploratory study suggests that blood plasma SERS analysis has potential to become a screening test for detecting both CRC and adenomas.

9704-40, Session 2

The road map to providing a robust Raman spectroscopy-based cancer diagnostic platform and integration into clinic

Katherine Lau, Ian Bell, Renishaw plc (United Kingdom); Martin E. Isabelle, Gavin R. Lloyd, Oliver Old, Gloucestershire Hospitals NHS Foundation Trust (United Kingdom); Jennifer Dorney, Univ. of Exeter (United Kingdom); Aaran Lewis, Riana Gaifulina, Manuel Rodriguez-Justo, Univ. College London (United Kingdom); Catherine A. Kendall, Gloucestershire Hospitals NHS Foundation Trust (United Kingdom); Nick Stone, Univ. of Exeter (United Kingdom); Geraint Thomas, Univ. College London (United Kingdom); David M. Reece, Renishaw plc (United Kingdom)

Raman spectroscopy has been shown to accurately differentiate cancerous tissue from non-cancerous tissues, as well as distinguishing the different stages of disease progression. Despite being shown as a promising tool for cancer diagnosis, Raman spectroscopy has yet to be incorporated into the clinical workflow during histopathology diagnosis of patient cases. Some of the challenges in its clinical integration lie in the differences between current tissue sample preparation procedures in Raman imaging and routine histopathology, the costs of “Raman suitable” substrates, as well as data reproducibility and transferability between instruments.

The Stratified Medicine through Advanced Raman Technologies (SMART) consortium, comprising of Renishaw plc, University of Exeter, Gloucestershire Hospitals NHS Foundation Trust and University College London, has begun to address the challenges mentioned above. In order to provide a robust Raman spectroscopy based diagnostic platform, we are working together to optimise the tissue sample preparation, spectral data collection, data transferability between instruments, data pre-processing, classification model building and validation.

At the end of the collaboration period, a robust user-friendly Raman tissue scanner, an experimental workflow, pathology classification models and a classifier building tool will be available. The platform aims to enable clinical researchers to review and stage oesophageal and colorectal cancer samples, define tumour margin, build cancer diagnostic models and discover novel disease biomarkers.

As a further step towards integrating the technology into the clinic, we aim to place the Raman imaging system in hospitals and clinics to encourage the clinical adoption of the technology.

9704-10, Session 3

Biophysical basis for noninvasive skin cancer detection using Raman spectroscopy

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Raman spectroscopy (RS) is proving to be a valuable tool for real time noninvasive skin cancer detection via optical fiber probe. However, current methods utilizing RS for skin cancer diagnosis rely on statistically based algorithms to provide tissue classification and do not elucidate the underlying biophysical changes of skin tissue. Therefore, we aim to use RS to explore skin biochemical and structural characteristics and then correlate the Raman spectrum of skin tissue with its disease state. We have built a custom confocal Raman microspectroscopy system with an 830nm laser light. The high resolution capability of the system allows us to measure spectroscopic features from individual tissue components in situ. Raman micro-images were collected from human skin samples from Mohs surgical biopsy, which were then compared with confocal laser scanning, two-photon fluorescence and hematoxylin and eosin-stained images to develop a linear model of skin tissue Raman spectra. In this model, macroscopic tissue spectra obtained from RS fiber probe were fit into a linear combination of individual basis spectra of primary skin constituents, including cell cytoplasm, cell nucleus, keratin, collagen, fat and cholesterol-like lipid. The fit coefficient of the model explains the biophysical changes spanning a range of normal and various disease states. The model allows for determining parameters similar to that a pathologist is familiar reading and will be a significant guidance in developing RS diagnostic decision schemes.

9704-11, Session 3

Endoscope-based beveled and volume fiber-optic Raman probes for the diagnosis of gastric dysplasia in vivo at endoscopy: a comparative study

Jianfeng Wang, Kan Lin, Wei Zheng, Zhiwei Huang, National Univ. of Singapore (Singapore)

Raman spectroscopy is a unique optical vibrational spectroscopic technique which is capable of probing biochemical and biomolecular structures and conformations associated with disease transformation. Raman probe is a key component to facilitate the in vivo tissue diagnosis by using Raman spectroscopy. The diagnostic performance of the two different endoscope-based fiber-optic Raman probe designs (i.e., beveled and volume Raman probes) were evaluated for real-time, in vivo diagnosis of gastric dysplasia at endoscopy. The beveled Raman probe provides approximately 2-fold improvements in tissue Raman to autofluorescence intensity ratios as compared to the use of volume Raman probe. The diagnostic accuracy of gastric dysplasia using beveled Raman probe is 93.0% (sensitivity of 92.5%; specificity of 93.1%), which is superior to the diagnostic performance (accuracy of 88.4%; sensitivity of 85.8%; specificity of 88.6%) using the volume Raman probe. Biomolecular modeling is finally employed to extract the different Raman active components interrogated by the two types of endoscope-based Raman probes.

9704-12, Session 3

Raman spectroscopy of oral cancer: investigations in pet canines

Piyush Kumar, Tanmoy Bhattacharjee, Pradeep Chaudhary, C. Murali Krishna, Advanced Ctr. for Treatment, Research &
Education in Cancer (India)

The oral cavity is a common site for a variety of cancers in pet canines, both benign and malignant. The most frequent benign oral tumors are epulides, involving the gingival tissue and malignant oral tumors are melanoma, squamous cell carcinoma and fibrosarcoma. The incidence of oral tumors accounts for 6% to 8% of all canine cancers. Currently available diagnostic techniques such as biopsy followed by histopathology have several disadvantages like inter-observer variation, tedious procedures and long output times. Therefore, there is a need for rapid, objective and sensitive techniques for diagnosis of oral cancer in canines. Several studies have shown feasibility of using Raman spectroscopy for diagnosis of cancers in humans as well as animals. The current study explores possibility of discriminating oral malignant tissues from oral normal using Raman spectroscopy ex vivo. Spectra were acquired from 8 malignant tissue samples and 7 normal tissue samples from as many animals, using HE 785 commercial Raman spectrometer. The spectral features in normal tissues suggested high lipid to protein ratio with Amide III bands at 1310 cm⁻¹, sharp 1450 cm⁻¹ and Amide I bands and ester bands (1750 cm⁻¹). Tumor spectra revealed dominance of protein features. Spectra were further processed and analyzed using multivariate statistical tool Principal Component Analysis (PCA). PCA shows distinct clusters of normal and tumor. Thus, results suggest feasibility of using Raman spectroscopy for diagnosis of oral cancer in canines.

9704-13, Session 3

Intra-operative on-line discrimination of kidney cancer from normal tissue by IR ATR spectroscopy of extra cellular fluid

Valdas Sabinškis, Vidita Urboniene, Martynas Velicika, Milda Pucetaite, Justinas Ceponkus, Feliksas Jankevičius M.D., Vilnius Univ. (Lithuania); Gerald Steiner, TU Dresden (Germany)

Spectroscopic imaging of biological tissue cryosection is conventional approach in application of IR absorption spectroscopy for detection of the cancerous tissue areas. Unfortunately, due to very small differences between the spectra of normal and cancerous tissue areas the samples were prepared by stamping of the kidney tissue on ATR diamond crystal. The spectral measurements were performed directly in OR during the surgery for 50 patients. In all the cases the large spectral differences were observed in the region between 700-1300 cm⁻¹, where spectral bands related to various vibrations of fatty acids, glycolipids and carbohydrates are located. The proposed method is instant and can be used in situ and even in vivo.

9704-39, Session 3

Evaluation of a multi-fibre needle Raman probe for tissue analysis

Leanne Fullwood, Univ. of Exeter (United Kingdom) and Gloucestershire Hospitals NHS Foundation Trust (United Kingdom); Ingeborg Iping-Pettersson, Univ. of Exeter (United Kingdom); Catherine A. Kendall, Gloucestershire Hospitals NHS Foundation Trust (United Kingdom); Charlie Hall, Gloucestershire Hospitals NHS Foundation Trust (United Kingdom); John Day, Univ. of Bristol (United Kingdom); Nick Stone, Univ. of Exeter (United Kingdom)

A novel Raman needle probe, with replaceable tips, will be described. It has been developed to be inserted through a 19 gauge needle for measurement of the disease specific changes in primary and secondary lymph node malignancies. The probe has a 6 collection fibre around illumination fibre geometry and filtration of unwanted optical signals from the optical fibres and elastic scattered light at a point proximal to the needle tip. Two small studies will be outlined:

1) The probes’ function for tissue discrimination is explored as a potential tool for localisation of the needle tip identified by the biochemical Raman spectra of the tissue at the tip.

2) Lymph nodes collected from oesophageal cancer surgery are snap frozen and stored until required for measurement. They are then probed and the resulting spectra compared to histopathology from the subsequently cut nodes.

Multivariate analysis of both sets of data is used to provide an understanding of the key differences observed.

9704-14, Session 4

Multimodal nonlinear microscopy of biopsy specimen: towards intraoperative diagnostics

Michael Schmitt, Friedrich-Schiller-Univ. Jena (Germany); Sandro Heuke, Tobias Meyer, Friedrich-Schiller-Univ. Jena (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany); Olga Chernavskaja, Leibniz-Institut für Photonische Technologien e.V. (Germany); Thomas W. Bocklitz, Friedrich-Schiller-Univ. Jena (Germany); Jürgen Popp, Friedrich-Schiller-Univ. Jena (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany)

The realization of label-free molecule specific imaging of morphology and chemical composition of tissue at subcellular spatial resolution in real time is crucial for many envisioned applications in medicine, e.g., precise surgical guidance and non-invasive histopathologic examination of tissue. Thus, new and advanced tools for a fast and reliable in vivo and near in vivo (ex corpore in vivo) tissue characterization to supplement routine pathological diagnostics is needed. Spectroscopic imaging approaches are particularly important since they have the potential to provide a pathologist with adequate support in the form of clinically-relevant information under both ex vivo and in vivo conditions. In this contribution it is demonstrated, that multimodal nonlinear microscopy combining coherent anti-Stokes Raman scattering (CARS), two photon excited fluorescence (TPEF) and second harmonic generation (SHG) enables the detection of characteristic structures and the accompanying molecular changes of widespread diseases, particularly of cancer and atherosclerosis. The detailed images enable an objective evaluation of the tissue samples for an early diagnosis of the disease status. Increasing the spectral resolution and analyzing CARS images at multiple Raman resonances improves the chemical specificity. To facilitate handling and interpretation of the image data characteristic properties can be automatically extracted by advanced image processing algorithms, e.g., for tissue classification. Overall, the presented examples show the great potential of multimodal imaging to augment standard intraoperative clinical assessment with functional multimodal CARS/SHG/TPEF images to highlight functional activity and tumor boundaries. It ensures fast, label-free and non-invasive intraoperative tissue classification paving the way towards in vivo optical pathology.
Hyperspectral imaging of cartilage by using multiplex CARS

Manabu Shiozawa, Hitachi, Ltd. (Japan)

Non-invasive cell analyses are increasingly important for the medical field. A CARS microscope is one of the non-invasive imaging equipments and enables to obtain images indicating molecular distribution. Some studies on discrimination of cell state by using CARS images of lipid are reported. However, due to low signal intensity, it is still challenging to obtain images of the fingerprint region (800-1800 cm⁻¹), in which many spectrum peaks correspond to compositions of a cell.

Here, to identify cell differentiation by using multiplex CARS, we investigated hyperspectral imaging of fingerprint region of living cells. To perform multiplex CARS, we used a prototype of a compact light source, which consists of a microchip laser, a single-mode fiber, and a photonic crystal fiber to generate supercontinuum light. Assuming application to regenerative medicine, we chose a cartilage cell, whose differentiation is difficult to be identified by change of the cell morphology. Because one of the major components of cartilage is collagen, we focused on distribution of proline, which accounts for approximately 20% of collagen in general. To validate a CARS image, both the CARS image and the stained image of the same cell were compared.

The periphery of the cartilage cell was highlighted in the CARS image of proline. Furthermore, the image showed a correlation with the image of stained collagen in the same cell. These results indicate that collagen was produced as extracellular matrix and suggest the availability of multiplex CARS for cell analyses.

Dental caries imaging using hyperspectral stimulated Raman scattering and multiphoton microscopy

Zi Wang, Wei Zheng, Jian Lin, Zhiwei Huang, National Univ. of Singapore (Singapore)

We report the development of a polarization-resolved hyperspectral stimulated Raman scattering (SRS) imaging technique based on a picosecond (ps) laser-pumped optical parametric oscillator system for label-free imaging of dental caries. In our imaging system, hyperspectral SRS images (512²512 pixels) in both fingerprint region (800-1800 cm⁻¹) and high-wavenumber region (2800-3600 cm⁻¹) are acquired in minutes by scanning the wavelength of OPO output, which is a thousand times faster than conventional confocal micro Raman imaging. SRS spectra variations from normal enamel to caries obtained from the hyperspectral SRS images show the loss of phosphate and carbonate in the carious region. While polarization resolved SRS images at 959 cm⁻¹ demonstrate that the caries has higher depolarization ratio. Our results demonstrate that the polarization resolved hyperspectral SRS imaging technique developed allows for rapid identification of the biochemical and structural changes of dental caries.

Raman imaging analysis reveals native-like bone repair of defects induced by porous strontium-loaded bioactive glass

Charalambos Kallepitis, Helene Autefage, Imperial College London (United Kingdom); Felix Allen, Allen E. Goodship, Univ. College London (United Kingdom); Julian R. Jones, Imperial College London (United Kingdom); Gordon Blunn, Univ. College London (United Kingdom); Molly M. Stevens, Imperial College London (United Kingdom)

The functional and mechanical properties of bone are dependent on the “quality” of the bone tissue, which is determined by various parameters such as macrostructure, collagen organization, biochemical composition and crystallinity. Raman spectroscopy imaging (RSI) is a vibrational analytical technique that has proven to provide comprehensive biochemical information of complex biological systems non-destructively. Due to its potential to concomitantly provide insights on the mineral and the organic matrix composition, RSI has emerged as a prominent tool to characterize bone in normal and pathological contexts and was used in this study as an innovative approach to explore the hypothesis that a novel porous bioactive glass would induce native bone repair in a defect. RSI was used to assess the “quality” of newly-formed bone chemistry, comprehensively, following biomaterials implantation. We developed a novel bioactive glass-based scaffold (pSrBG) with porous structure and strontium-releasing properties that showed enhanced bone repair when compared to the gold standard 45S5 bioactive glass after implantation in an ovine model in critical-sized defects. High resolution RSI analysis of the newly-formed bone regions, 3 months after implantation, demonstrated that the repaired tissue had similar characteristics to younger healthy bone for both implanted scaffolds. Our analyses further allowed identifying changes in the chemical signature of the synthetic scaffolds occurring at the bone-material interface. These data showed the potential of RSI in giving biochemical information in unrepresented detail and spatial resolution of a biological system and its potency as a characterization method in assessing the biomaterials fate and their effect in-vivo.
9704-19, Session 4

Rigorous comparison of the spectral SNR of FTIR and EC-QCL spectroscopy


FTIR spectroscopy using a thermal light source has been the dominant method for obtaining infrared spectra since the 1950’s. Unfortunately the limited surface brightness and low spatial coherence of black-body radiators limits the spectral SNR in microspectroscopy and stand-off detection. Two recent innovations are addressing this problem: a) FTIR instruments illuminated by high-spatial coherence broad-band supercontinuum sources and b) high spatial coherence narrow-band EC-QCL’s.

Here we ask whether these two approaches offer equivalent sensitivity. By noting an analogy with near-infrared optical coherence tomography we rigorously show that the high temporal coherence of the EC-QCL brings an additional, very large SNR advantage over an FTIR instrument illuminated by a supercontinuum source under otherwise matched conditions. Specifically if a spectrum containing N points is recorded by both instruments using the same illumination intensity and the same detector noise level, then the EC-QCL can deliver a given spectral SNR in a time ×N shorter than the FTIR instrument. This factor can reach ×1000, potentially even ×10000, in realistic applications.

We exploit the analogy with OCT further by developing a mid-infrared “swept laser”, using commercially available components, in which the tuning rate is much higher than in commercial EC-QCL devices. We use this swept laser to demonstrate the SNR advantage experimentally, using a custom-made EC-QCL spectrometer and PDMS polymer samples. We explore the potential upper limits on spectral acquisition rates, both from the fundamental kinetics of gain build-up in the external cavity and from likely mechanical limits on cavity tuning rates.

9704-41, Session 4

In vivo vibrational imaging: emerging platform for biology and medicine

Ji-Xin Cheng, Vibronix Inc. (United States) and Purdue Univ. (United States)

No Abstract Available

9704-500, Session HT

Stimulated nonlinear optical microscopy: imaging with a boost

Eric O. Potma, Univ. of California, Irvine (United States)

No Abstract Available

9704-1, Session 5

Combination of micro-dialysis and infrared spectroscopy: a multi-analyte assay for accurate biofluid analysis and patient monitoring

Sven Delbeck, Janpeter Budde, Lars Coccieri, Thorsten Vahlising, Dieter Ihrig, Herbert M. Heise, Fachhochschule Südwestfalen (Germany)

The combination of micro-dialysis and infrared spectroscopy has been proven to be applicable for patient monitoring, especially when several analytes are to be monitored. For analysis, multivariate calibration methods have been successfully applied for the determination of analytes such as glucose, lactate and urea. Due to application of recovery marker substances as part of the isotonic perfusate, even under variable dialysis recovery rates under push-pull operation during micro-dialysis, an accurate determination of compounds within the cell- and protein-free dialysate and, with application of the recovery rates, within the original bodyfluid can be achieved. While previously acetate had been employed as perfusate component, also mannitol has been recently tested as recovery marker.

Utilising this neutral monosaccharide is advantageous, as also pCO2 and bicarbonate can be determined and from their concentrations also a pH estimate of the biofluid sample is possible. For the in-vitro recovery experiments with human serum samples, different micro-dialysis catheters suited either for subcutaneous skin or vein implantation were tested under different constant flow-rates and a surrounding temperature of 37°C. By investigating, besides the analytes of clinical interest, the depletion of the marker substance from the perfusate simultaneously, micro-dialysis recovery rates were determined by infrared spectrometry. Results confirmed the theoretical nonlinear relationship between the relative acetate dialysate marker concentration, compared with the original perfusate concentration, and the recovery rates of glucose as predicted from the diffusion constants of the analytes of interest and a linear dependency for mannitol when used as alternative recovery marker substance.

9704-20, Session 5

In vivo confocal Raman spectroscopy study of the vitamin A derivative perfusion through human skin

Laurita dos Santos, Claudio A. S. Téllez, Priscila P. Fávero, Airton A. Martin, Univ. do Vale do Paraíba (Brazil)

In vivo confocal Raman spectroscopy is a powerful non-invasive technique able to track the perfusion of ingredients topically applied on human skin. In this work, this technique was applied to transdermal perfusion studies of the vitamin A derivative in human skin. The thickness of stratum corneum varies according to the body sites, from 12 μm (cheek region) up to 29 μm (back region). This first layer of skin is a lipid bilayer, predominantly composed by ceramides, cholesterol, and fatty acids. The composition of the lipid bilayer is decisive for the affinity and transport of the vitamin through skin. The vitamin A is significantly absorbed by human skin when applied with water in oil emulsion or hydro-alcoholic gel. The purpose of this study is to elucidate the behaviour of vitamin A derivative into human skin without the presence of enhancers. This study involved five healthy participants (women caucasian) with Fitzpatrick phototype I to II. The Raman spectrum was obtained for each depth (before and after application of the vitamin A derivative solution) from the skin surface down to 29 μm. The results showed that the intensity band of the derivative (around 1600 cm⁻¹), which represents the -C=O vibrational mode, was detected in different stratum corneum depths (up to 20 μm). This Raman peak of vitamin A derivative has non-coincident band with the Raman spectra of the skin epidermis, demonstrating that compound penetrated in forearm skin.

9704-21, Session 5

Raman spectroscopy of single extracellular vesicles reveals sub-populations with varying membrane content

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Exosomes are small (~100nm) membrane bound vesicles excreted by cells as part of their normal biological processes. These extracellular vesicles are currently an area of intense research, since they were recently found to carry functional mRNA that allows transfer of proteins and other cellular instructions between cells. Exosomes have been implicated in a wide range of diseases, including cancer. Cancer cells are known to have increased exosome production, and may use those exosomes to prepare remote environments for metastasis. Therefore, there is a strong need to develop characterization methods to help understand the structure and function of these vesicles. However, current techniques, such as proteomics and genomics technologies, rely on aggregating a large amount of exosome material and reporting on chemical content that is averaged over many millions of exosomes. Here we report on the use of laser-tweezers Raman spectroscopy (LTRS) to probe individual vesicles, discovering distinct heterogeneity among exosomes both within a cell line, as well as between different cell lines. Through principal components analysis followed by hierarchical clustering, we have identified four “subpopulations” of exosomes shared across seven cell lines. The key chemical differences between these subpopulations, as determined by spectral analysis of the principal component loadings, are primarily related to membrane composition. Specifically, the differences can be ascribed to cholesterol content, cholesterol to phospholipid ratio, and surface protein expression. Thus, we have shown LTRS to be a powerful method to probe the chemical content of single extracellular vesicles.

Detection of advanced glycation end products (AGEs) on human skin by in vivo confocal Raman spectroscopy

Airton A. Martin, Liliane P. Pereira, Syed M. Ali, Claudio A. Tellez, Priscila P. Fávero, Laurita dos Santos, Univ. do Vale paraiba (Brazil)

The aging process involves the reduction in the production of the major components of skin tissue. During intrinsic aging and photoaging processes, in dermis of human skin, fibroblasts become senescent and have decreased activity, which produce low levels of collagen. Moreover, there is accumulation of advanced glycation and products (AGEs). AGEs have incidence in the progression of age-related diseases, principally in diabetes mellitus and Alzheimer’s disease. AGEs causes intracellular damage and/or apoptosis leading to an increase of the free radicals, generating a crosslink with skin proteins and oxidative stress. The aim of this study is to detect AGEs markers on human skin by in vivo Confocal Raman spectroscopy. Spectra were obtained by using a Rivers Diagnostic System, 785 nm laser excitation and a CCD detector from the skin surface down to 120 µm depth. We analyzed the confocal Raman spectra of the skin dermis of 30 women volunteers divided into 3 groups: 10 volunteers with diabetes mellitus type II, 65–80 years old (DEW); 10 young healthy women, 20–33 years old (HYW); and 10 elderly healthy women, 65–80 years old (HEW). Pentosidine and glucosepane were the principally identified AGEs in the hydroxyproline and proline Raman spectral region (1000–800 cm–1), in the 1260–1320 cm–1 region assignable to alpha-helical amide II modes, and in the Amide I region. Pentosidine and glucosepane calculated vibrational spectra were performed through Density Functional Theory using the B3LYP functional with 3-21G basis set. Difference between the Raman spectra of diabetic elderly women and healthy young women, and between healthy elderly women and healthy young women were also obtained with the purpose of identifying AGEs Raman bands markers. AGEs peaks and collagen changes have been identified and used to quantify the glycation process in human skin.

Development of multivariate analysis methods for improved discrimination of inflammatory bowel disease in vivo

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Inflammatory bowel disease (IBD) is an idiopathic disease that is typically characterized by chronic inflammation of the gastrointestinal tract. IBD primarily consists of ulcerative colitis (UC) and Crohn’s disease (CD). Although UC and CD share many symptoms in common, they are two distinct types of IBD, and often require different medical treatments. In addition, up to 15% of IBD cases are categorized as indeterminate colitis (IC), a term indicating the lack of diagnosis of whether inflammation is due to UC or CD. Recently much effort has been devoted to the development of novel diagnostic tools that can assist physicians for fast, accurate, and automated diagnosis of the disease. Previous research based on Raman spectroscopy has shown promising results in differentiating UC and CD ex vivo. In the current study, we examined IBD and normal patients in vivo through a colonoscopy-coupled in vivo Raman system. The chemical-rich information provided by Raman spectra revealed compositional differences in the colon lining between normal, UC, and CD patients. Full-range spectra were analyzed using multiple multivariate statistical methods such as support vector machines and generalized linear models for IBD discrimination. Further, we incorporated several feature selection methods in machine learning into the classification model, which improved accuracy for disease differentiation from 65% to 89%. Our results showed that, compared to the classification using all the spectral features, inclusion of feature selection prior to classification yields more accurate discrimination between UC and CD.

Raman and surface-enhanced Raman spectroscopy for renal condition monitoring

Jingtian Li, Ming Li, Yong Du, Gregg M. Santos, Chandra Mohan, Wei-Chuan Shih, Univ. of Houston (United States)

Non- and minimally-invasive techniques can provide significant advantages in the monitoring and clinical diagnostics in renal diseases. Both acute and chronic nephritis account for substantial morbidity and mortality worldwide, partly due to the lack of reliable tools for detecting disease early and monitoring its progression. Although renal biopsy may be useful in establishing diagnosis in several diseases, it is an invasive approach and impractical for longitudinal disease monitoring. To address this unmet need, we have developed two techniques based on Raman spectroscopy. First, we have investigated the potential of staging nephritis by analyzing kidney tissue Raman spectra using multivariate techniques. We have built a line-scan Raman microscope which performs at a higher throughput compared to a single-point confocal Raman microscope. We demonstrate the feasibility of classifying kidney tissue samples into normal, mild nephritis, and severe nephritis. Secondly, we have developed a urine creatinine sensor based on surface-enhanced Raman spectroscopy. Since urine can be obtained non-invasively, the analysis of urine can provide metabolic information of the body and the condition of renal function with great convenience. Creatinine is one of the major components of human urine associated with muscle metabolism and is important for the renal clearance test, which can monitor the filtration function of kidney and health status. A simple device and protocol for creatinine sensing in urine samples can be
Identification of bacteria causing acute otitis media using Raman microspectroscopy

Oscar D. Ayala, Catherine A. Wakeman, Eric Skaar, Anita Mahadevan-Jansen, Vanderbilt Univ. (United States)

Otitis media (OM) is the leading cause of physician visits and prescription of antibiotics for children. Current standard techniques to diagnose acute otitis media (AOM) are limited by their ability to probe only changes in symptoms of the bacterial infection that causes AOM. Furthermore, they are not able to detect the presence of or identify bacteria causing AOM, which is important for diagnosis and proper antibiotic treatment. Our goal is to detect the presence of and identify the pathogens involved in causing AOM based on their biochemical profile using Raman spectroscopy (RS). An inVia confocal Raman microscope (Renishaw) at 785 nm was used to detect bacteria causing AOM in vitro. The three main bacteria that cause AOM, Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae were cultured in Mueller-Hinton agar plates to minimize Raman signal from the growth agar. The confocal Raman microscope system was then used to examine multiple spots on a single bacterial colony and multiple bacterial colonies from each bacterium. Preliminary results identified specific Raman spectral features characteristic of each bacterium causing AOM. A Raman peak ratio between 1450 cm⁻¹ and 1045 cm⁻¹ was significant in distinguishing each bacterium. Additional Raman measurements include live versus dead assays, effects of various strains, and the presence of other confounding factors. RS has the potential to accurately diagnose AOM, which will help in identifying the antibiotic that will be most beneficial for the patient and ultimately decrease the course of infection.

Discerning the differential molecular pathology of serous and mucoid fluids using Raman spectroscopy

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Middle ear infections - also known as otitis media - is the most common childhood illness; and accounts one third of all pediatric visits in the United States. Diagnosis of middle ear infection remains challenging in the clinical setting and suffers from significant observer variability and accuracy of physician diagnosis with currently available methods has been shown to be lacking with accurate diagnosis rates for otitis media ranging between 40 and 80%. Exploiting the endogenous molecular contrast may provide sufficient information to aid in obtaining objective and reproducible disease diagnoses. Serous otitis media is typically responsive to medical treatment as compared to mucoid otitis media, and the non-invasive differentiation of fluid type will enable the otolaryngologist in diagnosing the infection without rupturing the tympanic membrane. Here we present first technique that has the potential to non-invasively identify different types of otitis media by using biochemical signatures that can reveal latent information on pathological conditions. Identification of the spectral markers offer much-desired quantifiable data to enable early detection and longitudinal monitoring of middle ear infection that not only improves patient management but also serves as a basis for the molecular-level understanding of the infection.

Translation of infrared chemical imaging for cardiovascular evaluation

Saumya Tiwari, Jai Raman, Vijaya Reddy, Rohit Bhargava, Beckman Institute for Advanced Science and Technology (United States)

Infrared (IR) spectroscopic imaging has been applied to study histology of cardiovascular tissue using Fourier transform IR (FTIR) Imaging. Here we describe results for histologic imaging of cardiac biopsies using a fast, discrete frequency IR imaging system. The histologic classification of tissue is understood in terms of the constituent frequencies and speeded up by careful optimization of the data acquired. Results are compared for FTIR imaging in terms of the signal to noise ratio, acquisition time, accuracy of histologic recognition and information content.
immunohistochemical examination demonstrated positivity to anti-S100 cervical region. A histological examination described a benign process. An sample preparation. In this work, we report a case of a schwannoma in the techniques, FT-IR is one of the correnty techiniques which has been applied be improved if novel optical techniques are performed. Among these Schwannoma are rare benign neural neoplasia. The clinical diagnosis could on elderly participants with diabetes mellitus accelerate the formation of was centered around 1658 cm$^{-1}$. Hence, it is inferred that the hyperglycemia is accelerated when sugar in the human body is elevated, as in the case Glycation process is a non-enzymatic reaction between sugars and free amino groups of proteins, which leads to collagen cross-link. This process was accelerated when sugar in the human body is elevated, as in the case of uncontrolled diabetes. In this regard, the present study was aimed to analyze the changes arising from glycation in type I collagen in the dermis through the amide I band by confocal Raman spectroscopy. The analysis was performed in 30 subjects with skin type I and II, divided into 3 groups: 10 young healthy women in the age group of 20-30 years, 10 elderly healthy women and 10 elderly women with type I and II diabetes in age group of 56-81 years. The Raman spectra were collected from 70-120 µm depth. It was observed a softening in the amide I band, centered at 1664 cm$^{-1}$, for younger compared to the healthy elderly participants. For the elderly participants with Diabetes an even larger shift was observed and the peak was centered around 1658 cm$^{-1}$. Hence, it is inferred that the hyperglycemia on elderly participants with diabetes mellitus accelerate the formation of advanced glycation end products, which increase the degradation of type I collagen structure on human skin.

9704-29, Session PMon

Raman spectroscopic analysis of amide I band resulting from collagen glycation process


Glycation process is a non-enzymatic reaction between sugars and free amino groups of proteins, which leads to collagen cross-link. This process was accelerated when sugar in the human body is elevated, as in the case of uncontrolled diabetes. These findings corroborated with our previous ex vivo studies where dysplastic and SCC tissue spectra could be identified. These differences may be explored further to gain insights in oral carcinogenesis at cellular level. Findings suggest applicability of RS in distinguishing cell lines of different cancer stages as a label free, non-destructive and rapid technique.

9704-32, Session PMon

FT-IR spectroscopy characterization of schwannoma: a case study

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Schwannoma are rare benign neural neoplasia. The clinical diagnosis could be improved if novel optical techniques are performed. Among these techniques, FT-IR is one of the correnty techniques which has been applied for samples discrimination using biochemical information with minimum sample preparation. In this work, we report a case of a schwannoma in the cervical region. A histological examination described a benign process. An immunohistochemical examination demonstrated positivity to anti-S100 protein antibody, indicating a diagnosis of schwannoma. The aim of this analysis was to characterize FT-IR spectrum of the neoplastic and normal tissue in the fingerprint (1000-1800 cm$^{-1}$) and highwavenumber region (2800-3600 cm$^{-1}$). The IR spectra were collect from tumor tissue and normal nerve samples by a FT-IR spectrophotometer (Spotlight Perkin Elmer 400, USA) with 64 scans, and resolution of 4 cm$^{-1}$. A total of twenty spectra were recorded (10 from schwannoma and 10 from nerve). Multivariate Analysis was used to classify the data. Through average and standard deviation analysis we observed that the main spectral change occurs at ≈1600 cm$^{-1}$ (amide I) and ≈1400 cm$^{-1}$ (amide III) in the fingerprint region, and in CH2/CH3 protein-lipids and OH-water vibrations for the highwavenumber region. In conclusion, FT-IR could be used as a technique for schwannoma analysis helping to establish specific diagnostic.

9704-33, Session PMon

Raman spectroscopy of hamster buccal pouch carcinogenesis: investigating precancer changes due to confounding factors

Piyush Kumar, Mahazabeen Sayyed, C. Murali Krishna, Advanced Ctr. for Treatment, Research & Education in Cancer (India)

Raman spectroscopy (RS) has shown potential in identification of oral-carcinogenesis. Hamster buccal-pouch(BP) model for experimental oral-carcinogenesis shows tumor formation in 14 weeks on application of carcinogen 7, 12 Dimethylbenz(a)anthracene (DMBA). In our previous Raman study, a few vehicle and contralateral controls misclassified with precancers/ cancers which may be attributed to repeated mechanical-irritations due to forceps and indirect exposure of carcinogen. To investigate role of these confounding-factors, hamsters were randomized into 4 groups- Week0-controls (W0, n=4, sacrificed immediately), Untreated-controls (NT) Vehicle-controls (C) and DMBA-treated (D). Right BP of DR were topically administered with 0.5% DMBA, dissolved in edible oil. Left BP of these animals (DL) served as contralateral-controls. Right BP of vehicle controls (CR) were administered with edible oil. Animals were sacrificed at the end of 5 (NT5, CR5, DR5, DL5), 10 (NT10, CR10, DR10, DL10) and 14 (NT14, CR14, DR14, DL14) weeks (n = 4 in each case) and BP were excised out. Standard model was built using Raman spectra from W0, DR5, DR10, DR14 tissues and evaluated with remaining spectra. It was observed that in case of vehicle-controls, which undergone repeated irritation with forceps, one CR14 spectra showed misclassification with DR14. Contralateral-tissues(DL) undergo both forceps-handling and indirect exposure to DMBA and showed one DL10 and two DL10 spectra misclassified with DR14. Remaining groups did not misclassify with DR14, indicating the confounding-factors investigated may lead to abnormal changes; thus proper selection of controls is required for robust analysis. Further, the study also shows that RS can detect micro-changes in control tissues, which otherwise remain unnoticed.

9704-34, Session PMon

Biomarkers of chronic kidney disease in the urine of diabetic/hypertensive patients by means of Raman spectroscopy

Elzo E. S. Vieira, Jeyse A. M. Bispo, Adriana Barrinha Fernandes Moretti, Landulfo Silveira Jr., Univ. Camilo Castelo Branco (Brazil)

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) arisen from DM and AH. It has been used a dispersive Raman spectrometer (830nm, 250mW, 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 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.

9704-35, Session PMon

Development of an optical biosensor based on surface-enhanced Raman scattering for DNA analysis

Tugce Yigit, Ebru Akdogan, Isik Didem Karagoz, Mehmet Kahraman, Gaziantep Univ. (Turkey)

Rapid, accurate and sensitive DNA analysis is critically important for the diagnostic of genetic diseases. The most common method is fluorescence based microarrays when the DNA analysis methods are evaluated. However there are some disadvantages of the method such as the overlapping of the fluorescence emission wavelengths that can diminish the performance of multiplexing, needed to obtain fluorescence spectra from each dye and photo degradation. In this study, a novel approach based on SERS which is independent of SERS substrate properties and does not use any Raman active dyes for the DNA analysis is developed. First, single strand probe DNA is attached the SERS substrate and half of complimentary DNA is attached to gold nanoparticles. The surface and colloids are characterized by UV/Vis spectroscopy, atomic for microscopy (AFM) and dynamic light scattering (DLS). When the target DNA is present, the colloids are attached to the SERS substrate surface via hybridization of single strand target DNA. DNA analysis is demonstrated by peak shifting of certain peak of the small molecules attached to the SERS substrate surface instead of SERS spectrum obtained in the presence of target DNA from the Raman reporter molecules. The degree of peak shifting will be used for the quantification of target DNA in the sample. Plasmonic properties of SERS substrate and reproducibility issue will not be considerable due to the use of peak shifting instead of peak intensity for the qualitative analysis.

9704-36, Session PMon

Quantitative Raman characterization of cross-linked collagen thin films as a model system for diagnosing early osteoarthritis

Chao Wang, Columbia Univ. (United States); Krista Durney, Gregory Fomovsky, Gerard A. Ateshian, Sinisa Vukelic, Columbia Univ. (United States)

The onset of osteoarthritis in articular cartilage is characterized by degradation of extracellular matrix. Specifically, breakage of cross-links between collagen fibrils in the articular cartilage leads to loss of structural integrity of the bulk tissue. Currently, there are no broadly-accepted, non-invasive, label-free tools for diagnosing osteoarthritis at its early stage. Raman spectroscopy is therefore proposed in this work as a novel, non-destructive diagnostic tool for the quantitative identification of early stage osteoarthritis. In this study, collagen thin films were employed to act as a simplified model system of the cartilage collagen extracellular matrix. Cross-link formation was controlled via exposure to glutaraldehyde, by varying exposure time and concentration levels, and Raman spectral information was collected in order to quantitatively characterize the cross-link assignments imparted to the collagen thin films during treatment. Raman spectral maps of the collagen thin films were obtained and concentrations of cross-links assessed by analyzing Raman spectra with the proposed decomposition model. Raman spectral data correlates with glutaraldehyde treatment and therefore may be used as a proxy by which to measure loss of collagen cross-links in vivo. This study proposes a promising system of identifying osteoarthritis onset and may enable early intervention treatments which may serve to halt or prevent osteoarthritis progression.
Conference 9705: Microfluidics, BioMEMS, and Medical Microsystems XIV
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9705-1, Session 1

**Microfluidic approaches to separation of challenging biological particles and cells (Invited Paper)**

Bruce K. Gale, Kevin Petersen, Jiyoung Son, Matthew Hockin, John Nelson, Haidong Feng, Himanshu J. Sant, The Univ. of Utah (United States)

Cell and bioparticle separations have been a major focus of biologists and microfluidics experts for decades, yet there are a few particles that continue to challenge the existing tools for separations. Specifically, oddly shaped or delicate particles remain a challenge, as well as continuous separations with high resolution. In this presentation, results related to microfluidic separations of sperm, exosomes, chromosomes, and vesicles are reported. Techniques for these separations include: field flow fractionation, inertial microfluidics, and deterministic lateral displacement (DLD) arrays. Four specific experimental results will be presented: (1) Non-motile sperm have been separated from blood and other cellular contaminants generated from a micro-TESE surgery using a variety of inertial microfluidic and DLD arrays. The separated sperm have been collected and processed for cryopreservation. (2) Results of separations of intact metaphase chromosomes are compared between field flow fractionation, inertial microfluidics, and DLD arrays. (3) Exosome separations using a variety of FFF techniques are presented. Results for continuous separations of exosomes using a modified SPLITT system are likewise presented and compared to current techniques. Overall results show that these challenging particles can all be processed and separated using microfluidic approaches.

9705-2, Session 1

**A latchable thermally activated phase change actuator for microfluidic systems**

Christiane Richter, Kai Sachsenheimer, Bastian E. Rapp, Karlsruher Institut für Technologie (Germany)

Complex microfluidic systems often require a high number of individually controllable active components like valves and pumps. Introduction of microfluidic large-scale integration by Thorsen et al. (2002) allowed actuation of several thousand actuators by pneumatic pressure but they were not individually addressable. This can be achieved using electrical signals for controlling, e.g., the transformation of a voltage into heat in an ohmic resistor. Very promising concepts of such actuators are phase change actuators. Within these actuators a phase change material (PCM) is melted and the volume expansion of the PCM during phase transition or displacement of liquid PCM via an external pressure is used to deflect a membrane and close a microfluidic channel. For both concepts the amount of PCM determines the displacement of the actuator membrane. In our own experiments we showed that the amount of PCM should be as low as possible to achieve fast response times. Therefore we suggest a new concept of a latchable actuator termed “shift-gate actuator” for which the displacement of the actuator is independent of the used amount of PCM. We want to show the general working principle of the shift-gate actuator and demonstrate its general feasibility and scalability (a few to several dozen actuators) of the concept. Additionally we want to present the complete results of our studies to optimize the response time of the actuator - the influence of the heating power as well as the used PCM (melting temperature, purity) itself on melting and solidifying times.

9705-3, Session 1

**A focus-tunable liquid lens encapsulated by a membrane with aspherical cross-section for field curvature reduction at high diopters**

Hanyang Huang, Kang Wei, Yi Zhao, The Ohio State Univ. (United States)

Elastomer-liquid lens offers a simple solution to achieve tunable optical powers. This approach, however, suffers from substantial field curvature and thus leads to deteriorated resolution at high diopters given the undesirable lens profiles at large membrane deformations. In this study, a plano-convex elastomer-liquid lens is developed where the liquid is encapsulated by an elastomer membrane with an aspherical cross-section. The aspherical membrane is formed by replication from the deflection profile of another liquid lens encapsulated by a planar membrane. Mechanical and optical simulations show that such configuration allows for the lens profiles at high diopters to be close to spherical shapes by alleviating the edge-clamping effects. Resolution tests of a 6mm lens with optimized aspherical elastomeric membrane assuredly exhibits improved resolutions in both center and peripheral regions at 40 and 100 diopters than the lens with a planar membrane. It is the first report showing that aspherical membranes can reduce field curvature of elastomer-liquid lenses at high diopters, thus providing a new route of optimizing the imaging performance of adaptive liquid lenses for significant benefit in next-generation miniaturized autofocus and zoom lens design, and also a great potential in laser projection and processing, consumer and industrial illumination, machine vision, ophthalmology, and microscopy.

9705-4, Session 1

**One-layer microfluidic device for hydrodynamic 3D self-flow-focusing operating in low flow speed**

Yasaman Daghighi, Vaskar Gnyawali, Eric M. Strohm, Scott S. H. Tsai, Mickael C. Kolios, Ryerson Univ. (Canada)

Hydrodynamic 3D flow-focusing techniques in microfluidics are categorized as a) sheathless techniques which require high flow rates and long channels. Consequently, the cost of operating such systems is significant and their high flow rates are inappropriate for applications with flow rate limitations, and b) sheath-flow based techniques which usually require excessive sheath flow rate to achieve hydrodynamic 3D flow-focusing (e.g. core flow rate of 52µL/min while overall sheath flow rate is 787µL/min). Additionally, most of these devices use complicated fabrication methods to create multi-layer microchannels.

We have developed a sheath-flow based microfluidic device that is capable of hydrodynamic 3D self-flow-focusing, in which the core suspension of microparticles in a low speed flow, and a sheath flow, enter through two inlets and enter a 180 degree curved channel (300×300µm cross-section). Particles migrate outwards into the sheath-flow due to centrifugal effects and consequently, vertical focusing is achieved at the end of the curved channel. Then, two other sheath flows horizontally confine the main flow to achieve horizontal focusing. Thus, the core flow is three-dimensionally focused at the center of the channel at the downstream.

Using centrifugal force for 3D flow-focusing in a single-layer microchannel has been previously investigated by several groups. However, their proposed designs require high flow speed (>1m/s) which cannot be applied to live biomedical species. Here, we introduce a new design which is operational in
A two-fold control on both pressure and flow-rate for flow control and quality management in fluidic processes

Thibaut Thupnot, Benjamin Rouffet, Anne Le Nel, Fluigent (France); Nicolas Petit, ARMINES-ENSMP (France)

Conventional flow control systems, such as syringe and peristaltic pumps, are not well adapted to the control of flow in microchannels. They often result in long equilibration times, hysteresis and low stability. Herein, we present a new method to control the flows in microchannels based on a pressure actuation, by pressurization of reservoirs filled with fluids to be injected in the microstructure. The regulated pressure within the reservoirs generates pulse-free and very stable flows through the microchannels, with short settling time. To control the flow-rate with pressure actuation, highly precise flow sensors are implemented in the fluidic system and an algorithm has been developed to adjust automatically the pressure orders to reach the targeted flow-rates. Unlike a conventional PID regulation which is very sensitive to any transient behavior, our algorithm deals with any coupling effect between the different channels, and is designed to deliver the fastest and the most stable flow response. It calculates a matrix image of the microsystem with the relations between each actuated pressure channels and the measured flow-rates. Furthermore, the system can cope with any external disturbances of the system (presence of air bubble, partial clogging, variation of viscosity or temperature, etc...), and continuously re-adjust the applied pressures. The technology is perfectly suited for droplet manipulation experiments (among other applications) where we can generate 2pL water-in-oil droplets with very high monodispersity (1.63% CV) at an up to 12 kHz/s frequency. Only few seconds are needed to stop the droplets flow, reducing costs by a huge factor.

Tacky COC: a solvent bonding technique for fabrication microfluidic systems

Nico Keller, Tobias M. Nargang, Bastian E. Rapp, Karlsruher Institut für Technologie (Germany)

For bonding cyclic olefin copolymer (COC) several bonding methods have been developed such as thermal bonding or solvent bonding. Thermal fusion bonding requires high temperatures (about 95°C) and is not suitable if the microfluidic structure is temperature sensitive. For example if the structure is coated with proteins, the proteins will be denatured by high temperatures. Here, low temperature bonding methods are preferred. With our bonding method, we are able to bond protein-coated structures. Our method does not require any heat treatment. An additional advantage of our method over previous methods is that we expose only one substrate to the solvent, preferably a sealing lid. This also avoids problems with microstructure deformation upon solvent bonding. Our method relays on a short solvent immersion and subsequent drying which turns the surface of the COC tacky. The tacky lid was then pressed on the COC structure for a few seconds. A strong bond is obtained in less than three minutes overall. Such “tacky COC” lids can be used to bond a microfluidic chip made in COC. This process is significantly faster than the so-called solvent deposition, liquid injection, and encapsulation. As constituent materials, IZO coated convex glass, UV epoxy (NOA 68), DI water, and dodecane are used. The number of lenses on the fabricated panel is 10 by 10 and each lens has 1mm aperture with 1.6mm pitch between adjacent lenses. When the voltage is applied on the device, it is observed that each lens is changed from concave state to convex state. From the unique optical characteristics of curved array of liquid lenses such as controllable focal length and wide field of view, we can expect that it has potential applications in various fields such as medical diagnostics, surveillance systems, and light field photography.

Electrowetting liquid lens array on curved substrates for wide field of view image sensor

Yousung Bang, Muyoung Lee, Yong Hyub Won, KAIST (Korea, Republic of)

In this research, electrowetting liquid lens array on curved substrate is developed for wide field of view image sensor. In the conventional image sensing system, this lens array is usually in the form of solid state. However, in this state, the lens array which is similar to insect-like compound eyes in nature has several limitations such as quality of image degradation, narrow field of view because it cannot adjust focal length of lens. For implementation of the more enhanced system, the curved array of lens based on electrowetting effect is developed in this paper, which can adjust focal length of lens. The fabrication of curved lens array is conducted upon the several steps, including chamber fabrication, electrode & dielectric layer deposition, liquid injection, and encapsulation. As constituent materials, IZO coated convex glass, UV epoxy (NOA 68), DI water, and dodecane are used. The number of lenses on the fabricated panel is 10 by 10 and each lens has 1mm aperture with 1.6mm pitch between adjacent lenses. When the voltage is applied on the device, it is observed that each lens is changed from concave state to convex state. From the unique optical characteristics of curved array of liquid lenses such as controllable focal length and wide field of view, we can expect that it has potential applications in various fields such as medical diagnostics, surveillance systems, and light field photography.

Transfer molding processes for nano scale patterning of poly-L-lactic acid (PLLA) films

Rabin Dhakal, Akshit Peer, Rana Biswas, Jaeyoun Kim, Iowa State Univ. of Science and Technology (United States)

Periodically patterned structures or photonic crystals have made a tremendous impact in many diverse fields of sciences and has led to technical advances in synthesizing such structures in many dielectric materials.

Recently, there has been growing interest to explore the role of such artificially patterned structures in biomaterial. While the role of photon interaction is not important on such patterned biomaterials, the patterning itself has great potential to control diverse biological functions including - novel scaffolds for bone and tissue culture, nanorods on top of arrays of micro-pillars for super-wettability, microgrooves for promoting cell adhesion and Schwann cell alignment.

To evaluate the impact of patterning biodegradable polymers on their biological applications, we investigate economic, rapid and reproducible ways to create periodic microscale and nanoscale textures on Poly (L-lactic acid) (PLLA) surface. PLLA is a prototypical material commonly used for drug-eluting coronary stents and as a template for cell growth. Nano-texturing of these biodegradable films increase the available surface area so that it can be coated with larger doses of therapeutic agents. We demonstrate that soft lithography using a nano-transfer molding technique can be rapidly and reproducibly produce nanoscale patterns with a submicron pitch on the PLLA surfaces.

A master pattern consisting of a periodic array was first transferred to a PDMS mold and then to PLLA films using simple drop-casting and nanoimprinting methods. Drop-casting PLLA achieves the best transfer of patterns, with nanoarrays of holes and cones with pitch ~700 nm. Nanoimprinting the PLLA films results in shallower and less resolved features.
Improvement of mixing efficiency by using two sides of chip for inlets in a hybrid micromixer

Meisam Zaferani, Hamid Latifi, Zahra Saededian, Jalal Sadeghi, Shahid Beheshti Univ. (Iran, Islamic Republic of)

Many types of micromixers have been fabricated since the development of microfluidic applications. In conventional T-shape micromixers (T-mixers), at high Reynolds numbers (50< Re<150), the fluid vortices are symmetric thus the solute and solvent do not mix together.

In this paper we design and simulate a hybrid micromixer to obtain better mixing efficiency.

At first we use a spiral mixer cascaded to a T-mixer resulting in more efficient mixing by both molecular diffusion and chaotic advection. Then we present a new method of fluid entrance that uses the advantage of fluid feeding from two different sides. This posture resulting in an increment of interface in spiral channel and improves the interaction process in T-mixer part by introducing fluids to channel perpendicular to symmetrical axis.

Our hybrid model consist of a T-mixer surrounded by a spiral-shape mixer. Dimensions of channel cross section is 0.5mm*0.3mm. Length of T-mixer part is 1.5mm and spiral part has 4 rounds. Our model is compatible with miniaturization in microfluidics systems.

Numerical investigations of planar micromixer is carried out by using 3D Navier-stokes equation for laminar flows. Our simulations show that the mixing efficiency improves about 10% when using two sides of the chip for inlets in comparison with one side of chip for inlets.

Photothermal generation of microbubbles on plasmonic nanostructures for flow manipulation inside microfluidic channels

Jingtian Li, Ming Li, Greggy M. Santos, Fusheng Zhao, Wei-Chuan Shih, Univ. of Houston (United States)

In microfluidic devices, microbubbles have been widely utilized as micro-pumps, micro-mixers, micro-valves, micro-robots and surface cleaners. Among various microbubble generation techniques, hydrodynamic method is one of the most commonly used, relying on shear force between liquid and gas flows in T-junction, flow-focusing or co-focusing junction microchannels. High heat-flux pulse heating is another technique, where the bubble grows on the surface of resistive heaters due to the localized nucleate boiling effect. Another class of techniques for bubble generation utilizes high-power lasers, which relies on localized photothermal heating.

Metal films patterned in microchannels can act as photothermal heat sources when illuminated. As an alternative, noble metal nanoparticles of Au or Ag, hold great promise for being used as photothermal heaters. We present a technique to generate microbubbles using photothermal effects induced by continuous-wave laser irradiation of random nanoporous gold disk (NPGD) arrays covered microfluidic channels. The size of the microbubble can be controlled by adjusting the laser power. The dynamics of both bubble growth and shrinkage are studied. The potential application of flow manipulated in a Y-shaped microchannel is demonstrated. Using a spatial light modulator (SLM), simultaneous generation of multiple microbubbles at arbitrary locations with independent control has been demonstrated. The advantages of this technique are flexible bubble generation locations, long bubble lifetimes, no need for light-absorbing dyes, high controllability over bubble size, and low power consumption, etc. We expect this technique to provide new flow control functions in microfluidic devices.

The use of microfluidics and dielectrophoresis for separation, concentration, and identification of bacteria

Cynthia Hanson, Elizabeth Vargas, Karen Tew, Annelise Dykes, Michaela Salisbury, Utah State Univ. (United States)

Traditional bacterial identification methods take one to two days to complete, relying on large bacteria colonies for visual identification. This time-consuming analysis is unacceptable during time sensitive situations such as life threatening illnesses and bioterrorism. To address this need, several methods such as polymerase chain reaction, Raman or infrared spectroscopy, fluorescent microscopy, and micro-array testing have been used to identify bacteria. However, such methods require a pure sample or an extrinsic means for identification such as fluorescent tags, antibiotics,
or primers. Pure samples cannot be obtained directly and using extrinsic methods is costly due to the broad range of bacteria species and strains. To address these problems, a sorting mechanism based on characteristics of the sample itself is needed. One example of such a method is dielectrophoresis (DEP), which uses a non-uniform electric field to cause motion in particles based on the particle’s size and electrical properties. Researchers have used DEP to separate bacteria from sample debris and sort bacteria according to species. In this research, a microfluidic device was fabricated and tested for the purpose of using DEP to sort bacteria according to species for subsequent spectral identification by Raman spectroscopy.

9705-14, Session 3
Blister technology for the storage of liquid reagents in microfluidic devices
Suzanne Smith, Council for Scientific and Industrial Research (South Africa); René Sewart, microfluidic ChipShop GmbH (Germany); Kevin Land, Pieter Roux, Council for Scientific and Industrial Research (South Africa); Holger Becker, microfluidic ChipShop GmbH (Germany)

Blister pouches provide an elegant solution to implement reagent storage and release, and can be compressed either by means of an automated, mechanical actuation system or manually to enable the reagent to be dispensed into the device. However, there is little control over the volume of fluid that is released from the blister pouch, as fluid can be trapped in creases formed during the compression of the blister pack. Variations in reagent volumes in the blisters also occur as a result of the manufacturing and filling processes.

In a first version of a blood cell counting device, a white blood cell count was implemented using blister pouches to introduce a reagent to the device. From this work the need for precise and repeatable volume control of sample and reagent was evident for performing accurate blood cell counts. A blister pouch metering device was developed to ensure that reagents were introduced into the device in an accurate, repeatable, and contained manner. The microfluidic metering device consisted of a metering chamber with a pre-defined volume and a measurement channel, enabling volume measurements accurate to 0.45 μL to be made. Image processing was used along with known dimensions and gradations of the microdevice to determine the volume of reagent filling the channel. Blister pouches were filled with a standard white blood cell lysing and staining reagent containing acetic acid and crystal violet. The results from 15 blister metering devices show that accurate and repeatable reagent volumes were achieved.

9705-15, Session 4
Advances towards reliable identification and concentration determination of rare cells in peripheral blood (Invited Paper)
Daniel Hill, Univ. de Valência (Spain)

Through further development, integration and validation of micro-nano-bio and biophotonics systems FP7 CanDo is developing an instrument that will permit highly reproducible and reliable identification and concentration determination of rare cells in peripheral blood for two key societal challenges, early and low cost anti-cancer drug efficacy determination and cancer diagnosis/monitoring.

A cellular link between the primary malignant tumour and the peripheral metastases, responsible for 90% of cancer-related deaths, has been established in the form of circulating tumour cells (CTCs) in peripheral blood. Furthermore the relatively short survival time of CTCs in peripheral blood means that their detection is indicative of tumour progression thereby providing in addition to a prognostic value an evaluation of therapeutic efficacy and early recognition of tumour progression in theranostics. In cancer patients however blood concentrations are very low (=1 CTC/1E9 cells) and current detection strategies are too insensitive, limiting use to prognosis of only those with advanced metastatic cancer. Similarly problems occur in therapeutics with anti-cancer drug development leading to lengthy and costly trials often preventing access to market.

The novel cell separation/SERS analysis technologies plus nucleic acid based molecular characterization of the CanDo platform will provide an accurate CTC count with high throughput and high yield meeting both key societal challenges. Being beyond the state of art it will lead to substantial share gains not just in the high end markets of drug discovery and cancer diagnostics but due to modular technologies also in others.

Here we present some of the projects results to date.

9705-16, Session 4
Novel microfluidic system for online monitoring of biofilm dynamics by electrical impedance spectroscopy and amperometry
Julia Bruchmann, Kai Sachsenheimer, Thomas Schwartz, Bastian E. Rapp, Karlsruher Institut für Technologie (Germany)

Biofilm formation is ubiquitous in nature where microorganisms attach to surfaces and form highly adapted and protected communities. In technical and industrial systems like drinking water supply, food production or shipping industry biofilms are a major cause of product contamination, biofouling, and biocorrosion. Therefore, understanding of biofilm formation and means of preventing biofilm formation is important to develop novel biofilm treatment strategies. A system allowing directly online detection and monitoring biofilm formation is necessary. However, until today, there are little to none technical systems featuring a non-destructive real-time characterization of biofilm formation in a high-throughput manner.

This paper presents such a microfluidic system based on electrical impedance spectroscopy (EIS) and amperometric current measurement. The sensor consists of four modules, each housing 24 independent electrodes inside 12 microfluidic channels. Attached biomass on the electrodes is monitored as increased inhibition in charge transfer by EIS and a change in metabolic activity is measured as change in produced electric current by amperometry.

This modular sensor system is highly adaptable and suitable for a broad range of microbiological applications. Among others, biofilm formation processes can be characterized online, biofilm manipulation like inactivation or destabilization can be monitored in real-time and gene expression can be analyzed in parallel. The use of different electrode designs allows sensitive biofilm studies during all biofilm phases.

The whole system was recently extended by an integrated pneumatic microfluidic pump which enables easy handling procedures. Further developments of this pumping module will allow a fully-automated computer-controlled valving and pumping.

9705-17, Session 4
Rapidly reconfigurable monolithic laser/detector arrays with capillary fill microfluidics for chip-based flow cytometry
Robert Thomas, Cardiff Univ. (United Kingdom)

Low cost, portable chip based flow cytometry has great potential for applications in resource poor and point of care settings. Typical approaches utilise low cost silicon or glass substrates with light emission and detection
performed either off-chip using external equipment or incorporated on-chip using ‘pick and place’ diode lasers and photo-detectors. The former approach adds cost and limits portability while the sub-micron alignment tolerances imposed by the application make the latter impractical for all but the simplest of systems. Use of an optically active semiconductor substrate, on the other hand, overcomes these limitations by allowing multiple laser/detector arrays to be formed in the substrate itself using high resolution lithographic techniques.

The capacity for multiple emitters and detectors on a single chip not only enables parallel measurement for increased throughput but also allows multiple measurements to be performed on each cell as it passes through the system. Several different experiments can be performed simultaneously and throughput demand can be reduced with the facility for error checking. Furthermore, the fast switching times inherent with semiconductor lasers allows the active sections of the device to be reconfigured on a sub-microsecond time scale providing additional functionality. This is demonstrated here in a capillary fill system using pairs of laser/detectors that are operated in pulsed mode and alternated between lasing and detecting in an interleaved manner. Passing cells are alternately interrogated from opposing directions providing information that can be used to correct for differences in lateral cell position and ultimately differentiate blood cell type.

9705-19, Session 5

3D printing of microfluidic vascular channels in gels using commercial 3D printers (Invited Paper)

P. Ravi Selvaganapathy, Rana Attala, McMaster Univ. (Canada)

Lack of a simple and effective method to integrate vascular network with engineered scaffolds and tissue constructs remains one of the biggest challenges in true 3D tissue engineering. Here, we detail the use of a commercially available, low-cost, open-source 3D printer modified with a microfluidic print-head in order to develop a method for the generation of instantly perfusable vascular network integrated with gel scaffolds seeded with cells. The print-head features an integrated coaxial nozzle that allows the fabrication of hollow, calcium-polymerized alginate tubes that can be easily patterned using 3D-printing techniques. The diameter of the hollow channel can be precisely controlled and varied between 500 µm to 2mm by changing applied flow rates or print-head speed. These channels are integrated into gel layers with a thickness of 800 µm – 2.5 mm. The structural rigidity of these constructs allows the fabrication of multi-layered structures without causing the collapse of hollow channels in lower layers. The 3D printing method was directly characterized at a range of operating speeds (0-40 m/min) and corresponding flow rates (1-30 mL/min) were identified to produce precise definition. This microfluidic design also allows the incorporation of a wide range of scaffold materials as well as biological constituents such as cells, growth factors, and ECM material. Media perfusion of the channels causes a significant viability increase in the bulk of cell-laden structures. With this setup, gel constructs with embedded blood vessel networks. With this setup, gel constructs with embedded functionalization of 3D lab-on-chip devices (invited paper)

9705-20, Session 5

Femtosecond laser fabricated integrated chip for manipulation of single cells

Anusha Keloth, Melanie Jimenez, Helen Bridle, Lynn Paterson, Gerard H. Markx, Ajoy K. Kar, Heriot-Watt Univ. (United Kingdom)

Optical micromanipulation techniques and microfluidic techniques can be used in same platform for manipulating biological samples at single cell level. Novel microfluidic devices with integrated channels and waveguides fabricated using ultrafast laser inscription combined with selective chemical etching can be used to enable sorting and isolation of biological cells. In this paper we report the design and fabrication of a three dimensional chip that can be used to manipulate single cells in principle with a higher throughput than is possible using optical tweezers. The capability of ultrafast laser inscription followed by selective chemical etching to fabricate microstructures and waveguides have been utilised to fabricate the device presented in this paper. The complex three dimensional microfluidic structures within the device allow the injected cell population to focus in a hydrodynamic flow. A 1064 nm cw laser source, coupled to the integrated waveguide, is used to exert radiation pressure on the cells to be manipulated. As the cells in the focussed stream flow past the waveguide, optical scattering force induced by the laser beam pushes the cell from out of the focussed stream to the sheath fluid, which can be then collected at the outlet. Thus cells can be controllably deflected from the focussed flow to the side channel for downstream analysis or culture.

9705-21, Session 5

Maskless fabrication of a microfluidic device with interdigitated electrodes on PCB using laser ablation

Michael Contreras-Saenz, Christian Hassard, Rafael Vargas-Chacon, Jose Luis Gordillo, Sergio Camacho-León, Tecnológico de Monterrey (Mexico)

This paper reports the maskless fabrication of a microfluidic device with interdigitated electrodes (IDE) based on PCB-MEMS technology and laser ablation. The device has fire retardant (FR)-4 resin as substrate, cooper (Cu) as active material and SU-8 polymer as structural material. The geometric design for the electrodes and channels were patterned on each material from respective CAD layout files. Adjustment of the laser pulse frequency, average power and scanning velocity allowed to successfully define microchannels with 200 µm in width, 24 µm in depth and 800 µm in length, as well as IDEs with 50 µm in width, 25 µm in finger gap and 500 µm in finger overlap onto the FR-4 substrate. The measured surface roughness for SU-8, Cu and FR-4 was 0.28 µm, 0.374 µm and 6.32 µm, respectively. Surface roughness and dimensions were measured using confocal microscopy. A Nd:YAG G-switched laser operating at 355 nm with a Gaussian beam diameter of 15 µm was used in this work. It is shown that the ablation depth increases with an increasing average power or a decreasing pulse frequency for a fixed scanning velocity; whereas that for a fixed frequency and power, the depth decreases with an increasing scanning velocity. The resolution and repeatability achieved in this approach, along with the low cost of the involved materials, enable an affordable micromachining platform with a rapid fabrication-test cycle to develop active multiphase microdevices for potential applications in biosensing, cell culture, drug delivery, transport and sorting of molecules, among others.

9705-22, Session 5

Pyro-EHD ink-jet printing for direct functionalization of 3D lab-on-chip devices

Sara Coppola, Veronica Vespi, Vittorio Bianco, Laura Mecozzi, Michele Todino, Melania Paturzo, Istituto di Scienze applicata e Sistemi Intelligenti (Italy) and Consiglio Nazionale delle Ricerche (Italy); Pietro Ferraro, Istituto di Cibernetica Eduardo Caianiello (Italy) and Consiglio Nazionale delle Ricerche (Italy); Simonetta Grilli, Istituto di Scienze applicata e Sistemi Intelligenti (Italy) and Consiglio Nazionale delle Ricerche (Italy)

A challenging request in the fabrication of microfluidics and biomedical microsystems is a flexible on-demand ink-jet printing for breaking the rigidity of classical lithography. Electrohydrodynamic (EHD) has reached in
latest years exciting performances becoming the elite nano-tool for direct printing of lab-on-chip devices, from biomolecules to nano-electronics. However, it suffers of practical but severe limitations due to its intrinsic geometrical configuration. Recently a pyroelectric-EHD system has been presented, opening new scenarios for material packaging and assembling, overcoming material type limitations and adding much more flexibility to the fabrication process. The pyro-EHD system has proved challenging spatial resolution down to nanoscale, printing of high ordered geometrical patterns, capability of dispensing biological ink as DNA and protein array for biosensing fabrication, single cells printing and direct printing of nanoparticles. The method proposed appears as one of the most interesting polymer fabrication process in term of flexibility and viscosity of the material processed. In fact, high viscous polymer could be easily printed at high resolution in 2D or, even more, in 3D configuration. The pyro-EHD process has been also proved for the fabrication of biodegradable microneedles for trasdermal drug delivery. The microneedles have been realized on flexible patches of simple use in order to move the point of care directly to the patients. Other 3D polymer microstructures have been fabricated for collecting or distributing light signals in lab-on-a-chip optofluidic devices as potential 3D optical waveguides, opening way to innovative optogenesys studies, guiding light for generating or transporting optical/electronic signals from and to cells.

9705-23, Session 5

Enhancing defect tolerance in periodic post microfluidic channels

Glenn H Chapman, Bonnie L. Grey, Simon Fraser Univ (Canada)

Biomedical sensors using microfluidic channels are prone to blockage due to particles and bubbles in the fluid. The problem is to hold up the roof of the fluid channel a common design uses many parallel fluid channels separated by structural support walls, but these can be rapidly blocked by particulates. We have been studying an alternative “Cathedral Ceiling” design where the roof is support by periodic posts which creates many possible flow paths to bypass blockages. We use Monte Carlo modelling with iterative COMSOL fluid dynamics simulations to establish the stream lines, and particle velocities. Then a rules based methodology iterative places trapped particles based on the fluid paths created by the existing blockages, until the system becomes fully blocked. Previous work has shown that the periodic post design increases lifetime by allowing 6 to 7 times more blockages than can a parallel channel design. In this paper we first present mathematical analysis of our modelling results. We show why the lifetime is not very sensitive to the size of the support posts relative to the channel width. We also analyze why expanding the number of channels increases almost linearly the number of particles required for blockage, but increasing the channel length does not. In these we create general equations for application in microfluidic channel design. We discuss our experimental work to create test structures of cathedral ceiling design to verify the fluid flow modelling and analysis results. This includes attempts to confirm the stream lines of the simulations.

9705-24, Session 6

Silicon chip integrated photonic sensors for biological and chemical sensing (Invited Paper)

Swapnajit Chakravarty, Omega Optics, Inc. (United States); Ray T. Chen, The Univ. of Texas at Austin (United States); Naimei Tang, Omega Optics, Inc. (United States); Yi Zou, Wei-Cheng Lai, Hai Yan, Chun-Ju Yang, The Univ. of Texas at Austin (United States)

As sensing platforms migrate from bench-top to handheld, from rigid to wearable and implantable, silicon has become the material of choice for many applications. Due to superior electrical and optical characteristics, silicon electronic and photonic devices have found applications in micro-electro-mechanical-systems as well as integrated optics and electronics for chip-based communications and sensing. The silicon platform also benefits from a highly mature foundry processing technology that provides the potential for high volume manufacturing.

In this paper, we will review our work with silicon photonic crystal devices and their applications in chemical and biological sensing in near and mid-infrared wavelengths. In label free biosensors, we have experimentally demonstrated photonic crystal microcavity based biosensors with sensitivities down to 67 fg/ml. Sensors have demonstrated an order of magnitude better sensitivity compared to ELISA when detecting pancreatic cancer biomarkers in human serum samples. Miniaturization enables multiple sensors to be integrated on the same chip and measured simultaneously at the same instant of time from a patient sample, leading to high throughput assays, higher specificity and lower false positives. Applications of photonic crystal biosensors range from the detection of cancers and allergens, for drug discovery and biomarker discovery in medicine and life sciences, to food science and biodefense.

In on-chip optical absorption spectroscopy, slow light in silicon photonic crystals reduce the effective absorption path length and have enabled detection of chemical warfare simulant gases in the mid-infrared in low ppm and VOC pollutants in water in the near-infrared in low ppb concentrations.

9705-25, Session 6

Optofluidic lens(es) for switchable 2D and 3D imaging

Hanyang Huang, Kang Wei, Yi Zhao, The Ohio State Univ. (United States)

The stereoscopic image is often captured using two independent cameras arranged side-by-side, two static lenses connected to split channel optics, dual iris diaphragm, or biprism/mirrors to switch the optical path. The miniaturization of the overall size of stereoscope down to several millimeters is at a sacrifice of further size shrinkage of horizontally-separated optical elements. The limited light entry worsens the final image resolution and brightness. It is known that optofluidics offer good integration and reconfigurability where the deformation of elastomeric elements within microfluidics can modify the optical boundaries and yield light tunability. Leveraging this technique, we report a reconfigurable optofluidic system whose optical layout can be swapped between a pair of 3mm lenses and a 10mm single lens within the same optical channel. The binoculars capture stereoscopic images in a conventional way, while the singlet acquires a two-dimensional image yet at better resolution and brightness. Meanwhile, the Vari-focal binoculars and the singlet thus work interchangeably and complementarily to address the downside of stereoscope miniaturization. Coupling the characteristics of accommodation and binocular vision in a compact single-camera setting, this miniature device expects applications in machine vision, stereoscopic microscopy, and 3D endoscopic surface imaging.

9705-26, Session 6

Integrating opto-piezoelectric actuators and a two-mode excited linear ultrasonic motor for microfluidics transport of a biochip

Tsun-Hsu Chen, Hsin-Hu Wang, Yu-Hsiang Hsu, Chih-Kung Lee, National Taiwan Univ. (Taiwan)
Development of microfluidic devices for in situ investigation of cells using surface-enhanced Raman spectroscopy

Yu-Han Ho, Daniel D. Galvan, Qiuming Yu, Univ. of Washington (United States)

Surface-enhanced Raman spectroscopy (SERS) has emerged as a powerful analytical and sensing technique for many applications in biomedical diagnosis, life sciences, food safety, and environment monitoring because of its molecular specificity and high sensitivity. The inactive Raman scattering of water molecule makes SERS a suitable tool for studying biological systems. Microfluidic devices have also attracted a tremendous interest for the aforementioned applications. By integrating SERS-active substrates with microfluidic devices, it offers a new capability for in situ investigation of biological systems, their dynamic behaviors, and response to drugs or microenvironment changes. In this work, we designed and fabricated a microfluidic device with SERS-active substrates surrounding by cell traps in microfluidic channels for in situ study of live cells using SERS. The SERS-active substrates are quasi-3D plasmonic nanostructure array (Q3D-PNA) made in h-PDMS/PDMS with physically separated gold film with nanochannels on top and gold nanodisks at the bottom of nanowells. 3D finite-difference time-domain (3D-FDTD) electromagnetic simulations were performed to design Q3D-PNAs with the strongest local electric fields (hot spots) at the top or bottom water/Au interfaces for sensitive analysis of cells and small components, respectively. The Q3D-PNAs with the hot spots on top and bottom were placed at the up and down stream of the microfluidic channel, respectively. Each Q3D-PNA pattern was surrounded with cell trapping structures. The microfluidic device was fabricated via soft lithography. We demonstrated that normal (COS-7) and cancer (HepG2) cells were captured on the Q3D-PNAs and investigated in situ using SERS.

Optofluidics is a developing field of research emerged from the integration of optical components into Lab-on-a-Chip (LOC) microfluidic devices. Microfluidic devices obey the Navier-Stokes equations and in order to simulate their fluidic behavior, it is straightforward to solve these equations. Navier-Stokes equations provide us with the velocity field and the pressure distribution throughout the microchannel. If we aim at analyzing the optical properties of the microfluidic channel, we shall know its refractive index profile. The parameters which correlate the optical and fluidic properties are the “concentration” of different species present in the microchannel and their refractive indices. To obtain the concentration profile of each species, we use the mass balance equations.

The velocity vector can be obtained from Navier-Stokes equation and, then, can be inserted in the mass-balance equation to find the concentration profile. The next step is to use the concentration profile of the microfluidic channel to find the refractive index profile. In this study, we suggest to employ the Effective Medium Theory of Bruggeman which gives the effective optical properties of a mixed media based on the refractive index and volume filling fraction of its constituents. By implementing this theory, the refractive index profile of the microfluidic channel can be obtained and, then, by using Maxwell’s equation, the optical properties of a microfluidic channel can be extracted. As an example, we have carried out this procedure to analyze the liquid core/liquid cladding optofluidic waveguide.

A label free opto-fluidic micro flow cytometer based on optical fiber Fabry-Perot interrogation

Jalal Sadeghi, Hamid Latifi, Farnood Mirkhosravi, Mohsen Jamshidi, Hesamodin Khashei, Shahid Beheshti Univ. (Iran, Islamic Republic of)

This paper presents an integrated optical fiber Fabry-Perot interferometer (OFFPI) as a flow cytometer in order to carry out on-line detection of particles. The sensing mechanism is based on changes in the effective optical path length of the OFFPI due to passing particles. The OFFPI is the interrogating component in our optical set up and acts as an edge filter for converting a refractive index-induced wavelength variation into an optical power measurement. A Fiber Bragg Grating (FBG) is fixed on the approximately linear region of the interferometric pattern of the OFFPI at a wavelength of 1545nm. Due to the disparity in refractive indexes between the particles and water, there is no need for fluorescent labelling, and because of the integration of fiber optics there is no need for a bulky optical apparatus. This flow cytometer is used in counting of polystyrene beads at telecommunication wavelengths (1500nm -1600nm). Results showed that signal to noise ratio for optofluidic-interrogation system is 4 times more than optofluoroptic cytometer without the interrogation technique. In addition, Finite Element Method (FEM) is used for the simulation of optical coupling with different sizes of the polystyrene beads in the Fabry-Perot cavity. This is modeled in frequency domain over a range covering the expected resonances. We obtained experimental results that are in agreement with the expected shift direction under effective RI variation with particle passing.

Development of microfluidic-based cell collection devices for in vitro and in vivo use

Logan Butt, SUNY CNSE/SUNYIT (United States); Dave Entenberg, Albert Einstein College of Medicine (United States) and Gruss Lipper Biophotonics Ctr. (United States); Madhubhani Hemachandra, Matthew Strohmayer, SUNY
Due to the complexity of metastasis, it is essential to develop the ability to isolate and collect metastatic cells from the tumor microenvironment for study. An implantable microfluidic platform can be used to induce chemotaxis in the tumor and collect these invasive cells. There remain, however, unique challenges in the collection and extraction of metastatic cells, especially in regards to the shapes of the microfluidic channels within the device. In order to optimize the geometries involved for collection, a series of experiments has been performed for the characterization and evaluation of microfluidic features and their function. This characterization is performed with the Nano Intravital Device (NANIVID).

The NANIVID consists of a glass reservoir loaded with a hydrogel for sustained drug delivery and sealed to a glass cover using a polymer membrane. Using scanning electron microscopy, prototype device microchannels are characterized with regards to cross-sectional area and hydraulic diameter. Flow through the NANIVID was tested using pressure gradients to drive suspended cells into the device. Designs that show potential are evaluated in vitro and in vivo for collection through chemoatraction.

Incorporation of controllably activated microvalves allows for multiple enhanced functionalities of such devices. These include device sealing to prevent premature cell entry during implantation, and subsequent remote seal opening to enable cell migration into the device. Light-activation is ideal as it involves no change in temperature that may adversely affect the microenvironment. Azobenzene liquid-crystal polymers provide reversible photoactuation for powering the microvalve, thereby transitioning the NANIVID to an active microfluidic device.

### 9705-48, Session PSun

#### Using 3d-bio-printer make micro pattern

**Sang Mok Kim, Pusan National Univ. (Korea, Republic of)**

3D printer is easy to manufacture structures of many different methods. 3D printer builds structure by using metals and plastics. Many researchers use 3D bio-printer to their research purposes. 3D bio-printer’s ultimate objective is to constitute the human organs and cells. Micro and Nano patterns are created by using various lithography (optical methods, or pressure method.), and patterns have been used in many studies. Here we present new method that comes from 3D printer’s pattern making method to applicate 3D bio-printer. Existing 3D printer modules will produce to use 3D bio-printer. And we added the structure using by 3D printer which 3D bio-printer doesn’t have. We build syringe pump and set materials to the fluid and viruses. After set materials, syringe pump offers materials to the nozzle and build patterns to target where we want. Then We use Hotplate by 200-300? to remove fluid to gain pattern in real time. We can get various thickness of patterns by nozzle’s size. We make from 3D bio-printer is economical and pattern resolution is not bad. Because the 3D bio-printer which we made doesn’t make pattern to mask, we just need CAD pattern file. So we can make micro pattern without complicate lithography process.

### 9705-49, Session PSun

#### Decomplexification of positive blood cultures by acoustophoresis for rapid diagnosis of bloodstream infections

**Emilie Bisceglia, Lucien Talvard, M. H. Charles, J. Blaze, Fredric Pinston, Patrick Broyer, bioMérieux SA (France)**

Over the last decade, mainly through microfluidics expansion, acoustophoretic separation methods had been extensively used for separation of micrometer-sized particles. Nevertheless only few academic research groups focused on real biological samples, which is a key step to fit the medical need and to develop a strong added value extraction method for in vitro diagnosis. In this context, this study aims at developing and evaluating the potential of acoustophoresis-based separation method to extract efficiently micro-organisms from positive blood cultures, in order to shorten the current diagnosis of bloodstream infection and/or to have in-line analysis process after extraction.

To perform this study, we used a commonly described microfluidic architecture, where positive blood culture (BacT/Alert bottle with 10mL healthy blood and spiked with microorganisms) is laminated along the side walls of a separation channel by a buffer solution. Red blood cells, being significantly bigger than micro-organisms, will focused more efficiently in the centerline, since radiation force is proportional to the particle’s volume. Blood cells and micro-organisms can thus be recovered in different outlets at the end of the separation channel.

Acoustic extraction performances have been evaluated on blood cultures spiked with 3 different species (E. coli, S. aureus, C. albicans), illustrating the diversity of micro-organisms involved in bloodstream infections. Extraction efficiency has been quantified (between 9% and 50% depending on the species) with a sample flow rate of 607L/min, suitable for clinical applications (1mL of sample extracted in 15 minutes). These results emphasize the fact that the performances of this extraction step are highly dependent on the morphology of the target species.

Finally, we monitored the growth curve of micro-organisms following an acoustophoretic extraction step, in order to detect any effect of acoustic forces on the micro-organisms metabolism. The study suggests that acoustophoretic extraction does not interfere with micro-organisms growth afterwards.

To conclude, this study shows that separation based on acoustic forces could be an alternative method to extract micro-organisms from clinical samples while preserving their metabolisms. This would allow performing antibiotic susceptibility tests directly following the extraction, and thus dramatically improving the laboratory workflow efficiency.
Procalcitonin (PCT) is an early and highly specific biomarker in response to bacterial infection. The PCT-guided antibiotic therapy has demonstrated to be more efficient than standard therapy to reduce in antibiotic use without adverse outcome in mortality. The PCT detection in clinics is required to be highly sensitive with a sensitivity of 0.5 ng/ml. At present, the technologies for PCT detection are limited. This paper reported a highly sensitive nanoimprinted gold nanopillar array chip for PCT detection.

To achieve high sensitivity for PCT detection, the gold nanopillar array sensing chip was designed by plasmonic simulation and fabricated by high fidelity nanoimprinting technology. The gold nanopillars of 140 nm were nanoimprinted on glass substrate. A robust sandwich bioassay of capture antibody /PCT / quantum dot (QD) conjugated detection antibody was established on the gold nanopillar array chip to detect PCT. The nanopillars serve as localized surface plasmon resonance (LSPR) generators to enhance the fluorescent emission from QD. A limit of detection (LOD) of 0.5 ng/ml was achieved for PCT detection. This is the first time that PCT is detected with such high sensitivity by LSPR enhanced QD emission. By considering the low-cost, high sensitivity of the bioassay, as well as the inexpensive mass fabrication of the high quality chips, this novel nanoimprinted gold nanopillar array chip is particularly useful for developing a point-of-care system for PCT detection.

Dielectrophoresis assisted trapping of food pathogens in high conductive juice medium using microfluidic lab-on-chip device

Sekar Harikrishnan, Narjes Allahrabbabi, Suhanya Duraiswamy, Kian Meng Lim, Lin Yue Larry Yung, Heow Pueh Lee, National Univ. of Singapore (Singapore)

In the contemporary technologies, rapid detection and trapping of food borne pathogens using dielectrophoresis (DEP) technique enables faster, simple and efficient point of care diagnosis compared to the conventional laboratory based methods. Current paper discusses the trapping of food pathogens in highly conductive juice medium using dielectrophoresis techniques in different microfluidic device configurations. Inter-digitated, triangular electrodes and PDMS microchips were fabricated using soft lithography techniques and the DEP behaviour of the live Escherichia coli (E-Coli) cells were investigated in highly conductive commercial juice medium (0.35S/m), based on their response to the electrical field gradient generated. Indium Titanium Oxide (ITO) electrodes of width 150µm and inter-electrode spacing of 15µm and 100µm were fabricated and PDMS microchannels of depth 50µm were plasma bonded onto the electrodes to form the closed channels. Escherichia coli ATCC 1559 strain were cultured and spiked at different concentration in juice medium of different conductivities and the experiments were conducted. Capture voltage and cross over frequency of the cells were obtained for a wide range of voltage and frequencies and their dielectric properties were analysed. E-Coli cells were modelled as double shell spherical structure and a two dimensional simulation using COMSOL were performed to determine the dielectrophoretic force and the Clausius Mossotti factor was determined and compared with the experiment results. The trapping efficiency of each device was determined to be on an average 60%. This work would enhance the implementation of DEP biochip in wide range of applications involving bacterial diagnostics and label-free separation of target bio-particles.

Dielectrophoresis assisted trapping of food pathogens in high conductive juice medium using microfluidic lab-on-chip device

9705-31, Session 7

Dielectrophoresis assisted trapping of food pathogens in high conductive juice medium using microfluidic lab-on-chip device

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Over the past decade, there is a growing consensus among clinicians that tumors are highly patient-specific and likely to have different sensitivities to different types of anti-cancer agents. Most of the current research effort to personalized cancer therapy aims at developing biomarkers as statistical predictors of treatment response. A highly complementary approach would be to directly assess treatment response in vitro by testing the effectiveness of a drug on biopsied patient tissue. Yet, two challenges have impeded the development of these personalized in vitro cancer models: 1) maintaining biopsied tissue alive outside of the body for times sufficiently long to assay drug response and 2) finding an appropriate scoring metric to assess chemoresponse on chip. To address these issues, we tap into microfluidics unparalleled potential for the low cost, rapid, and integrated culture and analysis of cells and tissues on chip. In this presentation, we will introduce new microfluidic platforms under development in our laboratory to maintain alive and probe various kinds of 3D tumor samples, from spheroids to ex vivo human tumors. We will also discuss the challenge of directly assessing in vitro chemoresponse using on chip using three complementary techniques: confocal fluorescence imaging, fluorescence spectroscopy and flow cytometry. Given that the therapeutic effects of most drugs can only be felt after several weeks, this approach holds the potential of identifying non-responders early in a treatment to greatly reduce side effects for advanced cancer patients and save costs associated with no clinical benefit.

9705-33, Session 8
Microfluidics in-channel electrochemical imaging platform for neurotransmitter sensing
Amine Miled, Jesse Greener, Adnane Kara, Jessy Mathault, Arnaud Reitz, Martin Boisvert, Univ. Laval (Canada)

In this project we present the micro fabricated design of a complete microfluidic platform with integrated 200 electrodes for in situ screening and imaging of bio/chemical samples through a lab-on-chip system. For electrochemical imaging we used a novel method to incorporate electrochemical sensors array (20x10) connected to a PCB, a 200 µm height microfluidic channel was bonded to electrochemical sensors. The micro-channel contains 3 inlets where inlets in the edges are used to introduce phosphate buffer saline (PBS) and center inlet was to inject ferrocyanide. The flow rate of the 3 samples is controlled via LabVIEW interface and micro pumps; a multiplexer (Fig 1.b) was used to scan the 200 electrodes and perform individual cyclic voltamogrammes by a newly designed potentiotstat and pumping system. The behavior of the system was linear in term of variation of current versus concentration. A pseudo real time interface collected currents from each electrode. It was then analyzed with to detect different species, Fig.3 shows the electrochemical image generated by Matlab. A numerical calculation with COMSIL was achieved to solve Navier-Stocks equation to confirm experimental results. The designed system was also tested with different neurotransmitters such as serotonin, dopamine and glutamate.

9705-34, Session 8
Label-free detection and sizing of biomolecules using a UV-LED microfluidic platform
Pavan Kumar Challa, Yuewen Zhang, Jackie Carozza, Tuomas P. J. Knowles, Univ. of Cambridge (United Kingdom)

Studying conformational changes and folding pathways in biomolecules with extrinsic labelling via covalent or hydrophobic interactions is a widely used approach due to the high signal-to-noise ratios that it affords. However, this approach requires time-consuming labelling and purification steps; moreover, the interactions of extrinsic fluorophores with the structures of protein molecules can perturb their behaviour. Hence, the label-free detection techniques play a key role in studying conformational studies of proteins and other biomolecules. We have built a novel microfluidics-based system for the label-free detection and size measurement of biomolecules using a deep UV-LED (λ max = 280 nm). Using this platform, we demonstrate intrinsic fluorescence detection of proteins and combine detection with diffusional sizing to measure the dimensions of proteins in the solution phase in a label-free manner using a microfluidic co-flow geometry. Moreover, we show that our detection strategy is compatible with materials used in conventional soft lithography techniques, opening up the possibilities for routine use of label-free measurements on a chip.

9705-35, Session 8
Single-bead arrays for fluorescence-based immunoassays on capillary-driven microfluidic chips
Yuksel Temiz, Michel Lim, Emmanuel Delamarche, IBM Research - Zürich (Switzerland)

We report a concept for the simple fabrication of easy-to-use chips for immunoassays in the context of point-of-care diagnostics. The chip concept comprises mainly three features: (1) the efficient integration of reagents using beads functionalized with receptors, (2) the generation of capillary-driven liquid flows without using external pumps, and (3) a high-sensitivity detection of analytes using fluorescence microscopy. We fabricated prototype chips using dry etching of Si wafers. 4.5-µm-diameter beads were integrated into hexagonal arrays by sedimentation and removing the excess using a stream of water. We studied the effect of different parameters and showed that array occupancies from 30% to 50% can be achieved by pipetting a 250 nL droplet of 1% bead solution and allowing the beads sediment for 3 min. Chips with integrated beads were sealed using a 50-µm-thick dry-film resist laminated at 45 °C. Liquids pipetted to loading pads were autonomously pulled by capillary pumps at a rate of 0.35 nL/s for about 30 min. We studied ligand-receptor interactions and binding kinetics using time-lapse fluorescence microscopy and demonstrated 5 pM limit of detection (LOD) for an anti-biotin immunoassay. As a clinically-relevant example, we implemented an immunoassay to detect prostate specific antigen (PSA) and showed an LOD of 108 fM (i.e. 3.6 pg/mL). While a specific implementation is provided here for the detection of PSA, we believe that combining capillary-driven microfluidics with arrays of single beads and fluorescence readout to be very flexible and sufficiently sensitive for the detection of other clinically-relevant analytes.
9705-36, Session 9

Getting the most from microfluidic platforms in biomedical applications (Invited Paper)

Amy Shen, Okinawa Institute of Science and Technology (Japan)

Microfluidics has emerged in recent years as a versatile method of manipulating fluids at small length-scales, and in particular, for generating and manipulating micron size droplets with controllable size and functionality. For example, many research groups developed microfluidics devices for cell encapsulation, and synthesizing functionalized polymer microspheres and inorganic nanoparticles with precise control over their shapes and sizes. In this talk, I will showcase 2 microfluidic platforms to highlight their versatility and potential biomedical applications.

1) Droplet microfluidic platforms
   (a) A droplet microfluidics method to fabricate alginate microspheres while simultaneously immobilizing anti-Mycobacterium tuberculosis complex IgY and anti-Escherichia coli IgG antibodies primarily on the porous alginate carriers for specific binding and binding affinity tests. The binding affinity of antibodies is directly measured by fluorescence intensity of stained target bacteria on the microspheres. We demonstrate that the functionalized alginate microspheres yield specificity comparable with an enzyme-linked immunosorbent assay. We can easily modify the size and shape of alginate microspheres, and increase the concentration of functionalized alginate microspheres to further enhance binding kinetics and enable multiplexing.
   (b) A novel droplet microfluidics method to image oxygen in single islets (pancreatic cells) for glucose sensing. Individual islets and a fluorescent oxygen-sensitive dye were encased within a thin alginate polymer microcapsule for insulin secretion monitoring. The sensing system operated similarly from 2-48 hours following encapsulation, and viability and function of the islets were not significantly affected by the encapsulation process. This approach should be applicable to other cell types and dyes sensitive to other biologically important molecules.

2) A microfluidic chamber to perform uniform electric field stimulation in circular shaped culturewears
   A 3D computer-aided designed (CAD) polymeric insert is designed and retrofitted to circular shaped culturewears in an integrated microfluidic electrical stimulation platform to generate uniform EF with higher cell yields. In particular, NIH/3T3 mouse embryonic fibroblast cells are used to validate the performance of the 3D designed Poly(methyl methacrylate) (PMMA) inserts in a circular-shaped 6-well plate. The CAD based inserts can be easily scaled up to further increase effective stimulation area percentages, and also be implemented in commercially available culturewears for a wide variety of EF-related research such as EF-cell interaction and tissue regeneration studies.

9705-37, Session 9

Development of automated high throughput single molecular microfluidic detection platform for signal transduction analysis

Po-Jung Huang, Jun Kameoka, Texas A&M Univ. (United States); Hirohito Yamaguchi, Chao-Kai Chou, Mien-Chie Hung, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); Sina Baghbani-Kordmahale, Texas A&M Univ. (United States)

There have been advantages of single molecule microfluidic detections (SMMD) reported such as quantitative analysis of signal transductions or small sample volume requirements for post-translational modifications (PTM), protein-protein interactions (PPI), and protein-nucleic acid interaction (PNI); however, the throughput of this approach is very low and labor intensive. Therefore, we have demonstrated the development of the automated high throughput SMMD platform for a large number of molecular analyses. This platform consisted of microscope single molecule fluorescence detection system, microfluidic array device, automatic XY-stage and automated potential application system via relay and microcontroller. The microfluidic array device is made of quartz wafer that have 32 microchannels, gold electrodes at each reservoir at which biological sample solutions are dispensed. The other end of electrodes is connected to probes for potential applications for electroosmotic pumping. The microfluidic array is fixed on the automated XY-stage, which can control the position of microfluidic channels. The detection sequence includes 3 steps, (1) positioning the target microfluidic channel to the laser detection volume, (2) applying potential via relay system to target reservoirs for pumping, (3) acquiring molecular data. After acquiring 1000 molecular interaction data from the first microchannel, automated stage positions to focus the laser to the next microchannels. Meanwhile, relay starts applying the potential to the second microchannel for molecular detection. This sequence continues until all samples are characterized. As a result, we estimate that 32 samples can be analyzed for about 6 hours, contrary, conventional labor intensive approach can analyze 2 samples for 6 hours.

9705-38, Session 9

Cell analysis and sorting with Raman-tweezers in microfluidic system

Zden?k Pilát, Institute of Scientific Instruments of the ASCR, v.v.i. (Czech Republic)

We built an experimental apparatus for Raman analysis and sorting of optically trapped living cells (algae, microorganisms, cell cultures) in microfluidic environment. Optical trapping allowed non-contact spatial confinement and separation of the analyzed cells, while Raman spectroscopy was used for determination of the chemical parameters of the cells. Special microfluidic chip serves to deliver the cells to the area of Raman-tweezers and to separate and isolate the selected cells. The system allows fully automatic analysis and sorting of living microorganisms according to selected parameters of the Raman spectrum. The selected cells can be easily removed from the microfluidic chip in a form of cell-containing agarose plug. This system may find its use in various biomedical and biotechnological and medical disciplines. The research was supported by Ministry of Education, Youth and Sports CR (MEYS) (projects LO1212 and LD14069) and the research infrastructure by MEYS and EC (projects LOI212 and CZ.1.05/2.1.00/ 01.0017) and by Academy of Sciences of the Czech Republic (project RVO:68081731).

9705-39, Session 9

High-speed cell cytometry using nonlinear Brillouin imaging/sensing via time-resolved optical (BISTRO) measurements

Zhaokai Meng, Charles Ballman, Georgi I. Petrov, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Mechanical properties of living cells are often directly related to the cell types and their physiological conditions. Recent advances in atomic force microscopy (AFM) and optical tweezers have revealed the elasticity difference between healthy and diseased cells. However, all current detections are based on individual cells. The efficiencies of these techniques fundamentally impede their clinical applicability. Therefore, with the limited amount of samples, the elasticity of the cells cannot be statistically studied. Brillouin spectroscopy, an emerging technique in biomedical spectroscopy and imaging, is a non-invasive elasticity-specific probing technique based on inelastic light scattering originated from phonon-photon interactions. Nevertheless, the throughput of spontaneous Brillouin spectroscopy is also limited by the weak signal strength. In this report, by incorporating the...
9705-40, Session 10

Modular microfluidic cartridge-based universal diagnostic system for global health applications

Holger Becker, microfluidic ChipShop GmbH (Germany)

A central challenge for global health is a reliable and fast diagnosis of a broad range of diseases. We have developed a universal diagnostic system which, as a platform, can handle molecular, immunological and clinical chemistry tests on a single instrument platform in a low resource setting. One example for a molecular diagnostic test on this platform is the fully automated sample-in-answer-out cartridge for a rapid detection of mycobacterium tuberculosis (MTB). The injection-molded cartridge includes all reagents in liquid or dry form and fluid control elements such as valves. A family of cartridges with a common architecture for the different protocol types has been developed and model assays have been demonstrated. The system is conceptualized as an open platform, allowing companies to rapidly adopt their specific diagnostic assays onto this platform without the need for instrument modifications. This approach is specifically targeted towards reducing the costs and time required of developers and users to deploy new assays in low-resource settings.

9705-41, Session 10

Aqueous gradient by balancing diffusive and convective mass transport

Mohammed-Baker I. Habhab, Tania Ismail, Joe F. Lo, Arefa Haque, Univ. of Michigan-Dearborn (United States)

In wounds, cells secret biomolecules such as vascular endothelial growth factor (VEGF), a protein that controls many processes in healing. VEGF protein is expressed in a gradient in tissue, and its shape will be affected by the tissue injury sustained during wounding. In order to study the responses of keratinocyte cell migration to VEGF gradients and the geometric factors on wound healing, we designed a microfluidic gradient device that can generate large area gradients (1.5 cm in diameter) capable of mimicking arbitrary wound shapes. Microfluidic devices offer novel techniques to address biological and biomedical issues. Different from other gradient microfluidics, our device balances diffusion of biomolecules versus the convective clearance by a buffer flow on the opposite ends of the gradient. This allows us to create a large area gradient within shorter time scales by actively driving mass transport. In addition, the microfluidic device makes use of a porous filter membrane to create this balance as well as to deliver the resulting gradient to a culture of cells. The culture of cells are seeded above the gradient in a gasket chamber. However, Keratinocytes do not migrate effectively on filter paper. Therefore, in order to improve the motility of cells on the surface, we coated the filter paper with a 30µm thick layer of gelatin type B. After observation under the microscope we found that the gelatin coated sample showed cells with more spread out morphology, with 97% viability, suggesting better adhesion than the non-coated sample.
9706-1, Session 1

Adaptive enhancement and visualization techniques for 3D THz images of breast cancer tumors (Invited Paper)

Yuhao Wu, Tyler Bowman, John M. Gauch, Magda El-Shenawee, Univ. of Arkansas (United States)

This paper evaluates image enhancement and visualization techniques for pulsed terahertz (THz) images of tissue samples. Specifically, our research objective is to effectively differentiate between heterogeneous regions of breast tissues that contain tumors diagnosed as triple negative infiltrating ductal carcinoma (IDC). Tissue slices of varying thicknesses were prepared and scanned using our lab’s THz pulsed imaging system. One of the challenges we have encountered in visualizing the obtained images and differentiating between healthy and cancerous regions of the tissues is that most THz images have a low level of details and narrow contrast, making it difficult to accurately identify and measure the margins around the IDC. To overcome this problem, we have applied and evaluated a number of image processing techniques to the scanned 3D THz images. In particular, we employed various spatial filtering and intensity transformation to emphasize the small details in the images and adjust the image contrast. For each of these methods, we investigated how varying filter sizes and parameters affect the amount of enhancement applied to the images. To avoid over/under-enhancement, local image statistical features are calculated and considered to adaptively select the appropriate parameters used for the enhancement being applied. Our experimentation shows that several image processing techniques are effective in producing THz images of breast tissue samples that contain distinguishable details, making further segmentation of the different image regions promising.

9706-2, Session 1

Breast cancer margin detection with a single frequency terahertz imaging system (Invited Paper)

Sigfrid K. Yngvesson, Univ. of Massachusetts Amherst (United States); Andrew Karellas, Univ. of Massachusetts Medical School (United States); Stephen Glick, U.S. Dept. of Health & Human Services (United States) and U.S. Food and Drug Administration (United States); Ashraf Khan, Univ. of Massachusetts Medical School (United States); Paul R. Siqueira, Patrick A. Kelly, Univ. of Massachusetts Amherst (United States); Benjamin St. Peter, Spectral Sciences, Inc. (United States)

In breast conservation surgery (BCS), the surgeon attempts to remove malignant tissue with a surrounding margin of healthy tissue. The specimen is then submitted to pathology for evaluation to determine if the margins are clear of malignant tissue. In the pathological analysis it is often discovered that there are still positive margins and an average of 19-21% of BCS procedures need to be repeated. Thus, there is a real need for intra-operative tools that the surgeon can use to accurately assess tumor margins by imaging the specimen immediately after excision. In our research we explore the use of terahertz reflective imaging that has excellent potential for fast margin detection that could eventually be performed in the operating room. To test this hypothesis we have employed a prototype single frequency (CW) terahertz imaging system with a gas laser source and mechanically scanned 2x2 cm lumpectomy specimens under the laser beam. The images were correlated with optical histological micrographs of the same specimens, and a mean discrimination of 73% was found for five out of six samples using Receiver Operating Characteristic (ROC) analysis. The system design and characterization will be described in detail. The initial results are encouraging and we briefly discuss how further development of the technology as well as clinical evaluation could establish the feasibility of THz CW imaging in the clinical environment.

9706-3, Session 1

GaAs THz photoconductive sources with laser boresight alignment (Invited Paper)

Elliott R. Brown, Weidong Zhang, Wright State Univ. (United States)

A longstanding problem in the use of THz photoconductive sources is beam alignment. Usually these sources are coupled to free space using a planar antenna mounted on the backside of a low-loss dielectric (e.g., high-resistivity silicon) hyper-hemispherical lens. This often creates a high-directivity (D) THz beam after the lens, typically D ~25 dB or higher. The corresponding beam angle of ~1 deg or less makes the THz beams difficult to point, let alone track, in realistic scenarios such as imaging the skin of humans in a clinical setting. With recent advances in GaAs-based “extrinsic” photoconductors driven by 1550-nm lasers, roughly half of the 1550-nm power leaks through GaAs epitaxial layer and then propagates readily through the GaAs substrate and dielectric lens. Given good co-alignment of the antenna boresight with the optical axis of the lens, the THz and 1550-nm beams are nominally coaxial and the THz beam can be located in space by observing the 1550-nm beam using a laser viewing card or more sophisticated techniques. In this work we address the accuracy of this approach vs antenna directivity and lens radius, and consider sources of error such as antenna-lens misalignment. We also address the common need of blocking the 1550-nm radiation after geo-locating the THz beam using low-pass filters such as (absorptive) black polyethylene and (reflective) capacitive mesh.

9706-4, Session 1

Quasioptical imaging system design for THz medical imaging application

Shijun Sung, Zachary D. Taylor, Univ. of California, Los Angeles (United States)

In this presentation, a review and quasioptical imaging system and design considerations for an off-axis parabolic mirror based THz imaging systems are presented. It is shown that off-axis parabolic mirrors introduce off-axis intensity and polarization distortion. When a train of OAPs are used to relay THz beam, each distortions rapidly stacks to produce quite ugly beam and polarization profile. We show that the distortion of field distribution and intensity and polarization as a function of mirror curvature and focusing parameters. A brief review of design rules are shown to eliminate these distortions by symmetric configurations of off-axis parabolic mirror train. The detrimental distortion effects were cancelled out by orienting the final two mirrors in a way to that recovers the original source profile. Comparisons of field profiles between compensated and uncompensated design are shown and imaging performance on characterization targets presented. In addition to benefits in field and polarization distribution the improved design facilitates TD scanning with minimal change to overall optical path length.
Conference 9706: Optical Interactions with Tissue and Cells XXVII

9706-5, Session 1

**Design and analysis of a handheld galvanoscanner for terahertz time-domain spectroscopic imaging in clinical settings** *(Invited Paper)*

M. Hassan Arbab, Univ. of Washington (United States); Stefan Katletz, Research Ctr. for Non Destructive Testing GmbH (Austria); Zac Harris, Univ. of Washington (United States)

Terahertz imaging systems which are routinely built by raster-scanning at the focal point of the stationary imaging optics, are not suitable for clinical applications. We have designed a light-weight and handheld galvanoscanner to be incorporated in our fiber-coupled THz-TDS medical imaging system. To reduce the scanner weight, a gimbal mount is used to deflect the THz beam in two directions with a single mirror. This modification also has the advantage that the mirror and scanning lens form a telecentric optical system if the rotation point of the scanner is placed in the back focal point of the focusing lens. Consequently, the THz beam always stays parallel to the optical axis and optical path length differences and distortions can be avoided during scanning. The THz beam is focused onto the sample of one by the f-θ lens made from PTFE resulting in a linear relationship between the scanning angle and position of the focused THz beam in the focal plane. This concept has been proven to allow scanning of large or bulky objects without the need to translate either the target or the THz system. The scan size and resolution is determined by the diffraction limit. Also the improvement by using a gimbal mount will give constant signal amplitude and a flat scanning plane across the scan area. The maximum scan speed is 7 deg/s, therefore a line scan rate of 0.5 lines/s is achievable. The simulation and test results using tissue phantom studies will be presented.

9706-6, Session 1

**A millimeter-wave reflectometer for whole-body hydration sensing**

Weidong Zhang, Elliott R. Brown, Wright State Univ. (United States)

In recent years skin hydration has become a more important factor in evaluating the human state of health, approaching the stature of a “vital sign” in some circumstances. Through the use of THz sensing, hydration contrast has been the basis for imaging burns and skin carcinomas. And more recently, the remarkable sensitivity of THz reflectivity to hydration levels has been pursued for corneal health assessment. A drawback in most of these applications has been sensor detection or image acquisition time, which is usually measured in minutes. In the present work we have developed and demonstrated a contact hydration sensor that can be manually scanned over large regions of the body. It is based on a simple millimeter-wave reflectometer centered at 94 GHz (W Band) and incorporating a Gunn oscillator, a waveguide ferrite circulator, a Schottky rectifier, and a pyramidal horn having a flexible transparent window. A double amplitude-modulation and synchronous-detection scheme allows for measurements of the skin reflectance with an accuracy of ~2%, a spot size of ~1 cm diameter or less, and a modulation bandwidth of ~1 kHz. Large areas of the torso, arms, and legs can be manually mapped in less than one minute, and accurate calibration performed readily with human-skin surrogates. The system is demonstrated on human subjects and shown to correlate with their innate hydration levels.

9706-7, Session 1

**Surface roughness limited contrast to clutter ratios THz medical imaging**

Shijun Sung, Neha Bajwa, Jacob Goell, Zachary D. Taylor, Univ. of California, Los Angeles (United States)

The THz electromagnetic properties of rough surface are explored and their effect on the observed contrast in THz images is quantified. Rough surface scatter is a major source of clutter in THz imaging as the rough features of skin and other tissues result in non-trivial reflection signal modulation. Traditional approaches to data collection utilize dielectric windows to flatten surfaces for THz imaging. However, there is substantial interest surrounding window free imaging as contact measurements are not ideal for a range of candidate diseases and injuries.

In this work we investigate the variation in reflected signal in the specular direction from rough surfaces targets with known roughness parameters. Signal to clutter ratios are computed and compared with that predicted by Rayleigh Rough surface scattering theory. It is shown that Rayleigh rough surface scattering theory, developed for rough features larger than the interacting wavelength, holds acceptable at THz frequencies with rough features much smaller than the wavelength. Additionally, we present some biological tissue imaging examples to illustrate the impact of rough surface scattering in image quality.

9706-8, Session 1

**Morphological study of human sweat ducts for the investigation of THz-wave interaction** *(Invited Paper)*

Kodo Kawase, Saroj R. Tripathi, Nagoya Univ. (Japan)

Recently, some studies reported that the sweat ducts act as a low-Q-factor helical antenna due to their helical structure, and resonate in the terahertz frequency range according to their structural parameters. According to the antenna theory, when the duct works as a helical antenna, the dimension of the helix plays a key role to determine the frequency of resonance. Therefore, the accurate determination of structural parameters of sweat duct is crucially important to obtain the reliable frequency of resonance and modes of operations. Therefore, here we performed the optical coherence tomography (OCT) of human subjects on their palm and foot to investigate the density, distribution and morphological features of sweat ducts. Moreover, we measured the dielectric properties of stratum corneum using terahertz time domain spectroscopy and based upon this information, we determined the frequency of resonance. We recruited 32 subjects for the measurement and the average duct diameter was 95±11μm. Based upon this information on diameter of duct and THz dielectric properties of stratum corneum (?=5.1±1.3), we have calculated the frequency of resonance of sweat duct. Finally, we determined that the center frequency of resonance was 442±76 GHz. We believe that these findings will facilitate further investigation of the THz-skin interaction and provide guidelines for safety levels with respect to human exposure. We will also report on the EEG measurement while being shined by micro watt order THz waves.

9706-9, Session 1

**In situ monitoring of surgical flap viability using THz imaging**

Neha Bajwa, Shijun Sung, Warren S. Grundfest, Zachary D. Taylor, Ctr. for Advanced Surgical and Interventional Technology (United States)

This paper explores the utility of reflective THz imaging to assess the viability of surgical flaps. Flap surgery is a technique where tissue is
Elevations in tissue histamine levels have been observed during anaphylaxis reflective THz imaging. Histamine is a major contributor to allergic disease.

Grundfest, Ctr. for Advanced Surgical and Interventional Shijun Sung, Neha Bajwa, Warren S. Grundfest, Zachary THz imaging with applications to allergy Visualization of vasodynamics using 9706-11, Session 1 non-contact corneal imaging is feasible and indicate that signal acquired the drying of the tear film. The results suggest that clinically compatible, three times over the course of a few minutes with our novel imaging system. Significant differences in tissue water content were observed between rats over the experimental period. The results suggest that THz imaging may enable early assessment of flap viability.

9706-10, Session 1

Corneal tissue water content mapping with THz imaging: preliminary clinical results
Shijun Sung, Neha Bajwa, Sophie X. Deng, Zachary D. Taylor, Warren S. Grundfest, Univ. of California, Los Angeles (United States)

Well-regulated corneal water content is critical for ocular health and function and can be adversely affected by a number of diseases and injuries. Current clinical practice limits detection of unhealthy corneal water content levels to central corneal thickness measurements performed by ultrasound or optical coherence tomography. Trends revealing increasing or decreasing corneal thickness are fair indicators of corneal water content by individual measurements are highly inaccurate due to the poorly understood relationship between corneal thickness and natural physiologic variation.

Recently the utility of THz imaging to accurately measure corneal water content has been explored on with rabbit models. Preliminary experiments revealed that contact with dielectric windows confounded imaging data and made it nearly impossible to deconvolve thickness variations due to contact from thickness variations due to water content variation. A follow up study with a new optical design allowed the acquisition of rabbit data and the results suggest that the observed, time varying contrast was due entirely to the water dynamics of the cornea.

This paper presents the first ever in vivo images of human cornea. Five volunteers with healthy cornea were recruited and their eyes were imaged three times over the course of a few minutes with our novel imaging system. Noticeable changes in corneal reflectivity were observed and attributed to the drying of the tear film. The results suggest that clinically compatible, non-contact corneal imaging is feasible and indicate that signal acquired from non-contact imaging of the cornea is a complicated coupling of stromal water content and tear film.

9706-11, Session 1

Visualization of vasodynamics using THz imaging with applications to allergy testing
Shijun Sung, Neha Bajwa, Warren S. Grundfest, Zachary Grundfest, Ctr. for Advanced Surgical and Interventional Technology (United States)

This paper explores vasodynamics in response to histamine injection using reflective THz imaging. Histamine is a major contributor to allergic disease. Elevations in tissue histamine levels have been observed during anaphylaxis and experimental allergic responses of the skin, nose, and airways. In the skin specifically, vasodilation, vascular permeability, and pruritus is controlled by the release and resorption of histamine. These properties are leveraged in skin prick testing for allergies where histamine dihydrochloride is injected as a positive control to confirm allergen susceptibility prior to the administration of candidate allergens.

Subjective parameters such as skin coloration, irritation, and bulging as a consequence of histamine injection and histamine release are well characterized. However limited quantitative metrics on the body’s edematous response are available due to the lack of imaging diagnostics that can map surface tissue water content (TWC).

THz imaging was used to explore the utility of reflective THz imaging to quantify edematous responses to histamine. Rat models were injected with varying concentrations of histamine dihydrochloride and the resultant edematous response arising from perturbed vasodynamics was mapped. Significant build up and dissipation of surface tissue water content was observed and THz frequency contrast was seen to correlate with visual appearance in some cases and in others reveal tissue water content variations not discernable with the naked eye. The results suggest that THz imaging may be a valuable tool in quantifying the degree of allergic responses and assist in detecting hypersensitivity.

9706-12, Session 2

Understanding the tissue interaction of new treatment modalities in laparoscopic surgery in view of safe and effective application (Invited Paper)
Matthijs C. M. Grimbergen, John H. Klaessens, Albert J. van der Veen, Rudolf M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands)

During laparoscopic surgery, devices are require to either cut, ablate or coagulate tissue and veins with high precision and controlled lateral damage preferably in an one-for-all modality. The tissue interactions of 3 new treatment modalities were studied using special imaging techniques to obtain a better understanding the working mechanism in view of effective and safe application.

The Plasmajet produces a high temperature ionized gas ‘flame’ directed to the tissue surface at the tip of a 4 mm diameter rigid hand piece. The Lumenis DUO CO2 laser enables endoscopic laser energy delivery through a 1 mm outer diameter flexible hollow waveguide. The 2 μm ‘Thulium’ laser is delivered by (standard) 400 μm diameter optical fiber. Thermal imaging and Schlieren techniques were used to assess the superficial ablative and coagulation effects these surgical instruments scanning at preset velocities and distances from the surface of biological tissues and phantoms. The CO2 was very effective in tissue ablation even at a distance up to 10 mm due to a very small diverging beam from the hollow waveguide. In contrast, the Thulium laser showed less ablation and increasing coagulation at larger distance to the tissue. The gas ‘flame’ of the Plasmajet spread the thermal energy over the surface for effective superficial ablation and coagulation. However, the pressure of the gas flow is substantial on the tissue surface creating turbulence and even indirect cooling.

The specific ablation and coagulation effects of the three treatment modalities have to be appreciate and the effective and safe application will depend on the preference and skills of the surgeon.

9706-13, Session 2

Low-cost 420nm blue laser diode for tissue cutting and hemostasis
Kurt J. Linden, N2 Biomedical (United States)
This paper describes the use of a 420 nm blue laser diode for possible surgery and hemostasis. The optical absorption of blood-containing tissue is strongly determined by the absorption characteristics of blood. Blood is primarily comprised of plasma (yellowish extracellular fluid that is approximately 95% water by volume) and formed elements: red blood cells (RBCs), white blood cells (WBCs) and platelets. The RBCs (hemoglobin) are the most numerous, and due to the spectral absorption characteristics of hemoglobin, the optical absorption of blood has a strong relative maximum value in the 420 nm blue region of the optical spectrum. Small, low-cost laser diodes emitting at 420 nm with tens of watts of continuous wave (CW) optical power are becoming commercially available. Experiments on the use of such laser diodes for tissue cutting with simultaneous hemostasis were carried out and are here described. It was found that 1 mm deep x 1 mm wide cuts can be achieved in red meat at a focused laser power level of 3 W moving at a velocity of ~1 mm/s. The peripheral necrosis and thermal damage zone extended over a width of approximately 0.5 mm adjacent to the cuts. Preliminary hemostasis experiments were carried out with fresh equine blood in Tygon tubing, where it was demonstrated that cauterezation can occur in regions of intentional partial tubing puncture.

9706-14, Session 2

Light-assisted drying (LAD) of small volume biologics: a comparison of two IR light sources

Madison A. Young, Matthew P. Van Vorst, Gloria D. Elliott, Susan R. Trammell, The Univ. of North Carolina at Charlotte (United States)

Biopreservation is the science of achieving suspended animation of cells, tissues and other biologics for storage and later use. Cryopreservation methods followed by storage at cryogenic temperatures is the standard for the preservation of cells used for therapeutics and diagnostic assays. These cryogenic storage strategies can be challenging in many environments due to a lack of available resources. Anhydrous preservation, storage in a dry state, may provide an alternative to cold chain storage for biological samples. We are developing a novel processing method, light-assisted drying (LAD), to dehydrate cells suspended in a sugar (trehalose) solution for storage at supra-zero temperatures. Our technique selectively heats the water in small volume samples using near-IR light to speed dehydration which prevents sugar crystallization that can damage embedded cells. In this study, we compare the end moisture content (EMC) as a function of processing time of samples dried with two different light sources, Nd:YAG (1064 nm) and Thulium fiber (1850 nm) lasers. EMC is the ratio of water to dry weight in a sample and the lower the EMC the higher the possible storage temperature. LAD with the 1064 and 1850 nm lasers yielded 78% and 65% lower EMC, respectively, than standard air-drying. After 40 minutes of LAD with 1064 and 1850 nm sources, EMCs of 0.27±0.27 and 0.15±0.05 gH2O/gDryWeight were reached, which are near the desired value of 0.10 gH2O/gDryWeight that enables storage in a glassy state without refrigeration. LAD is a promising new technique for the preparation of biologics for anhydrous preservation.

9706-15, Session 2

Heating drug delivery to vascular wall with Rhodamine B and fluorescence labeled paclitaxel ranging 50 to 70°C: ex vivo study

Rie Homma, Machiko Shinozuka, Natsumi Shimazaki, Tsunenori Arai, Keio Univ. (Japan)

We studied heating drug delivery to vascular wall with Rhodamine B ranging 50 to 70°C by ex vivo study. Porcine carotid artery was dipped in the heated Rhodamine B solution in 15 s and then transferred to 37°C saline. Rhodamine B concentration distribution in the vascular wall was measured by the microscope with CCD camera using 550 nm excitation and 620 nm emission fluorescence. The total amount of measured fluorescence in the vascular wall over noise level was calculated as an indication of delivered Rhodamine B quantity. Delivered Rhodamine B quantity was increased with increasing heating temperature with 50 to 70°C. In the cases of 60 to 70°C heating, delivered Rhodamine B quantity was 3.1 to 23.3 fold by that of 50°C. Penetration of delivered Rhodamine B into the vascular wall was also significantly increased with the heating. To understand these drug delivery enhancement effects, scanning electron microscope was used to observe the heated vascular surface. We found some small holes less than 10 μm on the internal elastic lamina. We also studied heating drug delivery to the vascular wall with fluorescence labeled paclitaxel with 70°C in 15 & 60 s heating ex vivo. In both contact duration, delivered paclitaxel quantity was increased. We prospected that there might be relationships between collagen heating denaturation and drug delivery to vascular wall.

9706-16, Session 2

Influence of temperature on the myocardial cells death by an extracellular talaporfin sodium-induced photosensitization reaction

Emiyu Ogawa, Hiromi Takenoya, Tsunenori Arai, Keio Univ. (Japan)

We have proposed to apply the photosensitization reaction in myocardium interstitial fluid using talaporfin sodium to realize less-heated electrical conduction block for a tachyarrhythmia treatment. The cytotoxicity of the extracellular photosensitization reaction efficiency may change by the talaporfin sodium binding with serum proteins. These binding would change with solution temperature. We investigated the binding behavior of talaporfin sodium with human serum albumin (HSA), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) changing solution temperature from 17 to 37°C. We also studied the photocytotoxicity change by solution temperature of 17 and 37°C measuring cell survival by WST assay using fetal bovine serum. The binding ratio of talaporfin sodium with HDL and decreased 6.3% and 12.8% with temperature increasing from 17 to 37°C. There was no significant difference in the case of HSA. The cell survival was decreased about 30% with temperature increasing from 17 to 37°C. The myocardium tissue temperature increase was reported that less than 5°C in the case of our proposal catheter interventional ablotion. We think that the photocytotoxicity change by these temperature increasing would be negligible. We suggest that the temperature maintaining would be necessary to keep the photocytotoxicity efficiency in the case of the open surgery that would cause the tissue surface temperature decreasing.

9706-17, Session 2

Development of 2-micron nonlinear frequency conversion laser system and tissue interaction monitoring using optical coherence tomography

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In this study, we report on development of an optical parametric oscillator (OPO) based 2-micron laser system. This laser utilizes a periodically poled nonlinear crystal pumped by a Q-switched diode-pumped Nd:YAG laser with high average power operating at 10 KHz repetition rate. The laser creates 8 Watt average output at injection current of 20A from a quasi-phase-matched OPO using external cavity configuration. The laser tissue ablation efficiency is measured in terms of an optical fluence rate, wavelengths of the laser source and optical properties of target tissue. Tissue ablation volumes are quantified using three-dimensional imaging
Photothermal medical laser treatments are extremely dependent on the medical laser treatments system for controlling photothermal laser angioplasty. A non-contact temperature measurement range is effective for reducing thermal effects and selective ablation of the \( \mu \)m wavelength range. QCLs are relatively new types of semiconductor lasers that can emit mid-infrared range. They are sufficiently compact and considered to be useful for clinical application. However, large thermal effects were observed because conventional QCLs worked as quasi-continuous wave lasers due to their short pulse interval. Then we tried macro pulse irradiation (irradiation of pulses at intervals) of the QCL and achieved effective ablation with less-thermal effects than conventional QCLs worked as quasi-continuous wave lasers. And the ablation efficiency of the 2-micron laser was 0.5-12 ms. As a result, cutting difference was achieved between rabbit normal and atherosclerotic aortas in the oscillation wavelength of the QCL was 0.5 and 12 ms, respectively, because the thermal relaxation time of rabbit normal and atherosclerotic aortas in the oscillation wavelength of the QCL was 0.5-12 ms. As a result, cutting difference was achieved between rabbit atherosclerotic and normal aortas by the macro pulse irradiation. Therefore, macro pulse irradiation of a QCL in the 5.7 \( \mu \)m wavelength range is effective for reducing thermal effects and selective ablation of the atherosclerotic plaque. QCLs have the potential of realizing less-invasive laser angioplasty.

Selective ablation of rabbit atherosclerotic plaque with less thermal effect by the control of pulse structure of a quantum cascade laser in the 5.7 \( \mu \)m wavelength range

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Cholesteryl esters are main components of atherosclerotic plaques and have an absorption peak at the wavelength of 5.75 \( \mu \)m originated from C=O stretching vibration mode of ester bond. Our group achieved the selective ablation of atherosclerotic lesions using a quantum cascade laser (QCL) in the 5.7 \( \mu \)m wavelength range. QCLs are relatively new types of semiconductor lasers that can emit mid-infrared range. They are sufficiently compact and considered to be useful for clinical application. However, large thermal effects were observed because conventional QCLs worked as quasi-continuous wave lasers due to their short pulse interval. Then we tried macro pulse irradiation (irradiation of pulses at intervals) of the QCL and achieved effective ablation with less-thermal effects than conventional QCLs worked as quasi-continuous wave lasers. However, lesion selectivity might be changed by changing pulse structure. Therefore, in this study, irradiation effects of the macro pulse irradiation to rabbit atherosclerotic plaque and normal vessel were compared. The macro pulse width and the macro pulse interval were set to 0.5 and 12 ms, respectively, because the thermal relaxation time of rabbit normal and atherosclerotic aortas in the oscillation wavelength of the QCL was 0.5-12 ms. As a result, cutting difference was achieved between rabbit atherosclerotic and normal aortas by the macro pulse irradiation. Therefore, macro pulse irradiation of a QCL in the 5.7 \( \mu \)m wavelength range is effective for reducing thermal effects and selective ablation of the atherosclerotic plaque. QCLs have the potential of realizing less-invasive laser angioplasty.

A new analytical approach for heat generation in tissue due to laser excitation

Hakan Erkol, Farouk Nouizi, Alex T. Luk, Univ. of California, Irvine (United States); Mehmet B. Unlu, Bogaziçi Univ. (Turkey); Gultekin Gulsen, Univ. of California, Irvine (United States)

In this study, we present a fast analytical approach for laser induced temperature increase in biological tissue. The whole problem consists of two main steps. These steps are the light propagation and heat transfer in tissue. We first obtain a detailed analytical solution for the diffusion equation based on an integral approach for specific boundary conditions. Secondly, we also solve the Pennes' bio-heat transfer equation analytically using the separation of variables technique and obtain the temperature induced by optical absorption of tissue. Here, heat source term consists of the local absorption and photon density, which will be determined from the diffusion equation. We find a very comprehensive solution for the diffusion equation by using an integral method for the Robin boundary condition. In other words, we obtain a particular Green's function in a different way. Next, we use this solution as a source term in the Pennes' bio-heat equation by utilizing the heat convection boundary condition. It is important to note that these boundary conditions are good approximations for imaging of biological tissue. As a result, we obtain spatio-temporal temperature distribution inside the medium. First, our approach is validated by a numerical approach using a Finite Element Method (FEM). Next, we also validate our method by performing phantom and tissue experiments. Experimental data corresponding to spatio-temporal temperature distribution are recorded using magnetic resonance thermometry. The analytical results obtained by our method are in a very good agreement with ones obtained by the FEM and experiment.

A non-contact temperature measurement system for controlling photothermal medical laser treatments

Özgür Kaya, Murat Gülsoy, Bogaziçi Univ. (Turkey)

Photothermal medical laser treatments are extremely dependent on the generated tissue temperature. It is necessary to reach a certain temperature threshold to achieve successful results, whereas preventing to exceed an upper temperature value is required to avoid thermal damage. One method to overcome this problem is to use previously conducted dosimetry studies as a reference. Nevertheless, these results are acquired in controlled environments using uniform subjects. In the clinical environment, the optical and thermal characteristics (tissue color, composition and hydration level) vary dramatically among different patients. Therefore, the most reliable solution is to use a closed-loop feedback system that monitors the target tissue temperature to control laser exposure. In this study, we present a compact, non-contact temperature measurement system for the control of photothermal medical laser applications that is cost-efficient and simple to use. The temperature measurement is achieved using a focused, commercially available MOEMS infrared thermocouple sensor embedded in an off-axis arrangement on the laser beam delivery hand probe. The spot size of the temperature sensor is ca. 2.5 mm, reasonably smaller than the laser spot sizes used in photothermal medical laser applications. The temperature readout and laser control is realized using a microcontroller for fast operation. The utilization of the developed system may enable the adaptation of several medical laser treatments that are currently conducted only in controlled laboratory environments into the clinic. Laser tissue welding and cartilage reshaping are two of the techniques that are limited to laboratory research at the moment. This system will also ensure the safety and success of laser treatments aiming hyperthermia, coagulation and ablation, as well as LLLT and PDT.

Monitoring gold nanoparticle distribution in vivo with high resolution using photomagnetic imaging

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Gold nanoparticles have tunable and exceptionally strong optical absorption due to their plasma resonance. They are proved to be promising multi-functional platform because they can be used for many imaging and therapeutic functions. As a true multi-modality imaging technique, Photomagnetic Imaging (PMI) has a great potential to monitor the distribution of the gold nanoparticles non-invasively with MR resolution. With a simple add-on of a continuous wave laser to an existing MRI system, PMI converts the laser induced temperature increase measured by MR thermometry into tissue optical absorption map utilizing Finite Element Model (FEM) based numerical approach. FEM simulation models the photon distribution in the tissue and heat generation due to the absorption of the light and consequent heat diffusion. By using 3D MR temperature measurements, a dedicated PMI reconstruction algorithm has been developed to recover quantitative high resolution absorption maps superior compared to conventional diffuse optical tomography approaches. The key characteristic of the PMI is that as long as the temperature change is detectable by MR thermometry, the resolution is preserved at any depth. To make PMI suitable for diagnostic purposes, the laser powers has been kept under the American National Standard Institute maximum skin exposure limits in this study. Agar phantoms mimicking the small animal tissue has been used to validate the PMI technique. Current PMI prototype uses a single wavelength. We have been expanding this prototype to a multi-wavelength PMI system to quantitatively image gold nanoparticle distribution in tissue simulating phantoms and living animals bearing tumor models.

9706-22, Session 4
Femtosecond laser subsurface scleral treatment in cadaver human sclera and evaluation using two-photon and confocal microscopy
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Purpose: Glaucoma is the second-leading cause of blindness worldwide and is often associated with elevated intraocular pressure (IOP). Partial-thickness drainage channels can be created with femtosecond laser in the translucent sclera for the potential treatment of glaucoma. In this pilot study we demonstrate the creation of partial-thickness subsurface drainage channels with the femtosecond laser in the cadaver human eyeballs and describe the application of two-photon microscopy and confocal microscopy for noninvasive imaging of the femtosecond laser created partial-thickness scleral channels in cadaver human eyes.

Methods: A femtosecond laser operating at a wavelength of 1700 nm was scanned along a rectangular raster pattern to create the partial thickness subsurface drainage channels. IOP was measured before and 20 minutes after the laser treatment to evaluate the acute effect of the procedure. Analysis of the dimensions and location of these channels is important in understanding their effects. We describe the application of two-photon microscopy and confocal microscopy for noninvasive imaging of the femtosecond laser created partial-thickness scleral channels in cadaver human eyes.

Results: High-resolution images, hundreds of microns deep in the sclera, were obtained to allow determination of the shape and dimension of such partial thickness subsurface scleral channels. Comparison of pre- and postoperative IOP measurements in treated and control eyes revealed a reduction in the intraocular pressure due to the increased rate of aqueous humor (AH) outflow resulted in by the presence of the partial thickness scleral channels.

Conclusions: The two-photon and confocal microscopy can be used to investigate femtosecond laser created partial-thickness drainage channels in the sclera of cadaver human eyes. Our studies suggest that reduction in IOP achieved by the partial thickness channels which created by femtosecond laser suggests potential utility in the treatment of glaucoma.

9706-23, Session 4
Laser assisted bioprinting using a femtosecond laser with and without a gold transductive layer: a parametric study
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We are investigating whether a femtosecond laser pulse could be used for Laser-Assisted Bioprinting (LAB) with and without an absorptive layer. An industrial LAB workstation, was used to print, with a gold layer, different model viscous solutions (cell culture medium, a solution of water + glycerol, a solution of alginate + water + glycerol) and keratinocytes cells line HaCaT onto a plastic culture well plate. A test bench with a more powerful laser was used for LAB, without gold, to print similar model viscous solutions. Firstly, we measured printed diameter of different bioinks at 1 mm printing distance in function of fluence, using an absorptive layer of gold. 3D measurements by printing condition were performed. We also observed by shadowgraphy the laser-induced jet evolution in function of the same fluences and bioinks than the primary study and in function of laser distance defocalization.

Secondly, we identified the maximum high of the jet by shadowgraphy, in function of bioink viscosity, energy, focalization diameter and defocalization distance for LAB without an absorptive layer for 10 measurements by condition. We also measured printed diameters for 10 printing conditions. Solutions viscosities ranged between 1.3 to 146 mPa.s and pulse energies between 2 µJ to 3.5 µJ with gold and between 2 µJ to 22.5 µJ without gold. Also, we observed a good reproducibility of the process and the most adequate energy for cell printing was 3.5 µJ. Cell viability was maintained 24 h after printing according to Live/dead assays.

9706-24, Session 4
Precision resection of intestine using ultrashort laser pulses
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The surgical treatment of early stage colorectal cancer is typically based on diathermy and electrocautery tools. They imply restraints on precision and the risk of collateral thermal damage to the healthy tissue. We present the resection of intestine by means of picosecond laser pulses as a potential alternative to mitigate these limitations. High intensity ultrashort laser pulses enable nonlinear absorption processes, plasma generation, and as a consequence a predominantly non-thermal ablation regime. Robust process parameters for the laser resection are demonstrated using fresh in-vivo pig intestine samples. Square and circular cavities with comparable thickness to early stage colorectal cancers are removed for a wavelength of 1030 nm and 515 nm using a picosecond laser system. The corresponding histology sections exhibit in both cases only minimal collateral damage to the surrounding tissue. The resection depth can be controlled precisely by means of the pulse energy. Furthermore, by operating the laser at
The presented study introduces a novel technique for high-speed optical photography of cavitation bubble dynamics inside inhomogeneous media. In this case, the single effect is highly reproducible and, hence, the method of time-resolved photography is sufficiently applicable. In contrast, the reproducibility significantly decreases analyzing more solid and anisotropic media like biological tissue. Therefore, a high-speed photographic approach is necessary in this case. The presented study introduces a novel technique for high-speed photographic analysis of femtosecond laser-induced photodisruption, which is used for dissection of biological tissue with femtosecond laser pulses. In this study, we investigate the interaction between fiber-guided nanosecond pulses at 355 nm and blood, and fluoroscopy contrast media. The interaction between the radiation and the latter trickles pressure shockwaves, which may cause serious angiographic and clinical complications. Several methods are used to mitigate these effects (e.g., “flush-and-bathe” technique). However, drawbacks such as renal complications due to accumulation of contrast media, and the inability to use the contrast media in real-time imaging during the ablation, reduce the technique’s attractiveness. We propose that a third-harmonic Nd:YAG laser (355 nm wavelength) can be used as an alternative laser source. In previous studies, we investigated the delivery of nanosecond pulses at 355 nm through silica optical fibers, and succeeded in transmitting high fluence, sufficient for ablation of biological tissue. In this study, we investigate the interaction between fiber-guided nanosecond pulses at 355 nm and blood, saline, and fluoroscopy contrast media. We demonstrate that with this wavelength we can obtain reduced pressure shockwaves in contrast media, as opposed to the excimer laser. We further investigate ex-vivo disruption effects in porcine aorta. Numerical simulations based on the finite-elements method support the experimental findings. Our work indicates a feasibility of using a solid-state laser at 355 nm in percutaneous vascular laser interventions that include fluoroscopy contrast media.

9706-29, Session 5

A route to laser angioplasty in the presence of fluoroscopy contrast media using a nanosecond-pulsed 355nm laser

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Laser angioplasty is one of the foremost common techniques in the treatment of peripheral atrial disease, and includes a nanosecond pulsed Xenon monocloride excimer laser source. During the procedure, the laser radiation (at 308 nm wavelength) is guided to the occluded site through optical fibers, and encounters fluid-phase media such as saline, blood, and fluoroscopy contrast media. The interaction between the radiation and the latter two initiates pressure shockwaves, which may cause serious angiographic and clinical complications. Several methods are used to mitigate these effects (e.g., “flush-and-bathe” technique). However, drawbacks such as renal complications due to accumulation of contrast media, and the inability to use the contrast media in real-time imaging during the ablation, reduce the technique’s attractiveness. We propose that a third-harmonic Nd:YAG laser (355 nm wavelength) can be used as an alternative laser source. In previous studies, we investigated the delivery of nanosecond pulses at 355 nm through silica optical fibers, and succeeded in transmitting high fluence, sufficient for ablation of biological tissue. In this study, we investigate the interaction between fiber-guided nanosecond pulses at 355 nm and blood, saline, and fluoroscopy contrast media. We demonstrate that with this wavelength we can obtain reduced pressure shockwaves in contrast media, as opposed to the excimer laser. We further investigate ex-vivo disruption effects in porcine aorta. Numerical simulations based on the finite-elements method support the experimental findings. Our work indicates a feasibility of using a solid-state laser at 355 nm in percutaneous vascular laser interventions that include fluoroscopy contrast media.
related infection, or in opening venous occlusions that prevent the insertion of additional lead. In severe cases of fibrous encapsulation of the lead within a vein, laser-based cardiac LE has become one of the foremost methods of removal. In cases where the laser radiation (typically at 308 nm wavelength) interacts with the vein wall rather than with the fibrotic lesion, severe injury and subsequent bleeding may occur. Selective tissue ablation was previously demonstrated by a laser operating in the UV regime; however, it requires the use of sensitizers (e.g.: tetracycline). In this study, we present a preliminary examination of efficacy and safety aspects in the use of a nanosecond-pulsed solid-state laser radiation, at 355 nm wavelength, guided in a catheter consisting of optical fibers, in LE. Specifically, we demonstrate a correlation between the tissue elasticity and the catheter advancement rate, in ex-vivo experiments. Our results indicate a selectivity property for specific parameters of the laser radiation and catheter design. The selectivity is attributed to differences in the mechanical properties of the fibrotic tissue and a normal vein wall, leading to a different photomechanical response of the tissue's extracellular matrix. Furthermore, we performed successful in-vivo animal trials, providing a basic proof of concept for using the suggested scheme in LE. Selective operation using a 355 nm laser may reduce the risk of blood vessel perforation as well as the incidence of major adverse events.

9706-29, Session 5

Laser dosimetry for disabling anopheles stephensi mosquitoes in-flight


The Photonic Fence is a system designed to detect mosquitoes and other pestilent flying insects in an active region and to apply lethal doses of laser light to them. Previously, we determined lethal fluence levels for a variety of lasers and pulse conditions on anesthetized Anopheles stephensi mosquitoes. In this work, similar studies were performed while the bugs were freely flying within transparent cages. Dose-response curves were created for various beam diameter, pulse width, and power conditions at 455 nm, 532 nm, 1064nm, and 1540 nm wavelengths. Besides mortality outcomes, the flight behavior of the bugs and the performance of the tracking system were monitored for consistency and to ensure that they had no impact on the mortality outcomes. As in anesthetized experiments, the visible wavelengths required significantly less fluence than near infrared wavelengths to reliably disable bugs. For the visible wavelengths, lethal fluence values were generally equivalent to those found in anesthetized dosing, while near infrared wavelengths required approximately twice the fluence compared with anesthetized experiments. The performance of the optical tracking system remained highly stable throughout the experiments, and it was found not to influence mortality results for pulse widths up to 25 ms. In general, keeping energy constant while decreasing power and increasing pulse width reduced mortality levels. The results of this study further affirm the practicality of using optical approaches to protect people and crops from flying insects.

9706-30, Session 5

Exposure to nanosecond duration electrical pulses can induce electrodeformation

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Electrical pulses can induce membrane disruption and crops from flying insects.

9706-31, Session 5

High frequency application of nanosecond pulsed electric fields alters cellular membrane disruption and fluorescent dye uptake

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Cells exposed to nanosecond-pulsed electric fields (nSPEF) exhibit a wide variety of nonspecific effects, including blebbing, swelling, intracellular calcium bursts, apoptotic and necrotic cell death, formation of nanoparticles, and depletion of phosphatidylinositol 4,5-bisphosphate (PIP2) to induce activation of the inositol trisphosphate/diacylglycerol pathway. While several studies have taken place in which multiple pulses were delivered to cells, the effect of pulse repetition rate (PDR) is not well understood. To better understand the effects of PDR, a laser scanning confocal microscope was used to observe CHO-K1 cells exposed to ten 600ns, 200V pulses at varying repetition rates (5Hz up to 500KHz) in the presence of either FM 1-43, YO-PRO-1, or Propidium Iodide (PI) fluorescent dyes, probes frequently used to indicate nanoporation or permeabilization of the plasma membrane. Dye uptake was monitored for 30 seconds after pulse application at a rate of 1 image/second. In addition, a single long pulse of equivalent energy (200V, 6 us duration) was applied to test the hypothesis that very fast PDR will approximate the biological effects of a single long pulse of equal energy. Upon examination of the data, we found strong variation in the relationship between PDR and uptake in each of the three dyes. In particular, PI uptake showed little frequency dependence, FM 1-43 showed a strong inverse relationship between frequency and internal cell fluorescence, and YO-PRO-1 exhibited a “threshold” point of around 50 KHz, after which the inverse trend observed in FM 1-43 was seen to reverse itself. Further, a very high PDR of 500 KHz only approximated the biological effects of a single 6 us pulse in cells stained with YO-PRO-1, suggesting that uptake of different dyes may proceed by different physical mechanisms.

9706-57, Session PMon

Multi-channel photon migration study in visible Chinese human muscle for optical detection of deep vein thrombosis

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Deep vein thrombosis (DVT) always induced venous thrombosis. Most cases of venous thrombosis were induced by deep vein thrombosis (DVT), with high incidence rate of >60% in >60 years old people. Near-
infrared spectroscopy (NIRS) were reported recently to be an intriguing and potential technique in detecting DVT in clinics. However, the photon transport is still unclear, which is crucial for the image reconstruction of the updated development called as NIRS-based DVT imager. Here we employed the Monte Carlo simulation software for 3D voxelized media (MCVM) and the Visible Chinese Humen (VCH) model, which segmentation is finest in the world, to simulate multi-channel photon migration in calf muscle. And the image reconstruction of DVT hemodynamic distribution was achieved. This study, for the first time, provides the most realistic 3-D multichannel photon migration for NIRS study on DVT, and explored the image reconstruction for furtherly developing a NIRS-based DVT imager.

9706-58, Session PMon

Biophysical mechanism of transient retinal phototropism in rod photoreceptors

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Oblique light stimulation evoked transient retinal phototropism (TRP) has been recently detected in mouse and frog retinas. High resolution microscopy of freshly isolated retinas indicated that the TRP is predominantly by rod photoreceptors. Comparative confocal microscopy and optical coherence tomography (OCT) revealed that the TRP predominantly occurred from the photoreceptor outer segment (OS). However, biophysical mechanism of rod OS dynamics is still unknown. In this study, frog retinal slices, which open a cross section of retinal photoreceptor and other functional layers, are used to test the effect of light stimulation on rod OS. Near infrared light microscopy was employed to monitor photoreceptor changes in retinal slices stimulated by a rectangular-shaped visible light flash. Rapid rod OS length change was observed after the stimulation delivery. The magnitude and direction of the rod OS change varied with the position of the rods within the stimulated area. In the center of stimulated region the length of the retinal rods shrank, while in the peripheral region the rods swung towards center region in the plane perpendicular to the incident stimulus light. Our experimental result and theoretical analysis suggest that the observed TRP may reflect unbalanced disc-shape change due to localized pigment bleaching. Further investigation is required to understand biochemical mechanism of the observed rod OS kinetics. Better study of the TRP may provide a noninvasive biomarker to enable early detection of age-related macular degeneration (AMD) and other diseases that are known to produce retinal photoreceptor dysfunctions.

9706-60, Session PMon

Simulation of the dependence of spatial fluence profiles on tissue optical properties

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Medical laser applications are promoted as safe, effective treatments for a multiplicity of concerns, ranging from hyperthermal skin rejuvenation to subcutaneous tumor ablation. Chromophore and structural protein concentration and distribution within a patient’s tissue vary from patient to patient and dictate the interaction of incident radiative energy of a specific wavelength with the target tissue. Laser parameters must be matched to tissue optical and thermal properties in order to achieve the desired therapeutic results without inducing unnecessary tissue damage, although accurate tissue optical properties are not always measured prior to and during laser therapies. A weighted variable step size Monte Carlo simulation of laser irradiation of skin tissue was used to determine the effects of variations in absorption (\(\alpha\)) and scattering coefficients (\(\beta\)) and the degree of anisotropy (\(g\)) on the radiant energy transport per mm² in response to steady-state photon propagation. The three parameters were varied in a factorial experimental design for the ranges of 0.25/mm ≤ \(\alpha\) ≤ 2.0/mm, 30.0/mm ≤ \(\beta\) ≤ 140.0/mm, and 0.65 ≤ \(g\) ≤ 0.99 in order to isolate their impacts on the overall fluence distribution. Kernel density maps of the resulting fluence profiles were created and compared to identify ranges in which optical property variance could be considered to significantly impact the spatial variance of fluence within the simulation volume. Results indicated that accurate prediction of the fluence profiles that will be achieved by any given medical laser treatment is unlikely without pre-treatment assessment of the tissue optical properties of individual patients.

9706-59, Session PMon

Effects of snap freezing and formalin fixation on tissues during multimodal spectroscopic measurements

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Fiber based optical spectroscopy based on fluorescence, reflectance and Raman spectroscopies have been for tumor diagnosis in different types of tissues. For this type of spectroscopic measurements in-vivo or surgically excised in-vitro samples are preferred. In some cases, tissues snap frozen in liquid nitrogen are later thawed to room temperature and samples fixed in formalin are used for spectral investigations. This type tissue handling procedures could introduce distortions in the spectral intensity and line shape during spectroscopic measurements. Here, we report the effects of tissue handling procedures such as snap freezing and formalin fixation on rat muscle tissue during fluorescence at 378, 445 nm excitation and Raman spectroscopic measurements in comparison with freshly excised tissues. In the case of fluorescence at both the excitation wavelengths, a change in the spectral intensity was observed for wavelengths greater than 520 nm. The Raman spectral profile between freshly excised, snap frozen and formalin fixed rat muscle samples revealed a change in the spectral intensity at 440, 542, 663, 877, 1017 and 1491 cm⁻¹. Our results suggest a transformation in the spectral profile was observed in the wavenumber region 1200 to 1400 cm⁻¹. Furthermore, the temporal effects of freezing and thawing on multimodal spectroscopic measurements were studied and other biological endpoint insights due to snap freezing and formalin fixation will be presented.

9706-61, Session PMon

Laser photoactivation gibberellin molecules in the surface tissues of plants

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Using lasers (405 - 808 nm) has a positive effect on the content of pigments in leaves, increases the activity of catalase and peroxidase. In addition, reduced is afflicted plant pathogenic flora. The reason for increasing the yield of gymnosperms as a result of activation of the seed laser (compared to unexposed plants from seed) is higher germination and early seedling emergence, the best foliage, a higher rate of photosynthesis, accelerated development and maturation. Revealed the wavelength of the laser to ensure the best seed germination. This is due to the formation of an
enzyme that breaks down endospermy layer mechanically restricts the
growth of the embryo. Effects of laser cure to form linked growth promoter -
gibberellin, which activates germination. The irradiation of seeds with
specified energy leads to an increase in the rate of absorption of water by
changing the permeability of cell membranes.

9706-63, Session PMon

Determination of the effects of terahertz radiation on mitochondrial activity

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Due to increased use of terahertz (THz)-based technologies, more investigations are required to determine the biological effects associated with THz radiation. Reports have indicated that THz radiation can modulate membrane permeability and can cause a change in membrane potential in cells. However, it remains unknown if THz frequencies can influence the permeabilization and polarization/dipolarization of intracellular mitochondria. In this study, we examined how THz radiation at selected frequencies can affect mitochondrial activity. We initially examined the effect of THz irradiation on adenosine triphosphate (ATP) formation in mitochondria of exposed versus sham (unexposed) cells using an intracellular ATP concentration colorimetric assay. We next utilized a high-throughput oxygen and pH sensing system (Seahorse® XF24 extracellular flux analyzer) to assess the changes in mitochondrial bioenergetics. To monitor changes in mitochondrial membrane potential post THz irradiation, we stained the exposed and sham cells with fluorescent dyes and then compared changes in the accumulation of the dyes using optical microscopy and flow cytometry.

9706-64, Session PMon

Impact of terahertz frequencies on microtubule polymerization and dynamics

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Microtubules (MTs) are integral to the infrastructure and self-organization processes of eukaryotic cells. Polymerization is a stochastic polar process that leads to MT formation. The polar nature of alpha/beta tubulin heterodimers dictates overall polarity of MTs. MTs are believed to oscillate at unique natural frequencies spanning over the megahertz (MHz) to terahertz (THz) frequency spectrum and are capable of generating electromagnetic fields to influence morphogenesis and regional placement and behaviors of key biochemical pathways. In this study, we aimed to elucidate effects of deposition of external electromagnetic radiation at THz frequencies on MT structure focusing on three-dimensional protein molecular arrangement and dynamics, since THz waves have energies resonant with these biomolecular polymeric protein vibrations. We hypothesized that an exogenous stimulus, exhibiting a resonance within the vibrational frequency spectrum of tubulin’s subunits or its protofilaments could plausibly induce protein conformational change, polymer morphogenetic perturbation, and cellular function changes by modulating the MT lattice. To explore the THz effects on MT mechanics in vitro, a series of irradiations were executed at different stages in tubulin’s assembly to MTs. Cell studies using a mammalian cell line, expressing fluorescent tubulin, were also conducted to determine whether similar MT perturbations were conserved. Observations and quantification involved an ensemble of techniques including atomic force microscopy (AFM), dynamic light scattering (DLS) and confocal microscopy.

9706-65, Session PMon

Sensor structure concepts for the analysis or local radiation exposure of biological samples at Terahertz and Millimeter Wave Frequencies

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We have studied several sensor concepts for biomedical applications operating in the millimetre-wave and Terahertz range. On one hand, rectangular waveguide structure were designed and extended with microfluidic channels. In this way a simple analysis of aqueous solutions at various waveguide bands is possible. In our case, we focused on the frequency range between 75GHz and 110 GHz. On the other hand, planar sensor structures for aqueous solutions have been developed based on coplanar waveguides. With these planar sensors it is possible to concentrate the interaction volume on small sensor areas, which achieve a local exposure of the radiation to the sample. When equipping the sensor with microfluidic structures the sample volume could be reduced significantly and enabled a localized interaction with the sensor areas. The sensors are designed to exhibit a broadband behaviour up to 300 GHz. Narrow-band operation can also be achieved for potentially increased sensitivity by using resonant structures. Several tests with Glucose solved in water show promising results for the distinction of different glucose levels at millimeter wave frequencies. The planar structures can also be used for the exposure of biological cells or cell model systems like liposomes with electromagnetic radiation. Several studies are planned to distinguish on one hand the influence of millimeter wave exposure on biological systems and also to have a spectroscopic method which enables the analysis of cell processes, like membrane transport processes, with millimeter wave and terahertz frequencies by focussing the electric field directly on the analysing sample.

9706-66, Session PMon

Time of flight estimation for breast cancer margin thickness using embedded tumors

Tyler Bowman, Magda El-Shenawee, Univ. of Arkansas (United States); Lucas Campbell, Northwest Arkansas Pathology Associates, P.A. (United States)

This work aims to enact a quick and reasonable estimation of breast cancer margin thickness using time of flight analysis of embedded breast cancer tissue. The terahertz range has recently become a research area of great interest due to the creation of reliable terahertz sources. One application where terahertz imaging has been shown to be effective is in biomedical imaging of malignancies. In particular, terahertz signals have demonstrated the potential to differentiate between cancerous and non-cancerous tissue. Terahertz also has the unique distinction of having reasonable interaction into biological tissues while maintaining a usable depth resolution. This work makes use of a pulsed terahertz system to obtain reflection imaging scans from breast cancer tumors that are formalin-fixed and embedded in paraffin blocks. Based on the reflection imaging peaks obtained in this scan, time of flight analysis is applied in order to determine the signal path and the depth at which multiple reflections in the time domain signal occurred. The reflection patterns seen within the block are then compared to pathology sections and paraffin-embedded sections that are taken throughout the depth of the tumor block in order to determine how accurately the three-dimensional boundaries of the tumor can be estimated. Preliminary results thus far show that this estimate can be highly effective in determining tumor boundaries in the three-dimensional block. The time of flight analysis developed in this work to quickly obtain this depth estimate can then be applied to margin assessment of fresh tissue.
Regional spectroscopy of paraffin-embedded breast cancer tissue using pulsed terahertz transmission imaging

Tyler Bowman, Magda El-Shenawee, Univ. of Arkansas (United States); Lucas Campbell, Northwest Arkansas Pathology Associates, P.A. (United States)

The objective of this work is to establish a terahertz frequency characterization methodology for embedded breast cancer tumor tissue. The terahertz frequency range has recently emerged as an area of great research focus with one specific interest in biomedical imaging. In particular, it has shown great promise in the detection of malignant tumors. However, there is relatively little data on the electrical properties of different tissue types at terahertz frequencies. There are also problems in obtaining large enough samples of individual tissue types to be able to do transmission-based characterization of tissue. To address these concerns, this work seeks to obtain the properties of paraffin-embedded breast cancer tumors using transmission imaging and spectroscopy. Formalin-fixed and paraffin-embedded tumors are first sectioned into slices of 10 µm and 30 µm and placed between two tsurupica slides. The slides are then scanned in a pulsed terahertz system using transmission imaging. From the transmission scan it is possible to make a spectroscopy calculation at every point. In addition to the embedded tissue slices, 5 µm slices of tissue are taken and processed using standard hematoxylin and eosin staining in order to determine the regions of cancerous, fibroglandular, and fatty tissue present in the embedded tumor. The regions in the pathology section can then be compared to the transmission imaging scan in order to define a region of points over which to average the spectroscopy results from the scan.

Analytical terahertz spectroscopy and imaging of pharmaceutical crystals and cocrystals

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Terahertz (THz) spectroscopy is a powerful technique for distinguishing molecular interactions even in small pharmaceutical crystals, which have similar molecular compositions but differing crystal structures. Physicochemical properties, such as rate of dissolution and bioavailability, are manipulated by altering the crystal structure of an active pharmaceutical ingredient (API) through methods such as cosublimation. Here, analyses of pharmaceutical crystals and cocrystals using THz spectroscopy and imaging are presented. The cocrystal distribution in cellulose tablets was quantitatively determined. THz spectroscopy enables quantitative chemical imaging of APIs and offers a new analytical tool for researchers and developers in the biomedical field.

References

Investigation of superparamagnetic (Fe3O4) nanoparticle and magnetic field exposures on CHO-K1 cell line

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Rapid development in nanomaterial synthesis and functionalization has led to advanced studies in actuation and manipulation of cellular functions for biomedical applications. Often these actuation techniques employ externally applied magnetic fields to manipulate magnetic nanomaterials inside cell bodies in order to drive or trigger desired effects. While cellular interactions with low-frequency magnetic fields and nanoparticles have been extensively studied, the fundamental mechanisms behind these interactions remain poorly understood. Additionally, modern investigations on these concurrent exposure conditions have been limited in scope, and difficult to reproduce. This study presents an easily reproducible method of investigating the biological impact of concurrent magnetic field and nanoparticle exposure conditions using an in-vitro CHO-K1 cell line model, with the purpose of establishing grounds for in-depth fundamental studies of the mechanisms driving cellular-level interactions. Cells were cultured under various nanoparticle and magnetic field exposure conditions from 0 to 500 79/g/ml nanoparticle concentrations, and DC, 50 Hz, or 100 Hz magnetic fields with 2.0 mT flux density. Cells were then observed by confocal fluorescence microscopy, and subject to biological assays to determine the effects of concurrent extreme-low frequency magnetic field and nanoparticle exposures on cell-nanoparticle interactions, such as particle uptake and cell viability by MTT assay. Current results indicate little to no variation in effect on cell cultures based on magnetic field parameters alone; however, it is clear that deleterious synergistic effects of concurrent exposure conditions exist, based on a significant decrease in cell viability when exposed to high concentrations of nanoparticles and concurrent magnetic field.

A Monte Carlo simulator of PS-OCT local birefringence imaging

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Polarization-sensitive optical coherence tomography (PS-OCT) allows imaging of tissue birefringence. In practice, however, PS-OCT images are often confounded by high noise and confusing artifacts. A full understanding of the intrinsic and instrumentation-derived signal and noise properties of PS-OCT has not been developed. In this presentation, we describe a Monte Carlo (MC) simulator of PS-OCT local birefringence imaging that recapitulates the noise and signal properties observed in empirical images and, as such, can be used to understand and improve PS-OCT methods. The MC simulator builds upon a previously described MC methodology that supports arbitrary three-dimensional geometries. To this, we have added support for MC simulation of transverse speckle correlation. This is important because many of the noise sources in PS-OCT are driven by interactions with the speckle field. We have developed a method to support polarization-dependent measurements of birefringent tissues. Both additive (due to finite SNR) and polarization-mode dispersion noise can be incorporated. To demonstrate the utility of the simulator, we use it...
to reveal a previously unappreciated noise that results from the design of conventional local birefringence extraction algorithms, and we describe an improved method that lowers noise in both simulated and empirical datasets. We anticipate that this simulator will enable new explorations into the fidelity of PS-OCT measurements and accelerate the optimization of PS-OCT methods and algorithms.

9706-33, Session 6
Laser dosimetry planning tool for colonoscopic tumor resection
Maria Luisa Pelayo-Fernández, Félix Fanjul-Vélez, Irene Salas-García, Mihail Zverev, José Luis Arce-Diego, Univ. de Cantabria (Spain)

Gastrointestinal tumoral pathologies are quite common nowadays. Diseases such as gastric antral vascular ectasia (GAVE) or actinic proctitis may require endoscopic surgery. Argen Plasma Coagulated (APC) or radiofrequency are usually employed. However, they present disadvantages, such as the reduced treated area, magnetic resonance incompatibility, or an uncontrolled ablation depth.

Optical surgery could avoid these problems and contribute to a better and controlled treatment result, either ablative or coagulative, in a minimally invasive, non-contact and non-ionizing way. The treatment area could also be increased by adequate optical fiber probe design. In this work laser surgery is analyzed for resection of colonic tumors. A Monte Carlo model is employed to study optical propagation, and an optical ablation approach allows the estimation of the resected volume. The ablation approach is based on plasma-induced ablation, particularly taking into account the free-electron density generated in the tissue by the pulsed optical source. Several wavelengths, radiated malignant tissue types are considered, either healthy, adenomatous or even coagulated tissues. Optimum source parameters as a function of tumor geometry can be estimated for treatment planning.

9706-34, Session 6
Influence of the scattering phase function in numerical modelling of hyperspectral imaging
Matija Milani?, Univ. of Ljubljana (Slovenia) and Jo?ef Stefan Institute (Slovenia) and Norwegian Univ. of Science and Technology (Slovenia); Boris Majaron, Jo?ef Stefan Institute (Slovenia)

In simulations of a light transport in biological tissues and organs, knowledge of tissue optical properties is imperative for realism of the predicted effects. One factor, which is commonly overlooked is the choice of appropriate scattering phase function (PF). The Heneyy-Greenstein (H-P) PF is often implemented in Monte Carlo models, due to its suitability for analytical derivations and availability of the corresponding tissue anisotropy factors. At the same time, however, it is known that the H-G PF doesn’t match the angular distribution of scattering in many tissues. Recent studies demonstrated that the choice of PF can dramatically affect the predicted diffuse reflectance spectra (DRS) at short source-detector separations.

In here, we study the influence of the PF in 3D Monte Carlo (MC) simulations of hyperspectral imaging (HSI). For a simple geometrical (three-layered) model of skin with melanin volume fractions of 1-10% and discrete blood vessels at various depths, (0-500, 500-1000, 1000-1500) nm spectral range were simulated using the H-G, modified H-G, and Mie PF, respectively. The results are compared in the spatial and spectral domains. In addition, the effective tissue properties as determined from the simulated HSI using 1D inverse MC are compared with the input parameter values.

The results show that the choice of PF assumed in light transport models has a substantial impact on simulated HSI. Using an inappropriate PF can result in significantly altered HSI and considerable artifacts in extracted values of the skin parameters.

9706-35, Session 6
Light pattern preservation in rodent’s cortical tissue during optogenetic neurostimulation
Mehdi Azimipour, Farid Atry, Ramin Pashaie, Univ. of Wisconsin-Milwaukee (United States)

Optogenetics provides a tool for modulating activity of specific cell types by light pulses. Different light delivery mechanisms such as single optical fiber implanted on a skull or patterned illumination can be employed to direct light to a target area. For a highly scattering medium such as brain tissue, light distribution significantly depends on the scattering parameters of the tissue as well as the inherent inhomogeneity of the specimen. For in vivo studies, blood vessels which have considerable absorption coefficient in the visible spectrum play a major role in producing such inhomogeneity. Therefore, detailed information about brain optical properties and network of blood vessels which was ignored in previous studies is necessary to accurately predict light distribution and designing light delivery mechanism during optogenetic experiments to achieve the desired optical stimulation. In this paper, light pattern preservation while considering the impact of blood vessels is investigated in a rat cortex. First, the typical optical properties of rat cortical tissue were extracted by employing double integrated sphere technique, and then, optical coherence tomography was employed to obtain structure of blood vessels on the cortex. By combining the extracted optical properties and the network of blood vessels, a three-dimensional model of a rat cortical tissue was developed. Then, a Monte Carlo simulation code was used to predict light distribution in this model for different light source configurations and wavelengths. The results confirm that presence of vessels can significantly impact the light pattern in the tissue and affect the practical depth of penetration.

9706-36, Session 6
Coherent-wave Monte Carlo method for simulating light propagation in tissue
Maciej Kraszewski, Jerzy Pluci?ski, Gdansk Univ. of Technology (Poland)

Simulating propagation and scattering of coherent light in turbid media, such as biological tissues, is a complex problem. Numerical methods for solving Helmholtz or wave equation (e.g. finite-difference or finite-element methods) require large amount of computer memory and long computation time. This makes them impractical for simulating beam propagation into deep layers of tissue. Other group of methods, based on radiative-transfer equation, allows to simulate only propagation of light averaged over the ensemble of turbid medium realizations. This makes them useless for simulating phenomena connected to coherence properties of light.

We propose a new method for simulating propagation of coherent light (e.g. laser beam) in biological tissue, that we called Coherent-Wave Monte Carlo (CW-MC) method. This method, as finite difference or finite elements methods, is derived directly from Helmholtz equation. However, by direct computation of optical interaction between different parts of tissue, it allows to reduce amount of memory and computation time required for simulation. The CW-MC method uses Green functions approach to skip simulating of light propagation in space between scattering tissue elements. The main part of the method is a system of linear equations describing optical interaction between scatterers inside the tissue. Than, the Monte Carlo method is used to approximately solve this system of equations.

We present theoretical basis of the proposed method and conditions that ensure consistency between CW-MC and finite-difference frequency domain method for simulating propagation of light.
9706-37, Session 7

**Methods for focusing beams and variance reduction in Monte Carlo simulations**

Joel N. Bixler, Robert J. Thomas, Air Force Research Lab. (United States)

Monte Carlo methods have become the gold standard for analyzing light propagation in a turbid media, and are commonly used to investigate broad ranging problems including thermal dynamics, single- and multi-photon fluorescence, along with scattering dynamics. One well known problem associated with traditional Monte Carlo methods is the inability to incorporate the effects of diffraction, where photons instead propagate based on ray optics and focus to a spot that can be below the diffraction limits at the focal plane. Additionally, accurate modeling of complex systems can require a large number of photons to be simulated, and can thus be quite computationally intensive.

In this presentation, we will discuss the latest developments in methods for improving Monte Carlo simulations to include focusing into turbid media where photons follow optical paths derived from Gaussian optics along with techniques for variance reduction. Inclusion of Gaussian paths into traditional Monte Carlo simulations allows for diffraction limited spots to be accurately simulated within the framework of existing Monte Carlo models. Variance reduction techniques can be used to improve the efficiency of a Monte Carlo calculation and can be used to decrease the amount of time required to meet a specific tolerance. This concept will be demonstrated for several variance reduction techniques including quasi-random source sampling, forced interactions, along with distribution smoothing to reduce spatial variation due to sampling error. This computational beam analysis approach has been implemented in order to study the physical phenomenon and dimensional event space occurring during thermal diffusion, chemical changes, as well as ablation in biological tissues.

9706-39, Session 7

**Accurately modeling Gaussian beam propagation in the context of Monte Carlo techniques**

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Monte Carlo simulations are widely considered to be the gold standard for studying the propagation of light in turbid media. However, traditional Monte Carlo methods treat light as a particle, thus they are unable to include diffraction. This results in converging beams focusing to a point instead of a diffraction limited spot greatly effecting the accuracy of Monte Carlo simulations near a focal plane. Here, we present a technique capable of simulating a focusing beam in accordance to the rules of Gaussian optics, resulting in a diffraction limited focal spot. This is accomplished by splitting the photons into ballistic and scattered photons. Once a photon has scattered it is then propagated through the Monte Carlo simulation in a traditional manor. However, ballistic photons are propagated through the medium along trajectories predicted by Gaussian optics. This technique can be easily implemented into any traditional Monte Carlo simulation allowing existing models to be converted to include accurate focusing geometries with minimal effort. The ability to simulate accurate focusing geometries will greatly enhance the usefulness of Monte Carlo for countless applications, including studying laser tissue interactions in medical applications and light propagation through turbid media.

9706-38, Session 7

**Modeling intra-vital illumination of the lung**

Madeleine S. Durkee, Patrick J. Griffin, Landon D. Nash, Duncan J. Maitland, Texas A&M Univ. (United States); Jeffrey D. Cirillo, Texas A&M Health Science Ctr. (United States); Kristen C. Maitland, Texas A&M Univ. (United States)

Stochastic modeling techniques such as Monte Carlo simulations are powerful and well-defined tools for analyzing the propagation of light within biological tissues; however, they lack a robust method for analyzing complex tissue structures and non-uniform air-tissue interfaces, such as the intricate branching structure of the lung. In order to better understand the propagation of light within such tissues, we have developed a computational model of a mock airway structure in a ray tracing software, which easily incorporates these multifaceted structures and represents intra-vital illumination. Experimental validation of these simulations was accomplished by imaging tissue phantoms with controlled absorption and scattering characteristics and a designed structural void to represent the airway. We have created polymer phantoms with optical properties similar to that of mouse lung tissue for the validation of the ray-tracing simulation in a relevant animal model. Phantoms with these optical properties have been fabricated with a simple slab geometry as well as with a mock airway void to compare to the optical model and analyze the respective effects of optical properties and tissue structure on light propagation within lung tissue. The final optimized simulation will accurately describe the illumination profile of internal excitation of mouse lung to predict the probing depth of intra-vital micro-endoscopes.

9706-40, Session 7

**Noninvasive optical measurement of bone marrow lesions: a Monte Carlo study on visible human dataset**

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Bone marrow is both the main hematopoietic and important immune organ. Bone marrow lesions (BMLs) may cause a series of severe complications and even myeloma. The traditional diagnosis of BMLs rely on mostly bone marrow biopsiy/ puncture, and sometimes MRI, X-ray, and etc, which are either invasive and dangerous, or ionizing and costly. A diagnosis technology with advantages in noninvasive, safe, real-time continuous detection, and low cost is requested. Here we reported our preliminary exploration of feasibility verification of using near-infrared spectroscopy (NIRS) in clinical diagnosis of BMLs by Monte Carlo simulation study. We simulated and visualized the light propagation in the bone marrow quantitatively with a Monte Carlo simulation software for 3D voxelized media and Visible Chinese Human data set, which faithfully represents human anatomy. The result indicate that bone marrow actually has significant effects on light propagation. According to a sequence of simulation and data analysis, the optimal source-detector separation was suggested to be narrowed down to 2.8~3.5cm, at which separation the spatial sensitivity distribution of NIRS cover the most region of bone marrow with high signal-to-noise ratio. The display of the sources and detectors were optimized as well. This study investigated the light transport in spine and assisting to the BMLs detection issue and reported the feasibility of NIRS detection of BMLs noninvasively in theory. The optimized probe design of the coming NIRS-based BMLs detector is also provided.
A time-resolved subtraction method for evaluating the optical properties of layered turbid media

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The analysis of statistical moments of time-resolved (TR) diffuse optical signals can be used to evaluate the absorption and scattering coefficients of turbid media; however, this method requires careful measurement of the instrument response function. We propose an alternative approach that avoids this step by estimating the optical properties from the reference of TR measurements acquired at different source-detector separations. The efficiency of this method was validated using simulated data (from analytical model and Monte-Carlo simulations) and tissue-mimicking phantoms. Results for a homogenous and layered medium showed that the subtraction technique can accurately estimate the optical properties. Specifically, our preliminary results show that the method can estimate the optical properties of a homogeneous medium (simulated using $\alpha = 0.1$ mm$^{-1}$, $\beta = 10$ mm$^{-1}$) with an error less than 10 %. Accurate results were obtained at source-detector separations large enough (5 mm or greater) to resolve differences in the moments. Moreover, we also observed that the subtraction method has improved depth sensitivity compared to the classic method of moments. These results suggest that time-resolved subtraction is a simple but effective means of quantifying optical properties of turbid media, in addition to offering a new approach for obtaining spatially sensitive measurements, although additional studies are required to confirm the latter.

Quantification and analysis of tissue back-scattering in the sub-diffusive regime

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The quantification of tissue back-scattering in the sub-diffusive regime is mostly associated with the phase function related parameter ‘gamma’ or the scattering anisotropy ‘g’. Using an analytical solution to the radiative transfer equation, we study and demonstrate the significance of these two parameters for quantification of sub-diffusive back-scattering. For this analysis we make use of various scattering phase functions and reveal ambiguities related to both studied parameters. In consequence, we introduce an empirical parameter with higher specificity towards sub-diffusive back-scattering.

In order to also study sub-diffusive reflectance experimentally, we perform spatial frequency domain imaging at very high spatial frequencies. This allows for high surface sensitivity and increased detection of sub-diffusive light. At the same time, we bridge the gap between conventional spatial frequency domain imaging and structured illumination microscopy.

The former technique represents a diffuse optical imaging modality for quantification of tissue optical properties and the latter is an established high resolution microscopy technique based on ballistic light. With our sub-diffusive imaging approach, we aim for quantification on a microscopic scale and seek to relate quantified tissue properties to the tissue’s histology and histopathology. Consequently, our approach compromises high resolution while gaining averaging over sub-millimeter depth regimes encompassing multiple cellular layers.

In conclusion, our theoretical analysis is meant to elaborate on the correct interpretation of sub-diffusive back-scattering. The related experimental work makes use of these theoretical findings by quantification of sub-diffusive scattering and by relating the derived quantities to the tissue architecture.

Radiation absorption in different kinds of tissue analysis: ex vivo study with supercontinuum laser source

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The aim of this “ex vivo” study is to evaluate the transmission of wavelengths between 350 and 1700 nm in different kinds of tissues by using a supercontinuum broadband laser source coupled with an optical spectrum analyser.

Fifteen different kinds of tissues (nerve, skin, small intestine, lung, oesophagus, stomach, kidney, heart, spleen, liver, muscle, bone) from fresh Sprague Dawley rats have been analysed.

By the recording of the transmitted radiations and by supposing the reflected energy almost constant for all the tissues (generally it amounts to around about 3%), it was possible to calculate, by difference, the scattered and absorbed energies and consequently to know the efficacy of different lasers on different tissues.

The results showed that there are, for all the processed samples, two main absorption windows, the first in the visible portion, nearly corresponding to the so-called “therapeutic window” and related to the chromophores, the second in the infrared portion closer to the water absorption peak.

This study has confirmed that, even if each tissue has an affinity for some specific wavelengths, there are some of these which have a good absorption in all the sample tested.

While some of the lasers normally used in surgery and biostimulation (810, 980, 1064 nm) seem not to be particularly effective, some others, both in the visible and in the infra-red (450, 532, 1340, 1470 nm) have shown a good performance in the present study.

Transmittance of MCF-7 cancer cell line through visible spectrum

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The aim of this study is to evaluate and differentiate Breast cancer cells with different concentrations by applying and using Absorption/Reflection spectrophotometer. This was achieved by use of absorption and scattering characteristics of the cells, response to different wavelengths. Absorption and scattering of the light may give a key concept for the size of single cell or molecule of the cell’s surface leading to absorption and scattering properties. An absorption and reflection spectrophotometer (Zolix) has been used for measuring high quality of light transmittance performance. 6 different concentrations of MCF-7 cell line (5 ml medium each) have been prepared. Transmitted light has been detected by silicon detector which was connected to lock-in amplifier. Data were collected and evaluated by the Zolix Scan Basic software. Detected light intensity was regarded as reference and transmitted intensities of experimental groups were compared with the light and expressed as percent transmittance, i.e (100*experimental group light intensity) / light intensity at corresponding wavelength. It is
observed that there is significant difference within each concentration (p<0.05) at variable wavelengths.

9706-45, Session 8

Extraction of optical properties from hyperspectral images by Monte Carlo light propagation model

Matic Ivancic, Peter Naglic, Peter Usenik, Ale? Fidler, Univ. of Ljubljana (Slovenia); Franjo Perun?, Univ. of Ljubljana (Slovenia) and Sensum d.o.o. (Slovenia); Bo?tjan Likar, Univ. of Ljubljana (Slovenia) and Sensum d.o.o. (Slovenia); Miran Bürmen, Univ. of Ljubljana (Slovenia)

In diffuse reflectance spectroscopy the optical properties of turbid media are frequently determined from a single spectrum or multiple spectra captured by optical fiber probes with exactly defined source-detector fiber geometry. In this work, we extend the methodology to diffuse reflectance and transillumination hyperspectral imaging. Hyperspectral imaging systems have been shown to be of great value in numerous biomedical applications. The main advantage of hyperspectral imaging over optical fiber probe spectroscopy lies in the additional high resolution spatial information that is captured alongside the spectral information.

For the purpose of this study, a broadband visible and near infrared hyperspectral imaging system with collimated illumination was used. First, hyperspectral transillumination images of a collimated light beam propagating through different homogeneous layers were simulated by a modified version of the open source Monte Carlo (MC) for multi-layered tissues. Subsequently, the optical properties in terms of refractive index and absorption and reduced scattering coefficients were extracted from the simulated hyperspectral images by an inverse MC model based on a criterion function that exploits the spatially resolved information of hyperspectral images. The method was then validated on real hyperspectral images of turbid phantoms with exactly defined optical properties. Finally, we measured the optical properties of five different commercially available dental sealants by preparing homogeneous samples of various thicknesses.

9706-46, Session 9

Transcranial light-tissue interaction analysis

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The penetration depth of light plays a crucial role in therapeutic medical applications. In order to design effective medical photonic devices, an in-depth understanding of light’s ability to penetrate tissues, including bones, skin, and fat is necessary. The amount of light energy absorbed or scattered by tissues affects the intensity of light reaching an intended target in vivo. In this study, we examine the transmittance of light through a variety of cranial tissues for the purpose of determining the efficacy of neurostimulation using a transcranial laser. Tissue samples collected from a pig were irradiated with a pulsed laser. We first determine the optimal irradiation wavelength of the laser to be 808 nm. With varying peak and average power of the laser, we found an inverse and logarithmic relationship between the penetration depth and the intensity of the light. After penetrating the skin and skull of the pig, the light decreases in intensity at a rate of approximately 90.8 (±0.4) percent for every 5 mm of brain tissue penetrated. We also found the correlation between the irradiation time and dosage, using three different lasers (with peak power of 500 mW, 5000 mW, and 15000 mW, respectively). These data will help deduce what laser power is required to achieve a clinically-realistic model for a given irradiation time. This work is fundamental and the experimental data can be used to supplement existing and future research on the effects of laser light on brain tissue for the design of medical devices.

9706-47, Session 9

Extraction of optical properties in the sub-diffuse regime by spatially resolved reflectance spectroscopy

Peter Naglic, Franjo Perun?, Bo?tjan Likar, Miran Bürmen, Univ. of Ljubljana (Slovenia)

Spatially resolved reflectance spectroscopy, which utilizes optical fiber probes with multiple source-detector separations, can be used for quantitative and non-invasive determination of optical properties in biological tissues. Many of the recent studies have focused on optical fiber probes operating in the sub-diffuse regime, where the source-detector separation is small and similar to the mean free photon path length. The reflectance in sub-diffuse regime has been shown to significantly depend on the scattering phase function of the turbid medium.

In this paper, we first examine the influence of the phase function on Monte Carlo simulated reflectance spectra at small source-detector separations. Subsequently, the inverse Monte Carlo model based on absorption and reduced scattering coefficients is extended by a well-known similarity parameter, which carries additional information on the phase function. Finally, we propose a criterion function that enables extraction of the absorption and reduced scattering coefficients and phase function information encapsulated in from the reflectance measurements at multiple source-detector separations.

The performance of the extended inverse model was first evaluated by simulated reflectance spectra corresponding to various phase functions used in biomedical optics. Next, an experimental evaluation was conducted on tissue phantoms comprising molecular absorbers and polystyrene microspheres. A substantial increase in the accuracy of the extended inverse model that incorporates was observed. Lastly, we measured the reflectance spectra of common biological tissues and compared the extracted optical parameters to those available in the literature.

9706-48, Session 9

An improved analytic function for predicting light fluence rate in circular fields on a semi-infinite geometry

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Accurate in-vivo light dosimetry is crucial to ensure treatment efficacies of clinical and preclinical photodynamic therapy (PDT) treatment. This paper intends to study and compare how light behaves inside biological tissue, specifically a tcm laser, that would cover the range of optic properties in humans or mice (absorption constants (mua) from 0.1 to 1 and scattering constants (mus) from 2 to 40), in comparison with a broad beam. This was done through Monte-Carlo simulations for a semi-infinite turbid medium which serve to mimic the photon transport movement with an air/tissue surface based on probability density functions. A 6-parameter analytical expressions (1+b*exp(-lambda1*d))(C1*exp(-lambda2*d)+(C2*exp(-lambda3*d)))) was developed to fit the light distributions obtained from the Monte Carlo simulation with relative error of less than 5 % to the peak over the entire depth range. Then, each of these parameters would be expressed as a function of tissue optic properties. These results can then be compared against the 4 parameter expression available from literature for broad collimated beam for analysis in both accuracy and applicable range. Using the 6-parameter model, the accuracy for the light fluence rate through the entire range of depth and tissue optical properties is improved. The resulting expression can be used in light dosimetry in our preclinical studies in mice using the collimated beam, which is typically 1 cm in diameter.
Increased epidermal laser fluence through simultaneous ultrasonic microporation

Paul J. D. Whiteside, Jeff A. Chininis, Mason W. Schellenberg, Heather K. Hunt, Univ. of Missouri (United States)

Lasers have demonstrated widespread applicability in clinical dermatology as minimally invasive instruments that achieve photogenerated responses within tissue. However, before reaching its target, the incident light must first transmit through the epidermis, the surface layer of tissue, which is interspersed with chromophores (e.g. melanin) that preferentially absorb the light and may also generate negative tissue responses. These optical absorbers decrease the efficacy of such procedures. In order to ensure that the target receives a clinically relevant dose, most procedures simply increase the incident energy; however, this tends to exacerbate the negative complications of melanin absorption. Here, we present an alternative solution aimed at increasing epidermal energy fluence while mitigating excess absorption by unintended targets. Our technique involves the combination of a waveguide-based contact transmission modality with simultaneous high-frequency ultrasonic pulsation, which alters the optical properties of the tissue through the agglomeration of dissolved gasses into micro-bubbles within the tissue. Doing so effectively creates optically transparent pathways for the light to transmit unobstructed through the tissue, resulting in an increase in forward scattering and a decrease in absorption. To demonstrate this, Q-switched nanosecond-pulsed laser light at 532nm was delivered into pig skin samples using custom glass waveguides clad in titanium and silver. Light transmission through the tissue was measured with a photodiode and integrating sphere for tissue with and without continuous ultrasonic pulsation at 10MHz. The combination of these techniques has the potential to improve the efficiency of laser procedures while mitigating negative tissue effects caused by undesirable absorption.

Parameterized source term in the diffusion approximation for enhanced near-field modeling of collimated light

Mengyu Jia, Shuang Wang, Xueying Chen, Feng Gao, Huijuan Zhao, Tianjin Univ. (China)

Most analytical methods for describing light propagation in turbid medium exhibit low effectiveness in the near-field of a collimated source. Motivated by the Charge Simulation Method in electromagnetic theory as well as the established discrete source based modeling, we herein report on an improved explicit model for a semi-infinite geometry, referred to as “Virtual Source” (VS) diffuse approximation (DA), to fit for low-albedo medium and short source-detector separation. In this model, the collimated light in the standard DA is analogously approximated as multiple isotropic point sources (VS) distributed along the incident direction. For performance enhancement, a fitting procedure between the calculated and realistic reflectances is adopted in the near-field to optimize the VS parameters (intensities and locations). This parameterized scheme is proved to inherit the mathematical simplicity of the DA approximation while considerably extending its validity in modeling the near-field photon migration in low-albedo medium. The proposed VS-DA model was applied to the reconstruction of optical properties and validated through experiments.

Optical imaging of mitochondrial redox state in irradiated vs. non-irradiated rat hearts during ischemia and reperfusion

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Objective: In this study, the metabolic state of the heart tissue is studied in a rodent model of ischemia and reperfusion (IR) in rats exposed to irradiation injury using a cryo-reflectance imaging technique. Mitochondrial metabolic state is evaluated by autofluorescence of mitochondrial metabolic cofactors, NADH and FAD. The redox ratio (NADH/FAD) is a biochemical/metabolic marker of oxidative stress, before, during and after IR.

Materials and methods: Hearts were extracted from non-irradiated (control) and irradiated rats (Irr) given 15 Gy whole thorax irradiation rats (WTI). After 35 days, before the onset of radiation pneumonitis, these two groups of hearts were subjected to one of three treatments; Time control (TC; hearts perfused for the duration of the protocol without ischemia or IR), 25 minutes ischemia with no reperfusion and 25 minutes ischemia followed by 60 minutes reperfusion (IR). Hearts were removed from the Langendorff perfusion system and immediately snap frozen in liquid N2 to preserve the metabolic state after injury. 3-dimensional (3D) cryo-fluorescence imager was used to collect in fixed time NADH and FAD fluorescence images and their distribution across the entire ventricles. In this study, a 30-μm axial resolution was used resulting in 550 cross-section images per heart. The 3D images of the redox ratio and their respective histograms were calculated in the six groups of hearts.

Results: We compared the mean values of the redox ratio in each group, which demonstrate a reduced mitochondrial redox state in both irradiated and non-irradiated ischemic hearts and an oxidized mitochondrial redox state for both irradiated and non-irradiated ischemia-reperfusion hearts compared to control hearts. For non-irradiated hearts, ischemia and IR injuries resulted respectively in 61% increase and 54% decrease in redox ratio when compared with TC. For irradiated hearts, ischemia and IR injuries resulted respectively in 90% increase and 50% decrease in redox ratio when compared to TC.

Conclusion: The cryoimager is able to quantify ischemia and IR injuries. Cryoimaging is a unique 3D imaging tools that provides quantitative measurement of tissue metabolic state. Hearts that underwent irradiation indicates a more oxidized metabolic state in the tissue. This change persists across all three treatments.

Patency of heart blood vessels under photosensitization reaction shortly after intravenous injection of talaporfin sodium in canine model

Risa Hamada, Ryota Matsuzaki, Emiuyi Ogawa, Tsunenori Arai, Keio Univ. (Japan)

In order to investigate patency of heart blood vessels by photosensitization reaction shortly after intravenous injection of talaporfin sodium, we studied myocardium specimens one week after photosensitization reaction with 10 canine hearts. We also studied acute vascular endothelial cell lethality by the photosensitization reactions <I> in vitro</I>. Laser diffuser made of plastic optical fiber with 70 mm in length was used in the <I> in vivo</I> study. Red diode laser of 664nm wavelength was emitted from this diffuser with 171-42.9 mW/cm in 10 minutes. We estimated the fluence rate distribution by a ray-trace simulator using pre-measured optical coefficients, 7a; 0.12 mm<
irradiation of several dye/protein combinations has revealed effects on light in order to induce protein unfolding and alter its function. In the past, applications. One subset involves irradiating a photosensitizer with visible states)

Brancaleon, The Univ. of Texas at San Antonio (United States); Lorenzo Research Lab. (United States) and 711th HPW, Human Effectiveness Directorate (United States); Robert J. Thomas, Air Force (United States) and 711th HPW, Human Effectiveness Directorate (United States); Sarah C. Rozinek, The Univ. of Texas at San Antonio (United States)

9706-52, Session 10

Action spectrum for photochemical retinal pigment epithelium (RPE) disruption in an in vivo monkey model

Jie Zhang, Ranjani Sabarinathan, Tracy Bubel, David R. Williams, Jennifer J. Hunter, Univ. of Rochester (United States)

Observations of RPE disruption and autofluorescence (AF) photobleaching at light levels below the ANSI photochemical maximum permissible exposure (MPE) (Morgan et al., 2008) indicates a demand to modify future light safety standards to protect the retina from harm. To establish safe light exposures, we measured the visible light action spectrum for RPE disruption in an in vivo monkey model with fluorescence adaptive optics retinal imaging. Using this high resolution imaging modality can provide insight into the consequences of light on a cellular level and allow for longitudinal monitoring of retinal changes. The threshold retinal radiant exposures (RRE) for RPE disruption were determined for 4 wavelengths (460, 488, 544, and 594 nm). The anaesthetized macaque retina was exposed to a uniform 0.5º field of view (FOV). Imaging within a 2º field of view (FOV) was performed before, immediately after and at 2 week intervals for 10 weeks. At each wavelength, multiple RREs were tested with 4 repetitions each to determine the threshold for RPE disruption. For qualitative analysis, RPE disruption is defined as any detectable change from the pre exposure condition in the cell mosaic in the exposed region relative to the corresponding mosaic in the immediately surrounding area. We have tested several metrics to evaluate the RPE images obtained before and after exposure. The measured action spectrum for photochemical RPE disruption has a shallower slope than the current ANSI photochemical MPE for the same conditions and suggests that longer wavelength light is more hazardous than other measurements would suggest.

9706-53, Session 10

Quantification of protein-protein binding before and after photo-modification of albumin

Sarah C. Rozinek, The Univ. of Texas at San Antonio (United States) and 711th HPW, Human Effectiveness Directorate (United States); Robert J. Thomas, Air Force Research Lab. (United States) and 711th HPW, Human Effectiveness Directorate (United States); Lorenzo Brancalone, The Univ. of Texas at San Antonio (United States)

Bioeffects of directed-optical-energy encompasses a wide range of applications. One subset involves irradiating a photosensitizer with visible light in order to induce protein unfolding and alter its function. In the past, irradiation of several dye/protein combinations has revealed effects on protein structure. Beta lactoglobulin, human serum albumin (HSA) and tubulin have all been photo-modified with meso-tetra(4-sulfonatophenyl) porphyrin (TSPP) bound, but only for tubulin, has binding caused a verified loss of biological function (loss of ability to form microtubules) as a result of this light-induced structural change produced by regional TSPP binding. The current work questions if the photo-induced structural changes which occur to HSA, are enough to disable its biological function of binding to osteonectin. The albumin-binding protein, osteonectin, is about half the molecular weight of HSA, so the two proteins and their bound product may be separated and quantified by high performance liquid chromatography (HPLC) or non-denaturing gel electrophoresis followed by Western Blotting. With both techniques, TSPP bound to HSA will be irradiated, photo-modifying the structure of HSA. Then native HSA or photo-modified HSA (both with TSPP bound) will be investigated for binding activity to osteonectin. The quantity of HSA bound to osteonectin will be compared, in order to assess loss in HSA's binding ability as a result of light-induced structure modification.

9706-54, Session 10

Quantification of the effect of toxicants on the motility of mammary organoids by OCT fluctuation spectroscopy

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Mammary epithelial cell (MEC) organoids in 3D culture recapitulate features of breast ducts in vivo. OCT has the ability to monitor the evolution of MEC organoids non-invasively and longitudinally. The anti-cancer drug Doxorubicin (Dox) is able to inhibit proliferation of cancer cells and has been widely used for chemotherapy of breast cancers; while environmental toxins implicated in breast cancer such as estrogen regulates mammary tumor growth and stimulates the proliferation and metastatic potential of breast cells. Here we propose a quantitative method for measuring motility of breast cells in 3D cultures based upon OCT speckle fluctuation spectroscopy. The metrics of the inverse power-law exponent (\(\gamma\)) and fractional modulation amplitude (\(M\)) were extracted from speckle fluctuation spectra. These were used to quantify the responses of MEC organoids to Dox, and estrogen. We investigated MEC organoids comprised of two different MEC lines: MCF10DCIS.com exposed to Dox, and MCF7 exposed to estrogen. We found an increase (p<0.001) in \(\gamma\) of MEC along time (t=0, 1 hour, 24 hours, 48 hours and 6 days) at each dose of Dox (0, 1 \(\mu\)M and 10 \(\mu\)M), indicating lower fluctuation intensity at higher frequencies. We also observed a decrease (p<0.001) in \(M\) for increasing time. However, both \(\gamma\) and \(M\) of MCF7 treated with estrogen (0, 1 \(\mu\)M and 10 \(\mu\)M) exhibited the opposite trend along time. This novel technology provides rapid and non-invasive measurements of the effects of toxicants on MEC motility for understanding breast cancer development and assessing anti-cancer drugs.

9706-55, Session 10

Multi-scale analysis of engineered skin using serial optical coherence microscopy

Yujin An, Songyee Baek, Andrey Vavilin, Ulsan National Institute of Science and Technology (Korea, Republic of); Pill Un Kim, Oz-tec Co., Ltd. (Korea, Republic of); Haekwang Lee, AmorePacific Corp. (Korea, Republic of); Woonggyu Jung, Ulsan National Institute of Science and Technology (Korea, Republic of) and Institute of Basic Science (Korea, Republic of)

As biomimetic technology is advanced in recent years, various engineered tissues or organs have been investigated. Engineered skin is the one of representative tissues which can be immediately applied to biological
research, cosmetic, and medicine. In particular, the development of human like engineered skin is prospective, because it could be utilized as an alternative tool where the in vivo animal studies is prohibited or restricted. Even though the technology of engineered skin is getting mature, there is needs for further investigation to visualize engineered skin while offering comprehensive its structural and functional information. The desired imaging device is required to equip advanced capabilities such as dynamic monitoring, quantitative analysis and multi-scale imaging in large field-of-view.

Here, we introduce a novel optical imaging platform based on serial optical coherence microscopy which is specifically designed for engineered skin study. Our technique is integrated by optical coherence microscopy (OCM) and tissue sectioning which offers label-free, deep tissue imaging as well as high-resolution and throughput visualization of tissue for statistical analysis. To verify the performance of developed system, we applied it to various skins including home-built engineered skin, commercial engineered skin, and animal skin. We investigated volumetric morphology and their difference among skins such as layer composition, thickness and volume contraction in tissue-level. Also, our study involves the quantitative analysis of cells in the skin regarding to distribution, differentiation and proliferation. Our results show that serial OCM is very promising tool for multi-scale skin imaging as well as quantitative evaluation of volumetric engineered skin.

9706-70, Session 10

**Photothermal damage is correlated to the delivery rate of time-integrated temperature** *(Invited Paper)*

Michael L. Denton, Gary D. Noojin, Betsy Gamboa, Elharith Ahmed, Engility Corp. (United States); Benjamin A. Rockwell, Air Force Research Lab. (United States)

Temperature was measured at the boundary of cell death in response to various combinations of laser exposure and culture conditions. A specific rate of delivery of threshold time-integrated temperature (Tint), a measure of accumulated thermal dose, was correlated with damage occurrence over a broad range of laser exposure durations. Laser-dependent increase in Tint (?Tint) varied depending upon ambient temperature and degree of target pigmentation such that the observed threshold Tint value was delivered. We show that Tint data collected during laser exposure may be used to predict the final area of damage occurring 1 hr post exposure. Finally, we use the threshold thermal profiles taken from the boundaries of cell death in applications of the Arrhenius-based damage rate process model.
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9707-1, Session 1

Laser speckle micro rheology for micro-mechanical mapping of bio-materials (Invited Paper)
Zeinab Hajjarian Kashany, Harvard Medical School (United States); Shawn Ahn, Univ. of Illinois at Urbana-Champaign (United States); Hadi Tavakoli Nia, Harvard Medical School (United States); Diane M. Tshikudi, Massachusetts General Hospital (United States); Alan Grodzinsky, Massachusetts Institute of Technology (United States); Rakesh K. Jain, Seemantini K. Nadkarni, Harvard Medical School (United States)

Laser speckle Micro-rheology (LSM) is a novel optical tool for evaluating the viscoelastic properties of biomaterials. In LSM, a laser beam illuminates the specimen and scattered rays are collected through an objective by a high-speed CMOS camera. The self-interference of light rays forms a fluctuating speckle pattern captured by the CMOS sensor. Spatio-temporal correlation analysis of speckle images provides the intensity autocorrelation function, g2(t), for individual pixels. Next, the mean square displacements (MSD) of Brownian particles are deduced and substituted in the generalized Stokes-Einstein relation (GSER) to yield a 2D map of viscoelastic modulus, |G*(ω)|. To compare the accuracy, sensitivity, and dynamic range of LSM measurements with standard mechanical testing methods, homogeneous polyethylene glycol (PEG), agarose, and polyacrylamide (PA) gels, of assorted viscoelastic properties were fabricated and evaluated using LSM, shear rheology, and indentation-mode atomic force microscopy (AFM).

Results showed a statistically significant, strong correlation between |G*| values measured by LSM and shear rheology (R=0.94, p<5×10^-6) (|G*|: 30 Pa – 30 kPa at ω = 1 Hz). Likewise, strong correlation was observed between |G*| values measured by LSM and indentation moduli of AFM (R=0.94, p<0.05). Next, polyacrylamide substrates with micro-scale stiffness patterns were tested using LSM. The reconstructed |G*| maps illustrated the high sensitivity of LSM in resolving mechanical heterogeneities below 100 microns. These findings demonstrate the competent accuracy and sensitivity of LSM measurements. Moreover, the non-contact nature of LSM provides a major advantage over mechanical tests, making it suitable for in vivo studies in future.

9707-3, Session 1

Momentum transfer Monte Carlo model for the simulation of laser speckle contrast imaging
Caitlin Regan, Carole K. Hayakawa, Bernard Choi, Univ. of California, Irvine (United States)

Laser speckle imaging (LSI) enables measurement of relative blood flow in microvasculature and perfusion in tissues. To determine the impact of tissue optical properties and perfusion dynamics on speckle contrast, we developed a computational simulation of laser speckle contrast imaging. We used a discrete absorption-weighted Monte Carlo simulation to model the transport of light in tissue. We simulated optical excitation of a uniform flat light source and tracked the momentum transfer of photons as they propagated through a simulated tissue geometry. With knowledge of the probability distribution of momentum transfer occurring in various layers of the tissue, we calculated the expected laser speckle contrast arising with coherent excitation using both reflectance and transmission geometries. We simulated light transport in a single homogeneous tissue while independently varying either absorption (0.01-100mm^-1), reduced scattering (1-10mm^-1), or anisotropy (0.05-0.99) over a range of values relevant to blood and commonly imaged tissues. We observed that contrast decreased by 49% with an increase in optical scattering, and observed a 130% increase with absorption (exposure time = 1ms). We also explored how speckle contrast was affected by the depth (0-1mm) and flow speed (0-10mm/s) of a dynamic vascular inclusion. This model of speckle contrast is important to increase our understanding of how parameters such as perfusion dynamics, vessel depth, and tissue optical properties affect laser speckle imaging.

9707-4, Session 1

Effects of incident intensity on laser speckle contrast imaging
Mitchell A. Kirby, Kosar Khaksari, Sean J. Kirkpatrick, Michigan Technological Univ. (United States)

In this work the effects of incident intensity and effective camera dynamic range on image acquisition of both frozen and time-averaged dynamic speckle patterns, and their effects on laser speckle contrast imaging are addressed. A nematic liquid crystal, phase-only, spatial light modulator (SLM) was employed to generate laser speckle in a controlled and repeatable fashion. By addressing the calculated spatial contrast of frozen and time-averaged dynamic speckle patterns imaged across a wide range of intensities, we present a description of optimum intensity characteristics that should be observed when using LSCI. The results indicate the importance of assessing the intensity of the signal quantized by the camera in LSCI. By analyzing intensity PDF’s during image acquisition of speckle patterns used in LSCI, an optimum incident intensity can be detected when a single, polarized speckle frame displays the first order statistics characteristic of fully developed speckle. Our results indicate that there is a range of laser power densities where the ensuing imaged speckle exhibits optimum sensitivity to flow as well as relatively constant calculated contrast values. It is clear that at high intensities, high frequency information is lost due to camera saturation, resulting in a decrease in contrast. When imaging speckle at low intensity, there is a risk for loss of data during the digital quantization process. The results are presented in a generalized fashion, so they should be applicable to any LSCI system, regardless of incident laser power or camera depth.

9707-5, Session 1

New sensor for the stress measurement based on blood flow fluctuations analysis
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An effective stress management could have a dramatic impact on health care and preventive medicine. Thus a new stress marker has been developed and tested. We utilized a physiological fact that the mental stress affects the peripheral blood flow, elicits vascular vasodilation through increased arterial blood, sympathetic withdrawal and diminished noradrenaline release. Therefore, the blood flow response is an interplay of the oscillatory and stochastic blood flow responses coming from different type of vessels. A new miniaturized dynamic light scattering (mDLS) sensor was used to measure the laser speckle responses from the skin blood flow at wrist. Temporal spectral and correlation analysis of the measured speckle signals was utilized to differentiate between different types of the blood vessels contributions according to the shear rate of the microcirculation of blood. There was assumed that the lowest shear rate is associated with
the capillary blood and the pulsatile component is related to arterioles. We used such a multi-vessel representation for stochastic, fractal and oscillatory analysis of the blood flow fluctuations. In order to relate these new parameters to the stress level a group of subjects have been measured by mDLS sensor while different types of mental stress have been induced. More specifically, a so-called cognitive dissonance Stroop tests, mental arithmetics and sound tests have been used in order to active stress mechanisms. The physiological response was also assessed by using the heart rate variability analysis representation. There was demonstrated that the newly developed stress marker can be used for continuous monitoring applications.

9707-6, Session 1

Instrument to detect syncope and the onset of shock

Daniel R. McAdams, Noah J. Kolodziejski, Christopher J. Stapels, Daniel E. Fernandez, Matthew J. Podolsky, Dana Farkas, James F. Christian, Radiation Monitoring Devices, Inc. (United States); Michael J. Joyner, Christopher P. Johnson, Mayo Clinic (United States)

Currently the diagnosis of hemorrhagic shock is a subjective exercise, relying on the expertise of nurses and doctors. One of the first measurable physiological changes that marks the onset of hemorrhagic shock is a decrease in capillary blood flow. Diffuse correlation spectroscopy (DCS) quantifies this decrease. DCS collects and analyzes multiply scattered, coherent, near infrared light to assess relative blood flow. This work presents validation studies of a DCS instrument, including a preliminary study with human subjects using a lower body negative pressure (LBNP) protocol. This work builds on previous successful DCS instrumentation development and we believe it represents the first instance of a pre-clinical prototype integrated DCS instrument validated using a human model.

9707-7, Session 2

Mapping transverse capillary flow speed using time-varying OCT speckle signals (Invited Paper)

Woo June Choi, Ruikang K. Wang, Univ. of Washington (United States)

We present an optical coherence tomography (OCT) based method for mapping transverse red blood cell (RBC) flow speed at capillary. This OCT velocimetry utilizes a quantitative laser speckle temporal contrast analysis that estimates reliable speckle decorrelation time from the observed speckle contrast, which is related to microcirculatory flow velocity. For capillary speed measurement, we employ a home-built 1.3 µm MHz swept-source OCT (SS-OCT) system that can acquire OCT B-frames at a rate of 1.7 kHz. From the multiple B-frames obtained at the same location, intensity profiles with time-varying OCT speckle contrast are extracted at single capillaries using a capillary binary mask and then the transverse flow speed is calculated by adapting the profiles to the speckle contrast analytic model. Finally, a 3D speed map can be achieved for OCT volume imaging. To validate this method, we perform a systematic study using both phantom and in vivo rodent models. Result shows that our method is effective to measure transverse capillary flow speed.

9707-8, Session 2

Visualization and characterization of the acoustic radiation force assisted displacement of particles using an OCT technique

Marjan Razani, Azhar Zam, Nico J. J. Arezza, Yan J. Wang, Michael C. Kolios, Ryerson Univ. (Canada)

In this study, we present a technique to image the enhanced particle displacement generated using an acoustic radiation force (ARF) excitation source. A swept-source OCT (SS-OCT) system with a center wavelength of 1310nm, a bandwidth of -100nm, and a A-scan rate of 100 kHz (MEMS-VCSEL OCT Thorlabs) was used to detect gold nanoparticle (70nm in diameter) displacement. ARF was applied after the nanoparticles passed through a porous membrane and diffused into a collagen (6% collagen) matrix. B-mode, M-B mode, 3D and Speckle Variance (SV) images were acquired before and after the ARF beam was on. Differential OCT speckle variance images with and without the ARF were used to measure the particle displacement. The images were used to detect the microscopic enhancement of nanoparticle displacement generated by the ARF. Using this OCT imaging technique, the extravasation of particles though a porous membrane and characterization of the enhanced particle displacement in a collagen gel after using an ARF excitation was achieved.

9707-9, Session 2

Towards understanding speckle pattern formation in optical coherence tomography

Valentin Demidov, Univ. of Toronto (Canada); Igor Meglinski, Univ. of Oulu (Finland); Alexander Doronin, Yale Univ. (United States); I. Alex Vitkin, Univ. of Toronto (Canada) and Ontario Cancer Institute (Canada)

We consider the mechanism of speckle patterns formation in time-domain and swept source optical coherence tomography (OCT), and introduce a Monte Carlo based model for simulating OCT signals and images. The model takes into account polarization and coherent properties of light, mutual interference of the back-scattering light, and its interference with the reference beam. The developed model is employed to generate OCT images, and to analyze the resultant OCT speckle pattern properties. The model simulation results are compared with experimental measurements, and an interpretation of the speckle patterns formation in terms of its underlying physics is provided.

9707-10, Session 2

Comprehensive analysis of factors influencing the shadow-artifact in microcirculation imaging with optical coherence tomography

James McGrath, Hrebesh M. Subhash, Cerine Lal, Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Optical coherence tomography (OCT) based flow quantification and microcirculation mapping has become an active area of research, which aims to visualize the lumens and architecture of blood vessels in many clinical and fundamental areas of research, including cardiology, dermatology, neurology, ophthalmology, small animal imaging studies, and so forth. OCT based angiographic modalities and flow quantification techniques can provide non-invasive, label-free, high resolution depth
resolved mapping at clinically relevant speed and performance. However, there are many potential sources of artifacts which can degrade the quality and measurement accuracy of this modality. The “shadow-artifact” is one of the prevalent sources of noise in OCT-based blood flow imaging, which is attributed to multiple scattering in blood vessels with high hematocrit content. In this study, we present a comprehensive analysis of various flow parameters that can affect the shadow-artifact in a flow phantom and in vivo model, which include flow dimension, concentration of scatters, flow rate and Doppler angle. Moreover, we also present the influence of various system parameters such as numerical aperture (NA) and spatial coherence of the light source which can affect the shadow-artifact.

9707-11, Session 3

Capillary flux measurement using OCT microangiography

Utku Baran, Ruikang K. Wang, Univ. of Washington (United States)

Accurate visualization and quantification of the microvascular networks in the cerebral cortex are critical for evaluating therapies that treat neurovascular diseases, such as stroke, traumatic brain injury, vascular dementia, inflammation, and cancer. Neuron firing rates change with a wide range from -1 Hz to 200 Hz, depending on the activity and the part of the brain. This causes capillaries to exhibit heterogeneous and fluctuating red blood cell (RBC) dynamics. Optical coherence tomography (OCT) is a non-invasive method for imaging three-dimensional biological tissues with high resolution (~10 μm), without requiring the use of contrast agents. OCT signal can also be utilized to provide 3D blood angiography down to capillary level in vivo. However, RBC flux measurements in capillaries are challenging to be made using the standard OCT based microangiography methods. In this work, we present a technique based on OCT to quantify RBC flux over many capillaries at the same time in a 1 mm3 imaging volume. Moreover, we combined OCT flux maps with OCT microangiography images, including velocity measurements, to create a comprehensive visualization of microvascular dynamics during stroke on rodents. Our technique is applicable to traditional OCT systems with standard hardware. Enhanced capability of standard OCT systems will help understanding complex microvascular flow dynamics in healthy and diseased brain.

9707-12, Session 3

Blood flow changes after unilateral carotid artery ligation monitored by optical coherence tomography

Yushu Ma, Northeastern Univ. (China); Chengbo Liang, Yanyan Suo, Shenzhen Entry-Exit Inspection and Quarantine Bureau (China); Yuqian Zhao, Yi Wang, Northeastern Univ. (China); Tao Xu, Shenzhen Academy of Metrology and Quality Inspection (China); Ruikang K. Wang, Univ. of Washington (United States); Zhenhe Ma, Northeastern Univ. (China)

Unilateral carotid artery ligation which could induce adaptive improvement is a classic model that has been widely used to study pathology of ischemic disease. In those studies, blood flow is an important parameter to characterize the ischemia. Laser Doppler flowmetry is commonly used to measure the blood flow velocity, however, relatively low resolution refrains its application in this domain. Optical coherence tomography (OCT) is a powerful imaging modality which can provide depth resolved images in biological tissue with high spatial and temporal resolution. By calculating phase shift between OCT images, absolute measurement of blood flow velocity and the flow volume can be achieved. These advantages make OCT technology more and more popular on research of small animal model. In this study, blood flow volume was calculated based on the Doppler OCT image and the Doppler angle which is achieved by 3D structural image reconstruction. SPF rats was anesthetized with isoflurane and divided into two groups. The first group bilateral carotid artery was surgically exposed, and then left carotid artery was ligated. Blood flow changes of the contralateral carotid artery was monitored using high speed spectral domain optical coherence tomography, including the absolute flow velocity and the flow volume. In the other group, skull window was opened at the ipsilateral cerebral cortex of ligation, blood supply of small artery was measured before and after the ligation. The measured results demonstrate the blood supply compensation process after unilateral carotid artery ligation. Our work shows that OCT is an effective technology which has the potential for researches correlated with the model of unilateral carotid artery ligation.

9707-13, Session 3

Quantitative Mueller matrix microscope: theory, equipment, calibration, and applications (Invited Paper)

Hui Ma, Graduate School at Shenzhen, Tsinghua Univ. (China)

Polarized light imaging techniques can provide rich microstructural information on the samples. Among all the polarization techniques, Mueller matrix polarimetry provides a comprehensive description of the structural and optical properties of complex media, such as biomedical samples. In this paper, we develop a modular designed Mueller matrix microscope by adding polarization state generator and analyzer (PSG and PSA) to a commercial transmission optical microscope. The calibration process is also presented to minimize errors of the Mueller matrix elements to 0.01. A set of new polarization parameters transformed from the Mueller matrix is also introduced for quantitative characterization of the microstructure features. We apply the Mueller matrix microscope to a variety of samples, ranging from slices of human cancerous tissues to polymer films. The preliminary experimental results show that both the Mueller matrix elements and transformed Mueller matrix parameters can provide quantitative assessment on the characteristic microstructural features of different samples. Contrast mechanisms of the Mueller matrix images are also backed up by Monte Carlo simulations based on a sphere-cylinder birefringence model. The experimental and simulated data indicate that the microscopic polarization techniques can effectively distinguish the anisotropic features due to the fibrous scatterers and birefringent interstitial media, both of which are abundant in biological tissues. Mueller matrix microscope has the potential to become a powerful tool for clinical diagnosis.

9707-14, Session 3

Quantification of volumetric cerebral blood flow using hybrid laser speckle contract and optical coherence tomography (Invited Paper)

Niksa Valim, Andrew K. Dunn, The Univ. of Texas at Austin (United States)

Studying neurovascular blood flow function in cerebrovascular activities requires accurate visualization and characterization of blood flow volume as well as the dynamics of blood cells in microcirculation. In this study, we present a novel integration of laser speckle contrast imaging (LSCI) and spectral domain optical coherence tomography (SD-OCT) for rapid volumetric imaging of blood flow in cortical capillaries. LSCI uses the illumination of wide-field near infrared light (NIR) and monitors back scattered light to characterize the relative dynamics of blood flow in microcirculation. Absolute measurement of blood cells and blood volume requires high-resolution volumetric structural information. SD-OCT system uses coherence gating to measure scattered light from a small volume within high structural resolution. The structural imaging system rapidly
assesses large number of capillaries for spatio-temporal tracking of red blood cells (RBC). A very fast-ultra resolution SD-OCT system was developed for imaging high-resolution volumetric samples. The system employed an ultra wideband light source (1310 ± 200 nm in wavelength) corresponding to an axial resolution of 3 micrometers in tissue. The spectrometer of the SD-OCT was customized for a maximum scanning rate of 147,000 line/s. We demonstrated a fast volumetric OCT angiography algorithm to visualize large numbers of vessels in a 2-mm deep sample volume. A LSCI system that has been developed previously in our group was integrated to the imaging system for the characterization of dynamic blood cells. The conjunction data from LSCI and SD-OCT systems imply the feasibility of accurate quantification of absolute cortical blood flow.

9707-15, Session 4

Speckle fluctuations to probe dynamics on the macroscopic to microscopic scales (Keynote Presentation)

David A. Boas, Athinoula A. Martinos Ctr. for Biomedical Imaging (United States)

I will review recent advances and applications in Diffuse Correlation Spectroscopy, Laser Speckle Contrast Imaging, and Optical Coherence Tomography for measuring dynamics arising from cerebral blood flow and intra-cellular organelle motility.

9707-51, Session 4

Functional monitoring of blood flow dynamics in brain (Invited Paper)

Arjun G. Yodh, Univ. of Pennsylvania (United States)

Speckle fluctuations of multiply scattered light provide experimenters with information that enables measurement of the motions of light scattering tissue constituents. Arguably, the most important application of speckle techniques derives from their sensitivity to blood flow. Diffuse correlation spectroscopy (DCS), for example, employs temporal intensity correlation functions of diffusing light to investigate the blood flow biomarker in brain, cancer lesions and skeletal muscle. Recent studies from Penn will be described; they explore new clinical applications for DCS, develop strategies to isolate contributions of cerebral tissues, and improve sampling-rates for tissue monitoring.

9707-20, Session PSun

Quantitative assessment of reactive hyperemia using laser speckle contrast imaging at multiple wavelengths

Anthony Young, Karthik Vishwanath, Miami Univ. (United States)

Reactive hyperemia refers to an increase of blood flow in tissue post release of an occlusion in the local vasculature. Measuring this physiological response can help in studying microvascular diseases such as systemic sclerosis and diabetes. Laser speckle contrast imaging (LSCI) is an imaging technique capable of sensing superficial blood flow in tissue which can be used to quantitatively assess reactive hyperemia. Here, we employ LSCI using coherent sources in the blue, green and red wavelengths to evaluate reactive hyperemia in healthy human volunteers.

Blood flow in the forearms of subjects will be measured using LSCI to gauge reactive hyperemia triggered by the use of a pressure cuff applied to their biceps. Raw speckle images will be acquired and processed to yield quantitative blood-flow parameters from a region of interest before, during and after application of occlusion. Reactive hyperemia will be quantified via two measures - (1) By calculating the difference between the peak LSCI flow during the hyperemia and baseline flow, and (2) By measuring the amount of time elapsed from peak flow during hyperemia until flow returns to baseline values. These measurements will be acquired for each participant across a range of occlusion pressures and durations, for each laser wavelength employed. The studies will shed light on the utility of in vivo LSCI-based flow sensing for non-invasive assessment of reactive hyperemia responses and how they vary with the pressures and durations of vascular occlusion applied. They will also indicate whether the choice source wavelength influences the measured parameters.

9707-33, Session PSun

Demonstration of brain noise on human EEG signals in perception of bistable images

Vadim V. Grubov, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Anastasija E. Runnova, Saratov State Technical Univ. (Russian Federation); Maria K. Kurovskaia, Alexey A. Koronovskii, Alexey N. Pavlov, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Alexander E. Hramov, Saratov State Technical Univ. (Russian Federation)

In this report we studied human brain activity in the case of bistable visual perception. We proposed a new approach for quantitative characterization of this activity based on analysis of EEG oscillatory patterns and evoked potentials. Accordingly to theoretical background, obtained experimental EEG data and results of its analysis we introduced a concept of normalized noise intensity as a characteristic of brain activity during decision-making. We showed that theoretical calculations are in excellent agreement with results of EEG data analysis.

9707-34, Session PSun

Estimation of degree of synchronization in epileptic brain

Olga I. Moskalenko, Alexey A. Koronovskii, Alexey N. Pavlov, Alexander E. Hramov, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Maksim O Zhuravlev, Saratov State University (Russian Federation) and Saratov State Technical University (Russian Federation)

A method for calculation of zero conditional Lyapunov exponent from time series has been proposed. Such method is shown to define the degree of synchronization of the regime realized in the system. It has been applied to real experimental neurophysiological time series represented by electroencephalograms of WAG/Rij rats having genetic predisposition to absence-epilepsy. The degree of synchronization between different channels of epileptic brain has been quantified.
The competition of homophily and homeostasis mechanisms taking place in the multilayer network where several layers of connection topologies are simultaneously present as well as the interaction between layers is considered. We have shown that the competition of homophily and homeostasis leads in such networks to the formation of synchronous patterns within the different layers of the network, which may be both the distinct and identical.

9707-36, Session PSun

Analysis of the establishment of the global synchronization in complex networks with different topologies of links

Alexander A. Kharchenko, N.G. Chernyshevsky Saratov State Univ (Russian Federation); Vladimir V. Makarov, Olga I. Moskalenko, Alexey A. Koronovskii, Alexey N. Pavlov, Marina V. Khramova, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Syamal Dana, Indian Institute of Chemical Biology (India); Alexander E. Hramov, Saratov State Technical Univ (Russian Federation).

Analysis of the establishment of the global synchronization in complex networks with different topologies of links. We study the mechanism of the global synchronization onset through the formation of the synchronous clusters in complex networks with different topologies of links (scale-free networks, small-world networks, random networks). We consider the dependencies of integral characteristics of synchronous dynamics (synchronization measure, number of synchronous clusters, etc) on coupling strength between nodes. As a basic element of the node oscillator we consider Kuramoto phase oscillator and Van-der-Pole self-sustained oscillator. We have shown that global synchronization is established the most efficient for the scale-free network.

9707-37, Session PSun

THz-range generation frequency growth in semiconductor superlattice coupled to external high-quality resonator

Vladimir V. Makarov, Alexey A. Koronovskii, Vladimir A. Maksimenko, Alexey N. Pavlov, Marina V. Khramova, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Alexander E. Hramov, Saratov State Technical Univ. (Russian Federation).

We investigate effects of a linear resonator on spatial electron dynamics in semiconductor superlattice. We have shown that coupling the external resonant system to superlattice leads to occurrence of the additional area of negative differential conductance on the current-voltage characteristic, which doesn’t occur in autonomous system. Furthermore, this region shows great increase of generation frequency, that contains practical interest for biomedical applications concerned in terahertz detection of skin and epithelial cancers.

9707-38, Session PSun

Experimental study on synergistic effects of reflectance and transmittance for near infrared spectroscopy

Jingying Jiang, Jiajia Liu, Congcong Ma, Lin Li, Junsheng Lu, Kexin Xu, Tianjin Univ. (China).

Usually, diffused reflectance or diffused transmittance has been collected respectively when performing the near-infrared spectroscopic measurements. However, glucose-relative spectral signal is quiet weak due to the noises from the measuring system and the environment. Previous Monte-Carlo simulation results from our group showed that the spectral magnitude of both diffused reflectance and diffused transmittance can reach the same order. In this talk, it is our aim to further investigate the synergistic effect of diffused reflectance and diffused transmittance for Near Infrared spectral measurements. The diffused reflectance spectra and diffused transmittance spectra of human’s earlobe have been obtained simultaneously by home-made optical probe within the wavelength of 900-1700nm. Two processing methods are introduced to demonstrate the synergistic effect of reflectance and transmittance: Superposition Method and Division Method. Both of the processing methods are performed on diffused reflectance and diffused transmittance in accordance with corresponding wavelengths. The results show that the combination of diffuse reflectance and transmittance can effectively enhance SNR by reducing the interference caused by individual differences and measuring environmental factors. Moreover, comparatively, the Division Method has a more distinguished effect.

9707-39, Session PSun

Monte Carlo simulation study on the availability of the floating-reference theory to diffused transmittance spectra

Jingying Jiang, Lin Li, Congcong Ma, Jiajia Liu, Junsheng Lu, Kexin Xu, Tianjin Univ. (China).

Previous results revealed that the influences, caused by both individual differences and measuring environmental factors, can be reduced by using the floating-reference theory(FRT) for diffused reflectance spectra(DRS)-based blood glucose measurement by Near Infrared Spectroscopy(NIRS). The obtained signals can highlight the variation in light intensity which is brought only by the change of glucose concentration. The existing studies on FRT have mainly focused on the diffused reflectance spectra, but rarely involved the diffused transmittance spectra. In this talk, it is our aim to investigate the availability of FRT on the diffused transmittance spectra(DTS) on the basis of Monte Carlo(MC) simulation method. The MC simulations of DTS have been carried out with different wavelengths, glucose concentrations and skin tissue thicknesses. The simulation results show that the floating reference position point of DTS will disappear when the tissue thickness is greater than a certain value. Therefore, the FRT might be applied on thin tissue thickness model for DTS of NIRS.

9707-40, Session PSun

Quantitative relationship-established between skin optical properties and imaging performance using spectral domain optical coherence tomography

Rui Shi, Huazhong Univ. of Science and Technology (China); Li Guo, Zhejiang Univ. (China); Chao Zhang, Huazhong Univ. of Science and Technology (China); Peng Li, Zhihua Ding, Zhejiang Univ. (China); Dan Zhu,
Huazhong Univ. of Science and Technology (China)

Tissue optical clearing can reduce scattering and enhance the penetration of light in bio-tissue, which shows a promising potential in optimizing the imaging performance of various optical imaging techniques, e.g. contrast and resolution. Nevertheless, optical clearing methods introduced in changes in tissue parameters has been seldom quantitatively assessed, especially for in vivo. Here, a spectral domain optical coherence tomography (SD-OCT) was applied to quantitatively evaluate FPT-induced skin optical clearing, in terms of scattering coefficient, refractive index mismatching extent, optical clearing degree and rate. Results showed that FPT could induce 24% and 12.8% decreases in scattering coefficient and refractive index mismatching extent, respectively. Moreover, the corresponding imaging performance optimization in imaging depth, signal intensity, resolution and contrast were also deduced. Results showed that FPT could induce 32.3% and 72.6% increases in SD-OCT imaging depth and signal intensity, respectively. Further analysis indicated that the relationship between FPT-induced imaging performance optimization and changes in skin tissue parameters was established. This study would be able to explain the essential reason about how tissue optical clearing improves the imaging performance.

9707-41, Session PSun

A rapid and reversible skull optical clearing method for monitoring cortical blood flow

Chao Zhang, Yanjie Zhao, Rui Shi, Dan Zhu, Huazhong Univ. of Science and Technology (China)

In vivo cortex optical imaging is of great important for revealing both structural and functional architecture of brain with high temporal-spatial resolution. To reduce the limitation of turbid skull, researchers had to establish various skull windows or directly expose cortex through craniotomy. Here we developed a skull optical clearing method to make skull transparent. Laser speckle contrast imaging (LSCI) technique was used to monitor the cortical blood flow after topical treatment with the optical clearing agents. The results indicated that the image contrast increased gradually, and then maintained at a high level after 15 min for adult mice, which made the image quality and resolution of micro-vessels nearly approximate to those of exposed cortex. Both the cortical blood flow velocity almost kept constant after skull became transparent. Besides, the treatment of physiological saline on the skull could make skull return to the initial state again. Based on the laser speckle contrast images, we also implemented arteriovenous segmentation accurately, which was impossible on the condition of intact skull. Thus, we could conclude that the skull optical clearing method was rapid, valid, reversible and safe, which provide us available approach for performing the cortical structural and functional imaging at high temporal-spatial resolution.

9707-42, Session PSun

Recognition of short-term changes in physiological signals with the wavelet-based multifractal formalism

Alexey N. Pavlov, Olga S. Sindeeva, Sergey S. Sindeev, Olga N. Pavlova, Elena V Rybalova, Oxana V. Semyachkina-Glushkovskaya, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

Based on the laser speckle contrast imaging and wavelet-analysis we study a latent (“hidden”) stage of the development of intracranial hemorrhages in newborn rats. We apply two measures based on the continuous wavelet-transform of the blood flow velocity in the sagittal sinus, namely, the spectral energy and a multiscale degree quantifying complexity of experimental data. We show that the wavelet-based multifractal formalism reveals changes in the cerebrovascular blood flow at the development of intracranial hemorrhage. Complexity measure quantifying the width of the singularity spectrum represents a quite sensitive characteristics of the hidden stage of intracranial hemorrhage. Based on this measure, a reduction of complexity was revealed. The latter can be treated as a marker of pathological changes in cerebrovascular blood flow.

9707-43, Session PSun

Speech signal denoising with wavelet-transforms and the mean opinion score characterizing the filtering quality

Alaudeen S. Yaseen, Alexey N. Pavlov, Alexander E. Hramov, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

We study the possibility of improving the quality of optical image denoising with wavelet-based techniques. For this purpose, approaches based on the classical discrete wavelet transform (DWT) and the complex wavelet transform (CWT) are considered. Within the first approach, Daubechies wavelets with the hard and the soft thresholding are used. It is shown that the soft thresholding increases denoising performances of 2-D DWT compared with the hard thresholding. Nevertheless, selection of the threshold level becomes of high importance. Statistical analysis performed for different optical images allowed us to reach better noise reduction with the soft thresholding approach. Denoising abilities of CWT are studied that is a nearly shift invariant method. The latter means that image resizing does not essentially influence the wavelet coefficient patterns. The performed analysis has shown that CWT reduces error of image denoising compared with the classical 2-D DWT.

9707-44, Session PSun

Full-field tracking and measurement of the motion of particles in capillary vessels by using time-varying laser speckle

Hongxian Zhou, Zhenhe Ma, Yi Wang, Northeastern Univ. at Qinhuangdao (China)

We propose a semi-random perturbation model to describe the variations of laser speckle patterns caused by moving particles in capillary vessels. When passing through probing volume, moving particles encode random perturbations into observed laser speckle patterns. We extract the perturbation envelopes of time-varying laser speckles for tracking the motion of single particle. And, the full-field transverse velocities of flowing particles are obtained by using cross-correlation between the perturbation envelopes. The proposed method is experimentally verified by the use of polymer microsphere suspension in a glass capillary.

9707-45, Session PSun

Quantitative measurement of particle concentration by using optical coherence tomography

Luying Zhang, Foshan Univ. (China); Zhenhe Ma, Yi Wang, Northeastern Univ. at Qinhuangdao (China)

We demonstrate the statistic characteristic of the intensity fluctuations of the light backscattered by particle flow using an optical coherence tomography (OCT) system. When passing through a detection volume, particles encode their moving information into OCT signals. The fluctuating
Conference 9707: Dynamics and Fluctuations in Biomedical Photonics XIII

9707-46, Session PSun

OCT as the convenient tool to assess the quality and application of the organotypic retinal samples

Ying Yang, Nicholas Khoshnaw, Rachel Gater, Keele Univ. (United Kingdom)

Diseases such as retinal detachment and macular degeneration have profound consequences on the quality of human life. Without treatment, these diseases could lead to loss of sight. To develop better treatments for retinal diseases, including cell therapies and drug intervention, establishment of an efficient and reproducible 3D native retinal tissue system, enabling over a prolonged culture duration, will be very valuable. The retina is a very complex tissue, consisting of ten layers with different density and cellular composition to each. Uniquely, as a light transmitting tissue, retinal refraction of light differs among the layers, forming the good basis to use optical coherence tomography (OCT) in assessing the layered structure of the retina and its change during the culture and experimentation. In this study, we develop a new methodology to generate retinal organotypic tissues, and use three substrates which mimic supportive vitreous: filter paper, collagen hydrogel and amnion membrane to culture the organotypic tissue. Freshly slaughtered pig eyes have been obtained for use in this study. The layered morphology of intact retinal tissue in eyecup and organotypic retinal tissue in culture under above three substrates have been examined by an spectral domain OCT. The viability of the tissues have been examined by live/dead fluorescence dye kit to correlate the OCT images. For the first time, it is demonstrated that vitreous-like substrates support the viability of retinal organotypic tissue, capable of prolonged culture up to 15 days. Overall, OCT is a convenient tool for appraising the quality and application of organotypic retinal samples and is crucial in the development of current organotypic models.

9707-47, Session PSun

Measurement of cerebral blood flow rate and its relationship with brain function using optical coherence tomography

Jian Liu, Zhenhe Ma, Yi Wang, Yuqian Zhao, Northeastern Univ. at Qinhuangdao (China); Shidan Dou, Northeastern Univ. (China); Yushu Ma, Northeastern Univ. at Qinhuangdao (China)

Activity of brain neurons will lead to changes in local blood flow rate. Thus, it is important to measure the local blood flow rate of cerebral cortex on research of neuron activity in vivo, such as rehabilitation evaluation after stroke, etc. Currently, laser Doppler flowmetry is commonly used for blood flow measurement, however, relatively low resolution limits its application. Optical coherence tomography (OCT) is a powerful noninvasive 3D imaging modality with high temporal and spatial resolutions. Furthermore, OCT can provide flow distribution image by calculating Doppler frequency shift which makes it possible for blood flow rate measurement. In this paper, we applied OCT to measure the blood flow rate of the primary motor cortex in rats. The animal was immobilized and anesthetized with isoflurane, an incision was made along the sagittal suture, and bone was exposed. A skull window was opened on the primary motor cortex. Then, blood flow rate changes in the primary motor cortex were monitored by our homemade spectral domain OCT with a stimulation of the passive movement of the front legs. Finally, we established the relationship between blood flow rate and the test design. The aim is to demonstrate the potential of OCT in the evaluation of cerebral cortex function.

9707-48, Session PSun

Phototoxicity of cationic porphyrins and nanocomposites of anisotropic silver

Grigor V. Gyulkhandanyan, Institute of Biochemistry (Armenia); Robert K. Ghazaryan, Yerevan State Medical Univ. (Armenia); Anna G. Gyulkhandanyan, Institute of Biochemistry (Armenia); Marina H. Paronyan, Scientific and Production Ctr. Armbiotechnology (Armenia); Marina A. Sheyryan, Yerevan State Univ. (Armenia); Aram G. Gyulkhandanyan, Institute of Biochemistry (Armenia); Elena S. Tuchina, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Valery V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation) and Institute of Precision Mechanics and Control (Russian Federation) and National Research Tomsk State Univ. (Russian Federation)

Photodynamic inactivation of microorganisms by photosensitizers is one of the most promising trends for the destruction of the antibiotic-resistant microorganisms. We have studied the photodynamic inactivation some Gram (+) and Gram (-) microorganisms (St. aureus, E.coli) by cationic porphyrins. Investigations of phototoxicity of porphyrinic preparations were performed at sufficiently low concentrations at which they are no longer toxic without light exposure. Thus, when studying the photodynamic action of metalloporphyrin Zn-TBu4PyP on strain E. coli K12 the phototoxicity study should be conducted at concentrations of 5 μg/ml or less, for Zn-TOE4PyP - at concentrations of 20 μg/ml or less. Investigations of photodynamic activity have shown that efficiency of Zn-metalloporphyrins was 3-5 times higher than metal-free porphyrins, and microorganisms completely were destroyed at concentrations of 2-3 μg/ml. Previously we have shown that for destruction of microorganism St. aureus 209P and methicillin-resistant strains (MRSA) concentrations of Zn-metalloporphyrins needed 0.1 μg/ml or less. Efficacy of photosensitizers against microorganisms increases significantly by using nanocontainers and in particular of anisotropic silver nanoparticles (ASN). Photodynamic efficiency of such nanocomposites [ASN + Zn-metalloporphyrin] compared to Zn-metalloporphyrins is significantly higher. Addition into solution of small amounts of monovalent or divalent salts (NaCl, CaCl2) leads to complete desorption of porphyrins and metalloporphyrins from silver nanoparticles and a significant increase in the local concentration of the photosensitizer in the vicinity of the cell wall or inside bacteria. In combination with the effect of plasmon resonance such nanocomposites can be highly effective agents for struggling against a wide spectrum of microorganisms.

9707-49, Session PSun

Photodynamic damages of red blood cells membranes

Natalia V. Tkachenko, Alexander B. Pravdin, Valery V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Nikita A. Navolokin, Natalia V. Polukonova, Saratov State Medical Univ. (Russian Federation); Alexander A. Serov, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

Process of photodynamic haemolysis is a rather convenient model for monitoring sensitized photodestructive processes in cell membranes, including the effect of ambient conditions variation. The dynamics and severity of photodestruction depend on different factors, the most important physical and chemical factor being the amount of reactive oxygen...
may either dissolve and release the drug, or an external signal must trigger the release of drug-loaded particulate carrier systems. In the hair follicles the particles with diameters of approximately 600 nm in diameter were found to penetrate best into the hair follicles, where they can be stored for maximally 10 days. Their retention time in the hair follicles exceeds that in the stratum corneum by almost one order of magnitude. Particles penetrate more efficiently into the hair follicles than non-particulate substances.

Topical application of nanoparticles: prospects and safety aspects *(Invited Paper)*

Jürgen M. Lademann, Helike Richter, Sora Jung, Martina C. Meinke, Charité Universitätsmedizin Berlin (Germany); Eckart Rühl, Ulrike Alexiev, Marcelo Calderon, Freie Univ. Berlin (Germany); Alexa Patzelt, Charité Universitätsmedizin Berlin (Germany)

The requirements on nanoparticles for cosmetic and medical applications are very different. While nanoparticles applied in sunscreens shall remain on the skin surface or in the upper cell layers of the stratum corneum, nanoparticles for medical drug delivery shall penetrate through the skin barrier to the target structures in the living cells.

Under the Collaborative Research Project 1112 various methods are used at the CCP to investigate the cutaneous penetration and storage of nanoparticles, hair follicles being in the focus of attention. Human hair follicles are ideal target structures for drug delivery. Hosting both the stem and dendritic cells, they are surrounded by a dense network of blood vessels. Investigating nanoparticles of different size and materials, particles of approximately 600 nm in diameter were found to penetrate best into the hair follicles, where they can be stored for maximally 10 days. Their retention time in the hair follicles exceeds that in the stratum corneum by almost one order of magnitude. Particles penetrate more efficiently into the hair follicles than non-particulate substances. For particles from 40 nm-1 μm in diameter, however, no follicular penetration has been detectable if the skin barrier was intact. This is plausible as the hair follicle has its own barrier.

It will be demonstrated that the best way for drug delivery is the application of drug-loaded particulate carrier systems. In the hair follicles the particles may either dissolve and release the drug, or an external signal must trigger the drug release from the particle.

Detection of dermal systemic sclerosis using noncontact optical coherence elastography

Chih-Hao Liu, Yong Du, Mamunohan Singh, Jiasong Li, Chen Wu, Zhaolong Han, Raksha Raghunathan, Thomas Hsu, Shezaan Noorani, Anthony Chang, Chandra Mohan, Kirill V. Larin, Univ. of Houston (United States)

Systemic sclerosis (SSc) is a connective tissue disease where fibrosis arises due to excessive collagen accumulation in skin or other internal organs and is commonly diagnosed by the presentation of fibrotic skin. The modified Rodnan skin score (mRSS) is considered as the benchmark for monitoring SSc progression by assessing the severity of dermal fibrosis. Contact-based mechanical testing devices such as the Vesometer and Durometer have been proposed for detecting SSc. However, inter-observer variability can bias the mRSS measurement, and the sensitivity of mechanical testing is insufficient for detecting SSc in its early stages. Therefore, an objective, accurate, and highly sensitive technique that can overcome these limitations could provide a more robust basis for early SSc detection. Optical coherence elastography (OCE) is an emerging technique that can image mechanical contrast in tissue with micrometer scale spatial resolution and may be able to overcome the aforementioned limitations. In this work, we present the first use of OCE to differentiate SSc in murine skin in vitro and in vivo. Our results demonstrate that OCE was able to distinguish healthy and fibrotic skin (P<0.05), and the OCE results were in good agreement with histopathological and skin thickness assessments. Therefore, OCE is potentially useful for aiding in SSc diagnosis.
Noncontact imaging of plethysmographic pulsation and spontaneous low-frequency oscillation in skin perfusion with a digital red-green-blue camera

Izumi Nishidate, Akira Hoshi, Yuta Aoki, Tokyo Univ. of Agriculture and Technology (Japan); Kazuya Nakano, Tokyo Univ. of Science (Japan); Kyuichi Niizeki, Yamagata Univ. (Japan); Yoshihisa Aizu, Muroran Institute of Technology (Japan)

Plethysmogram is the change in blood volume due to the cardiac pulse traveling through the body. Vasomotion is the spontaneous oscillation in the diameter of blood vessels with 1-4 cycles/min (alpha wave) and 4-8 cycles/min (beta wave), independent of the cardiac pulse or respiration. It is said that the plethysmogram and vasomotion are related to the autonomic nervous system. We propose a non-contact imaging method to evaluate plethysmogram and vasomotion with a digital red-green-blue camera, based on the Monte Carlo simulation (MCS) of light transport in skin tissue. In the method, the RGB-values are converted into the tristimulus values in CIE XYZ color space which is a device-independent color system and compatible with the common RGB working spaces. MCS is used to specify a relation among the tristimulus XYZ-values and the concentrations of oxygenated hemoglobin (ChbO) and deoxygenated hemoglobin (ChbR). Once we determine the empirical formula, images of ChbO and ChbR are reconstructed without the MCS. The image of total hemoglobin concentration (ChbT) can be simply calculated as a sum of ChbO and ChbR. Applying the fast Fourier transform band pass filters to each pixel of the sequential images for ChbT along the time line, two-dimentional plethysmogram and vasomotion can be reconstructed. The frequency bands of the FFT filters were 0.7-3.0 Hz for plethysmogram and 0.009-0.2 Hz for vasomotion. In vivo experiments with human skin before and during mental stress induced by the Stroop color-word test demonstrated the feasibility of the method to evaluate the activities of autonomic nervous systems.

Breath air measurement using wide-band frequency tuning IR laser photo-acoustic spectroscopy

Yury V. Kistenev, Alexey V. Borisov, National Research Tomsk State Univ. (Russian Federation); Dmitry A. Kuzmin, Siberian State Medical Univ. (Russian Federation); Andrey A. Boyko, Nadezhda Y. Kostyukova, Alexey A. Karapuzikov, Special Technologies, Ltd. (Russian Federation); Anna A. Bulanova, Siberian State Medical Univ. (Russian Federation)

The results of measuring of biomarkers in breath air of patients with broncho-pulmonary diseases using wide-band frequency tuning IR laser photo-acoustic spectroscopy and the methods of data mining are presented. We will consider the original experimental equipment based on photo-acoustic detector and wide-band frequency tuning IR laser source with optical parametric oscillator (OPO). The analysis of the data was based on preprocessing of the experimental spectra, dimension reducing of the feature space and classification of the data. The examples of application of Principal Component Analysis and Canonical Correlation Analysis to component analysis of the breath air spectra will be presented.

The work was carried out with partial financial support of the FCPIR contract No 14.578.21.0082 (ID RFMEFI57814X0082).

Imaging pulse wave velocity in mouse retina using swept-source optical coherence tomography

Shaozhen Song, Wei Wei, Ruikang K. Wang, Univ. of Washington (United States)

Blood vessel dynamics has been a significant subject in cardiology and internal medicine, and pulse wave velocity (PWV) on artery vessels is a classic evaluation of arterial distensibility, and has never been ascertained as a cardiovascular risk marker. The aim of this study is to develop a high speed imaging technique to capture the pulsatile motion on mouse retina arteries with the ability to quantify PWV on any arterial vessels. We demonstrate a new non-invasive method to assess the vessel dynamics on mouse retina arteries with repeated B-scans are obtained on mouse retina capillary bed, and the mouse oxymeter signal is recorded simultaneously. The pulse wave on artery and vein are resolved, and with the synchronized heart beat signal, the temporal delay on different vessel locations is determined. The vessel specific measurement of PWV is achieved for the first time with SS-OCT, for pulse waves propagating more than 100 cm/s. Using the novel methodology of PWV assessment, it is hoped that the clinical OCT scans can provide extended diagnostic information of cardiology functionalities.
Wavelet-based multifractal analysis of dynamic infrared thermograms and X-ray mammograms to assist in early breast cancer diagnosis

Alain Arneodo, École Normale Supérieure de Lyon (France)

Breast cancer is the most common type of cancer among women and despite recent advances in the medical field, there are still some inherent limitations in currently used screening techniques. The radiological interpretation of X-ray mammograms often leads to over-diagnosis and to unnecessary traumatic and painful biopsies. We use the 2D Wavelet Transform Modulus Maxima (WTMM) method to reveal changes in skin temperature dynamics of women breasts with and without malignant tumor. We show that the statistics of temperature temporal fluctuations about the cardiogenic and vasomotor perfusion oscillations do not change across time-scales for cancerous breasts as the signature of homogeneous monofractal fluctuations. This contrasts with the continuous change of temperature fluctuation statistics observed for healthy breasts as the hallmark of complex multifractal scaling. When using the 2D WTMM method to analyze the roughness fluctuations of X-ray mammograms, we reveal some drastic loss of roughness spatial correlations that likely results from deep architectural change in the tumor microenvironment. This local breast disorganisation may deeply affect heat transfer and thermomechanics in the breast tissue and in turn explain the loss of multifractal complexity of temperature temporal fluctuations previously observed in mammary glands with malignant tumor. These promising findings could lead to the future use of combined wavelet-based multifractal processing of dynamic IR thermograms and X-ray mammograms to help identifying women with high risk of breast cancer prior to more traumatic examinations. Besides potential clinical impact, these results shed a new light on physiological changes preceding anatomical alterations in breast cancer development.

Fluorescence polarization imaging for detecting breast cancer at cellular level

Anna N. Yaroslavsky, Xin Feng, Univ. of Massachusetts Lowell (United States)

Breast cancer is the most common non-skin-related cancer among women in the United States. At present breast cancer is diagnosed using invasive biopsy followed by histological evaluation of tissue and cells based on morphology. As some benign cells/lesions closely mimic appearance of breast carcinoma, there is a need for rapid, objective, and sensitive technique of diagnosing cancer at the cellular level. The goal of this study was to investigate the feasibility of discriminating cancer cells quantitatively using Methylene Blue (MB) dye and exogenous fluorescence polarization imaging.

Biodynamic profiling of three-dimensional tissue growth techniques (Invited Paper)

Hao Sun, Daniel A. Merrill, David D. Nolte, John J. Turek, Purdue Univ. (United States)

Three-dimensional tissue culture is replacing conventional two-dimensional cell culture in pharmaceutical applications because it presents a more biologically-relevant environment in which to perform drug development. However, there are several popular growth methods that are not equivalent and may not lead to biologically-relevant tissue models on which to screen drugs. For instance, growth in rotating bioreactors takes many weeks during which the tissue is able to evolve natural extracellular matrix with high adhesion density. At the other extreme is the U-bottom multiwell plate that has become one of the dominant growth techniques for 3D tissue growth. The assembly into a spheroidal tissue takes only several days, and can be easily parallelized to provide many samples for high-throughput screening. However, the rapid aggregation is not equivalent to slow growth in the bioreactor, and U-bottom tumor spheroids lack significant ECM and have low adhesion density. One of the challenges to unravelling whether these growth techniques may provide high-accuracy drug screens has been the difficulty of obtaining high-content information from inside three dimensional tissue. A new high-content 3D imaging approach that is helping to meet this challenge is biodynamic imaging. Biodynamic imaging probes three-dimensional living tissue with low-coherence scattered light to measure subcellular motions inside their natural microenvironments. The information content of biodynamic imaging is displayed through tissue dynamics spectroscopy (TDS), which captures and displays the changes in the Doppler signatures from intracellular constituents in response to applied therapeutics. In this paper, we use biodynamic imaging to study three different types of three-dimensional multicellular spheroid growth: rotating bioreactor, hanging-drop and plate-
grown spheroids. These three growth techniques show systematic variations among properties such as tissue cohesiveness and intracellular activity, and they display different pharmacokinetics and pharmacodynamics under identical drug dose conditions. These results demonstrate that not all three-dimensional tissue culture are equivalent, and that drug-response studies must take into account the method of growth.

9707-27, Session 7

Subcellular metabolic contrast in living tissue using dynamic full field OCT (D-FFOCT)

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Cells shape or density is an important marker of tissues pathology. However, individual cells are difficult to observe in thick tissues frequently presenting highly scattering structures such as collagen fibers. Endogenous techniques struggle to image cells in these conditions. Moreover, exogenous contrast agents like dyes, fluorophores or nanoparticles cannot always be used, especially if non-invasive imaging is required.

Scatterers motion happening down to the millisecond scale, much faster than the fix and highly scattering structures (global motion of the tissue), allowed us to develop a new approach based on the time dependence of the FF-OCT signals. This method reveals hidden cells after a spatiotemporal analysis based on singular value decomposition and wavelet analysis concepts. It does also give us access to local dynamics of imaged scatterers. This dynamic information is linked with the local metabolic activity that drives these scatterers.

Our technique can explore subcellular scales with micrometric resolution and dynamics ranging from the millisecond to seconds. By this mean we studied a wide range of tissues, animal and human in both normal and pathological conditions (cancer, ischemia, osmotic shock...) in different organs such as liver, kidney, and brain among others. Different cells, undetectable with FF-OCT, were identified (erythrocytes, hepatocytes...). Different scatterer clusters express different characteristic times and thus can be related to different mechanisms that we identify with metabolic functions.

We are confident that the D-FFOCT, by accessing to a new spatiotemporal metabolic contrast, will be a leading technique on tissue imaging and could lead to better medical diagnosis.

9707-28, Session 7

The relationship between decorrelation time and sample thickness in brain tissue

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The optical opacity of biological tissue has long been a challenge in biomedical optics due to the strong scattering nature of tissue in the optical regime. While most conventional optical techniques attempt to gate out multiply scattered light and use only unscattered light, new approaches in the field of wavefront shaping exploit the time reversible symmetry of optical scattering in order to focus light inside or through scattering media. While these approaches have been demonstrated effectively on static samples, it has proven difficult to apply them to dynamic biological samples since even small changes in the relative positions of the scatterers within will cause the time symmetry that wavefront shaping relies upon to decorrelate.

In this paper we investigate the decorrelation curves of acute rat brain slices for thicknesses in the range 1-3 mm (1/e decorrelation time on the order of seconds) using multi-speckle diffusing wave spectroscopy (MSDWS) and compare the results with theoretical predictions. The results of this study demonstrate that the 1/L^2 relationship between decorrelation time and thickness predicted by diffusing wave spectroscopy provides a good rule of thumb for estimating how the decorrelation of a sample will change with increasing thickness. Understanding this relationship will provide insight to guide the future development of biophotonic wavefront shaping tools by giving an estimate of how fast wavefront shaping systems need to operate to overcome the dynamic nature of biological samples.

9707-29, Session 7

Measuring intracellular motion in cancer cell using optical coherence tomography

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Optical coherence tomography (OCT) is an emerging biomedical imaging technique that can perform cellular-resolution imaging in situ and in real-time. In this study, we demonstrate that OCT speckle decorrelation techniques can be used to probe intracellular motion in cancer cells.

Spheroids and cell pellets were used as a model to probe intracellular motion. ZnCl2 was used to inhibit mitochondrial motion within the cells. The longitudinal acquisition of in situ cell spheroid and cell pellet B-Scans and volumetric scans were done using high speed MEMS-VCSEL Swept Source OCT from Thorlabs (100nm @ 1300nm Swept Source Laser operating at 100,000 A-scan/s). OCT system axial resolution is ~12µm lateral resolution is ~25µm. The OCT images (B-scans) consist of 100 A-scans (lines) acquired over 0.5 and 1 mm to provide high frame rate (500 Frames/s acquisition speed) required for our analysis. The volumetric scans were acquired in repetitive 3D and Speckle Variance over a time period of 21 days. In this paper we present speckle decorrelation and motility map analysis of the cancer cell spheroid and pellets. The results reveal the changes in intracellular motion during the spheroid growth phase and as pellets were exposed to ZnCl2. The speckle decorrelation time during the growth phase of spheroids decreased by 35 ms over 21 days and 25 ms during inhibition of mitochondrial motion 10 minutes after exposure to ZnCl2.

9707-30, Session 7

DoFP polarimeter based polarization microscope for biomedical applications

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Polarization microscope is a useful technique to observe the optical anisotropic nature of biomedical specimens and can provide more microstructural information than the conventional microscope. In this paper, we present a division of focal plane (DoFP) polarimeter based polarization microscope which is capable of imaging both the Stokes vector and the 3 ? 4 Mueller matrix of the sample. The Mueller matrix measurement can help us completely understand the polarization properties of the sample and the Stokes vector measurement is a simultaneous technology. First, we calibrate a DoFP polarimeter using the polarization data reduction method for accurate Stokes vector measurements. Second, as the Stokes vector computation for all pixels using the calibrated instrument matrix is usually time consuming, we develop a GPU acceleration algorithm for real time Stokes vector calculations. Third, based on the accurate and fast Stokes vector calculation, we present an optimal 4-states of polarization (4-SoP) illumination scheme for Mueller matrix measurement using the DoFP polarimeter. Finally, we demonstrate the biomedical applications of the DoFP polarimeter based polarization microscope. Experimental results show that the characteristic features of many biomedical samples can be observed in the polarization staining images using the circularly polarized light as illumination. In this way, combined with GPU acceleration algorithm, the DoFP polarization microscope has the capacity for real time polarization monitoring of dynamic processes in biological samples.
Deep tissue ablation using ultrafast laser with optical clearing

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Background: Deep tissue ablation is the next challenge in ultrafast laser microsurgery. By focusing ultrafast pulses below the tissue surface one can create an ablation void confined to the focal volume. However, as the ablation depth increases in a scattering tissue, increase in the required power can trigger undesired nonlinear phenomena out of focus that restricts our ability to ablate beyond a maximum ablation depth of few scattering lengths. Optical clearing (OC) might reduce the intensity threshold and increase the maximal ablation depth by lowering the refractive index mismatch, and therefore reducing scattering. Some efforts to ablate deeper showed out of focus damage, while others used brutal mechanical methods for clearing. Our clinical goal is to create voids in the scarred vocal folds and inject a biomaterial to bring back the tissue elasticity and restore phonation.

Materials and methods: Fresh porcine vocal folds were excised and immersed in a biocompatible OC agent (75% glycerol). Collimated transmittance was monitored. The tissue was optically cleared and put under the microscope for imaging and ablation 60 microns deep. Threshold energy was also recorded.

Results: With the same energy as in control samples, the OC tissue ablation depth increased by 80%. Respectively, for the same depth, pulse energy giving the same results was reduced. Undesired damaged was not observed. Scattering length was almost doubled, corresponding to collimated transmittance measurements.

Conclusion: Optical clearing can reduce the threshold energy for ablation and increase the maximal ablation depth. This technique can potentially improve clinical microsurgery.

Longitudinal optical characterization of bacterial biofilm growth and dynamics

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Bacterial biofilms are prevalent in many natural, industrial, and medical settings. Due to their robust nature and natural resilience to antibiotics, biofilms that negatively impact human health are currently of great interest and concern in medicine and surgery. Biofilms are complex communities of multiple species of bacteria residing within an exo-polymeric matrix, and can form and thrive on many surfaces such as on sutures, endotracheal tubes, catheters, prosthetics, and even on tissues within the body.

In previous studies, our group has used handheld in vivo optical coherence tomography (OCT) to image biofilm structures affixed to the tympanic membrane in patients with chronic or recurrent cases of otitis media (OM). OM is an extremely common infection of the middle ear that affects up to 95% of children. To better assess the appearance, structure, and optical properties of these biofilm structures in vivo, single and mixed strain otopathogenic bacterial biofilms were grown in vitro using a rocker plate within an incubation chamber at 37 ºC and 5% CO2. Biofilm growth dynamics were imaged and tracked with OCT over the course of several days, and measurements, including bulk refractive index and biomass thickness, were acquired. These findings help to clarify not only how biofilms appear under OCT, but also to calculate a more accurate biofilm thickness when observed in vivo. Generally, this information will help to accurately visualize longitudinal biofilm growth and regression in vivo, as well as various clinical interventions, such as antibiotic treatment or surgical intervention.
Overtone absorption-based intravascular (IVPA) catheter is a promising technology for quantifying the amount of lipid and its spatial distribution inside the arterial wall. Thus far, the clinical translation of IVPA technology is limited by its slow imaging speed due to lack of a high-power and high-repetition-rate laser source for lipid-specific excitation at 1.7 μm. Here, we demonstrate a potassium titanyl phosphate-based optical parametric oscillator (OPO) with output pulse energy up to 2 mJ at a wavelength of 1724 nm and with a repetition rate of 500 Hz. This OPO enabled IVPA imaging at 1 frame per sec, which is about 50-fold faster than previously reported IVPA systems. The IVPA imaging system was characterized by a pencil lead and a lipid-mimicking phantom for its imaging resolution, sensitivity, and specificity, respectively. Its performance was further validated by ex vivo study of an atherosclerotic human femoral artery and comparison to gold standard histology.

Vascular elastic photoacoustic tomography in humans

Pengfei Hai, Yong Zhou, Jinyang Liang, Chiye Li, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Elastic properties of biological tissue are related to their structures and functions and are often altered in pathological states. In blood vessels, the elastic properties are strongly affected by abnormal hemodynamic states induced by thrombosis, which can lead to severe conditions such as acute myocardial infarction, stroke, and pulmonary embolism. Thus, quantification of vascular elastic properties can contribute to the detection of thrombosis and prevention of potential life threatening conditions. Here, we propose vascular elastic photoacoustic tomography (VE-PAT) to measure blood vessel compliance in humans. VE-PAT was developed by incorporating a linear-array-based photoacoustic computed tomography system with a customized compression stage. By measuring the deformation of blood vessels under uniaxial loading, VE-PAT was able to quantify the vascular compliance. We first demonstrated the feasibility of VE-PAT in blood vessel phantoms. In large vessel phantoms, VE-PAT detected a decrease in vascular compliance due to simulated thrombosis, which was validated by a standard compression test. In small blood vessel phantoms embedded 3 mm deep in gelatin, VE-PAT detected changes at depths that are difficult to image using other elasticity imaging techniques, such as ultrasound elastography or optical coherence elastography. We then applied VE-PAT to assess vascular compliance in a human subject and detected a decrease in vascular compliance when an occlusion occurred downstream from the measurement point, demonstrating the potential of VE-PAT in clinical applications such as detection of deep venous thrombosis.

High-speed intravascular photoacoustic imaging at 1.7 μm

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Lipid deposition inside the arterial wall is a hallmark of plaque vulnerability. Overtone absorption-based intravascular photoacoustic (IVPA) catheter is a promising technology for quantifying the amount of lipid and its spatial distribution inside the arterial wall. Thus far, the clinical translation of IVPA technology is limited by its slow imaging speed due to lack of a high-power and high-repetition-rate laser source for lipid-specific excitation at 1.7 μm. Here, we demonstrate a potassium titanyl phosphate-based optical parametric oscillator (OPO) with output pulse energy up to 2 mJ at a wavelength of 1724 nm and with a repetition rate of 500 Hz. This OPO enabled IVPA imaging at 1 frame per sec, which is about 50-fold faster than previously reported IVPA systems. The IVPA imaging system was characterized by a pencil lead and a lipid-mimicking phantom for its imaging resolution, sensitivity, and specificity, respectively. Its performance was further validated by ex vivo study of an atherosclerotic human femoral artery and comparison to gold standard histology.

Clinical real-time photoacoustic/ultrasound imaging system at POSTECH

Jeesu Kim, Sara Park, Yuhan Jung, Pohang Univ. of Science and Technology (Korea, Republic of); Yumiao Zhang, Jonathan Lovell, The State Univ. of New York (United States); Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

We have successfully developed a clinical real-time photoacoustic/ultrasound (PA/US) imaging system. The combined PA/US imaging system was adapted with a FDA approved commercial US imaging system (ALPINION Medical) and a portable pulsed laser system. All image processing and display tasks were performed in the US imaging system, and thus no post-processing was required. Not only a linear US array transducer but also other types such as a convex array and a phased array was implemented. The US transducer probe was integrated with a bifurcated optical fiber bundles. We have tested the performance of the developed system by measuring the signal-to-noise-ratios, noise-equivalent sensitivities, and spatial resolutions using organic naphthalocyanine nanoformulations (nanonaps) in biological tissues. The maximum penetration depth was ~4.5 cm with a laser pulse energy of 6 mJ/cm² in the near-infrared region. The noise equivalent concentration at ~2-cm-depth was 0.36 mg/mL. The axial resolution was maintained around 200 μm at all imaging depths. By applying a linear scanning along one transverse direction, volumetric PA/US imaging was performed. We have successfully acquired the volumetric PA/US images of gastro intestinal tracts in rats in vivo after oral administration of organic nanonaps. We believe that the developed clinical real-time PA/US imaging system can be utilized in various preclinical and clinical studies in the near future.
tumor thickness can be accurately determined in vivo with a handheld photoacoustic probe. In that study, a single ultrasonic transducer and a 10 Hz laser were employed, limiting the imaging speed to around one frame every ten seconds. In addition, only the horizontal boundaries of the tumor could be detected due to the limited view angle of the focused transducer. To address these limitations, we applied a linear-array-based photoacoustic probe to detect the tumor depth and volume of melanin-containing melanoma in nude mice in vivo. This system can image melanomas at five frames per second (fps), which is much faster than our previous handheld single transducer system (0.1 fps). We first theoretically show that, in addition to the higher frame rate, almost the entire boundary of the melanoma can be detected by the linear-array-based probe, while only the horizontal boundary could be detected by the previous system. Then we demonstrate the ability of this linear-array-based system in measuring both the depth and volume of melanoma through phantom, ex vivo, and in vivo experiments. The volume detection ability also enables us to accurately calculate the rate of growth of the tumor, which is an important parameter in quantifying the tumor activity. Our results show that this system can be used for clinical melanoma diagnosis and treatment in humans at the bedside.

9708-5, Session 1

Photoacoustic imaging system for small-vessel imaging based on clinical ultrasound technology
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One of the features of photoacoustic (PA) imaging is small-vessel visualization realized without injection of contrast agent or exposure to X-ray. For carrying out clinical study in this field, prototype PA imaging system has been developed. The PA imaging system utilizes technological platform of FUJIFILM’s clinical ultrasound (US) imaging system mounting manycore MPU for enhancing the image quality of US B and Doppler modes which can be superposed to PA images. By evaluating the PA images and the US Doppler images of the prototyped system, applicability of the prototype system to small-vessel visualization has been discussed.

The light source for PA imaging was on a compact cart of US unit and emitted 750 nm wavelength laser pulses. The laser light was transferred to illumination optics in hand-held US transducer which was connected to the US unit. Obtained PA rf data are reconstructed to PA images in the US unit. 3D images were obtained by scanning a mechanical stage which the transducer is attached to.

Several peripheral parts like finger and foot were observed by PA and US Doppler imaging. As for small arteries, US Doppler images were better than PA images for visualizing, for example, arc-shaped artery in the tip of finger. As for small veins, PA images showed a good resolution and connectivity. Therefore, superposed images of the PA and US Doppler images can visualize both the small arteries and veins, which should be a differentiating feature of US/PA combined technology from other clinical vascular imaging modalities.

9708-6, Session 1

The application of differential frequency-domain photoacoustics for characterizing arterial vessels
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According to Statistics Canada, in year 2011, 60,910 deaths were recorded due to cardiovascular related diseases. This number accounts for 25.2% of total deaths in Canada in that year. Among the most important cardiovascular diseases is atherosclerosis, a chronic disease occurred with gradual build-up of lipid rich plaques in the inner layer of the arterial wall. Current methods of diagnosis of atherosclerosis consist of angiography, intravascular ultrasound (IVUS) and optical coherence tomography (OCT). The sensitivity of all these methods is inadequate, as the ideal technique should be capable of both depth profiling, as well as functional imaging. An alternative technique is photoacoustics (PA) which can perform deep imaging and spectroscopy. The presented study explores the application of the wavelength-modulated differential photoacoustic radar (WM-DPAR) for characterizing arterial vessels. The wavelength-modulated differential photoacoustic technique was shown to be able to increase the dynamic range and sensitivity of hemoglobin oxygenation level. This technique was used with a very high frequency modulation range (12-17 MHz). To perform spectrosopic PA imaging, at least two wavelengths are required. The selected wavelengths for this work were 1210 nm and 980 nm. 1210 nm corresponds to the maximum optical absorption coefficient of cholesterol and cholesteryl esters which are the main constituent of plaques. Since water, elastin and collagen also have high absorption coefficients at 1210 nm, therefore this wavelength alone cannot provide a very high sensitivity. The additional wavelength, 980 nm, corresponds to high absorption coefficient of those constituents in healthy artery tissue. The simultaneous application of the abovementioned wavelengths can provide higher sensitivity in detecting lipids in the arterial vessels.

9708-7, Session 1

An automated breast ultrasound scanner with integrated multispectral photoacoustic tomography
Corey Kelly, Hamid Moradi, Tim E. Salcudean, The Univ. of British Columbia (Canada)

We have integrated photoacoustic imaging into an automated breast ultrasound scanner to simultaneously perform ultrasound (US) and multispectral photoacoustic tomography (PAT). This was accomplished with minimal change to the existing automated scanner by coupling laser light into an optical fibre for flexible and robust delivery.

By illuminating locally (near the current acquisition slice) rather than globally, this approach reduces overall light exposure at the tissue surface, allowing higher light intensity per acquisition (which translates to higher absorber contrast), while remaining below safe exposure thresholds. Precise knowledge of the optical fibre geometry allows the spatial profile of the illumination in the tissue to be deconvolved from the reconstructed initial pressure distribution. This provides accurate localization of absorbers and minimizes background noise.

We present preliminary phantom data acquired with this setup, and compare potential weighting schemes for deconvolving the illumination geometry. We employ a novel, deconvolution-based reconstruction technique which can account for array directivity and limited view. We also present time-domain simulations used to validate the data and associated reconstruction scheme.

9708-8, Session 2

Integrated transrectal probe for translational ultrasound-photoacoustic imaging
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Previously we demonstrated advantages of using photoacoustic imaging for visualization of brachytherapy seeds over pure ultrasound imaging. However, these previous studies used handheld linear array probes with external illumination and were not suitable for clinical translation. We report the development of a custom trans-rectal probe for ultrasound and photoacoustic imaging. The probe houses a 192-element 5 MHz linear array.
Interventional multi-spectral photoacoustic imaging in laparoscopic surgery

Emma R. Hill, Wenfeng Xia, Daniil I. Nikitichev, Matthew J. Clarkson, Crispin Schneider, Univ. College London (United Kingdom); Kurinchi Gurusamy, Univ. College London (United Kingdom); Paul C. Beard, David J. Hawkes, Brian R. Davidson, Adrien E. Desjardins, Univ. College London (United Kingdom)

Laparoscopic liver resection can be an attractive treatment option for liver cancer, with a shortened hospital stay and reduced morbidity compared to open surgery. One of the central challenges of this technique is visualisation of concealed critical structures in the liver, including the hepatic artery, the portal vein and the bile duct. As photoacoustic (PA) imaging can provide contrast for haemoglobin and bilirubin in real time, it may be well suited to guiding laparoscopic procedures in order to avoid inadvertent trauma to vascular structures. In this study, a clinical laparoscopic ultrasound probe was used to receive ultrasound for PA imaging and to obtain co-registered B-mode ultrasound (US) images. Pulsed excitation light in the wavelength range of 750-900 nm was delivered to the tissue via a fibre bundle. Monte Carlo simulations were performed to optimise the light delivery geometry for photoacoustic signal generation, and a 3D-printed mount was used to position the fibre bundle relative to the transducer according to the simulation results. The performance of the photoacoustic laparoscope imaging system was evaluated with phantoms and a porcine model. Multi-spectral PA images were processed to provide contrast for blood oxygenation. The clinical potential of hybrid PA-US imaging to improve the guidance of laparoscopic liver surgery is discussed.

Interventional multispectral photoacoustic imaging with a clinical linear array ultrasound probe for guiding nerve blocks

Wenfeng Xia, Simeon J. West, Univ. College London (United Kingdom); Daniil I. Nikitichev, Paul C. Beard, Adrien E. Desjardins, Univ. College London (United Kingdom)

Accurate identification of tissue structures such as nerves and blood vessels is critically important for interventional procedures such as nerve blocks. Ultrasound imaging is widely used as a guidance modality to visualize anatomical structures in real-time. However, identification of nerves and small blood vessels can be very challenging, and accidental intra-vascular or intra-vascular injections can result in significant complications. Multi-spectral photoacoustic imaging can provide high sensitivity and specificity for discriminating hemoglobin- and lipid-rich tissues. However, conventional surface-illumination-based photoacoustic systems suffer from limited sensitivity at large depths. In this study, for the first time, an interventional multispectral photoacoustic imaging (IMPA) system was used to image nerves in a swine model in vivo. Pulsed excitation light with wavelengths in the ranges of 750 – 900 nm and 1150 – 1300 nm was delivered inside the body through an optical fiber positioned within the cannula of an injection needle. Ultrasound waves were received at the tissue surface using a clinical linear array imaging probe. Co-registered B-mode ultrasound images were acquired using the same imaging probe. Nerve identification was performed using a combination of B-mode ultrasound imaging and electrical stimulation. Using a linear model, spectral-unmixing of the photoacoustic data was performed to provide image contrast for oxygenated and de-oxygenated hemoglobin, water and lipids. Good correspondence between the nerve locations and the lipid-rich regions in the photoacoustic images was observed. The results indicate that IMPA is a promising modality for guiding nerve blocks and other interventional procedures. Challenges involved with clinical translation will be discussed.

Minimum energy and fiber diameter requirements for safe photoacoustic guidance of endonasal neurosurgeries

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Endonasal transsphenoidal surgery, an effective approach for pituitary adenoma resection, poses the serious risk of injury to the internal carotid arteries hidden by bone. Intraoperative photoacoustic imaging implemented with an optical fiber attached to the surgical tool and transducer placed on the temple has the potential to eliminate this risk, requiring light and sound transmission through the sphenoid and temporal bones, respectively. To investigate energy requirements for vessel visualization, experiments were conducted with a 4.8 mm inner diameter blood-filled tube, 0.5-2.0 mm thick human adult cadaveric bone specimens, and an ex vivo sheep brain tissue. An Ultrasonix SonixTouch ultrasound scanner and operated at 760 nm wavelength. The fiber bundle output energy was varied from 1.2 to 9.3 mJ. Ten photoacoustic images acquired at each energy level were summed prior to beamforming. After image formation, signal contrast was measured as a function of energy for each sphenoid-temporal bone combination, and these data were fit to polynomial curves. The minimum energy required to visualize the vessel boundaries with at least 4.5 dB contrast ranged 12.5-50 mJ for 0.15 mm sphenoid and 1 mm temporal bone thicknesses, increasing to 4.2-5.9 mJ for 0-0.5 mm sphenoid and 1.5-2.0 mm temporal bone thicknesses. Associated laser beam diameter requirements were derived from ANSI maximum permissible exposure guidelines by assuming a circular exposure surface area. Results indicate that minimum beam diameters of 2.4-5.3 mm are required to maintain safety within the current guidelines for skin (26.4 mJ/cm²), which is likely a conservative estimate for the proposed application. Nonetheless, these safety conditions may be achieved with multiple fibers surrounding the surgical tool.

VHF-induced thermoacoustic imaging of fresh human prostates using a clinical ultrasound transducer array

Sarah K. Patch, Univ. of Wisconsin-Milwaukee (United States)
we examined the capacity of volumetric optoacoustic tomography (VOT) for success hereby depends on accurate real-time monitoring of lesion progression and characteristics of the vessel wall further showing correlation with the temperature elevation in the area adjacent to the ablation spot.

9708-14, Session 2
In vivo cryoablation of prostate tissue with real-time optoacoustic monitoring
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Cryoablation of prostate cancer is a clinically approved procedure, which involves repetitive rapid cooling of lesion to lethal temperatures of ~40oC and below. The major drawback of the technique is insufficient control over the fast thermal processes that may result in severe complications (hemorrhage, incontinence, and perforation of the rectal wall) and morbidity. The developed optoacoustic imaging technique provides non-invasive real-time temperature mapping and monitoring of the iceball and enables more efficient control over the procedure, which is necessary to reduce side effects and accelerate physician’s learning curve. In these studies we successfully demonstrated optoacoustic thermography of prostate and adjacent rectal wall using real-time transrectal optoacoustic imaging during prostate cryoablation in live canine models. The technique employed previously discovered invariance of the temperature-dependent normalized optoacoustic response of blood. Nanosecond-pulse radiation of Ti-sapphire laser tuned to the isosbestic point of hemoglobin (805 nm) was delivered via fiberoptic illuminators assembled on both sides of the linear array of the standard transrectal ultrasound probe. Temperature readouts at discrete locations inside and nearby prostate were also performed using standard transperinidal needle sensors. The analysis of collected data showed that in vivo accuracy using non-invasive optoacoustic temperature mapping was in agreement with thermocouples up to +4oC for the entire range of temperatures from +34 to -16oC. Optoacoustic imaging was also able to delineate boundaries of the developing ice ball, with leading edge correlated well on both optoacoustic and ultrasound imaging modalities, the latter used as the clinically accepted standard.

9708-15, Session 3
A full-field illumination approach with multiple speckle for optical-resolution photoacoustic endoscopy
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Optical-resolution photoacoustic endomicroscopy (OR-PAE) allows going beyond the limited penetration depth of conventional optical-resolution photoacoustic systems. Recently, it has been shown that OR-PAE may be performed through minimally invasive multimode fibers, by raster scanning a focus spot with optical wavefront shaping (1). Here we introduce for the first time an approach to perform OR-PAE through a multimode fiber with a full-field illumination approach. By using multiple known speckle patterns, we show that it is possible to obtain optical-diffraction limited photoacoustic images, with the same resolution as that obtained by raster scanning a focus spot, i.e. that of the speckle grain size. The fluctuations patterns of the photoacoustic amplitude at each pixel in the sample plane with the series of multiple speckle illumination were used to encode each pixel. This approach with known speckle illumination requires an initial calibration stage, that consists in learn a set of fluctuation patterns pixel by pixel, which will encode patterns each pixel of the scanned area. A point-like absorber was scanned across the filed-of-view during the calibration stage to acquire the reference patterns. Image reconstruction may be carried out

VOT is capable of monitoring lesion progression and characteristics of the vessel wall further showing correlation with the temperature elevation in the area adjacent to the ablation spot.
by cross-correlating the series of photoacoustic amplitude measured with the sample to the reference patterns obtained during the calibration stage. In this work, the approach above was carried out both theoretically with Monte-carlo simulations and experimentally through a multi-mode fiber with samples made of absorbing spheres.


9708-16, Session 3
Prostate cancer characterization by optical contrast enhanced photoacoustics
Guan Xu, Univ. of Michigan Medical School (United States); Ming Qin, Univ. of Michigan (United States); Javed Siddiqui, Scott A. Tomlins, Univ. of Michigan Medical School (United States); Raoul Kopelman, Univ. of Michigan (United States); Xueling Wang, Univ. of Michigan Medical School (United States)

During the past decades, prostate cancer (PCa), with an annual incidence rate much higher than any other cancer, is the most commonly diagnosed cancer in American men. PCa has a relatively low progression rate yet the survival percentage decreases dramatically once the cancer has metastasized. Identifying aggressive from indolent PCa to prevent metastasis and death is critical to improving outcomes for patients with PCa. Standard procedure for assessing the aggressiveness of PCa involves the removal of tumor tissues by transrectal (TR) ultrasound (US) guided needle biopsy. The microscopic architecture of the biopsied tissue is visualized by histological or immunohistochemical staining procedures. The heterogeneity of the microscopic architecture is characterized by a Gleason score, a quantitative description of the aggressiveness of PCa. Due to the inability to identify the cancer cells, most noninvasive imaging modalities can only provide diagnosis of PCa at limited accuracy. This study investigates the feasibility of identifying PCa tumors and characterizing the aggressiveness of PCa by photoacoustic imaging assisted by cancer targeting polyacrylamide (PAA) nanoparticles (NPs). PAA is a biocompatible material used in clinics for the past 20 years. PAA NPs can protect capsulated optical contrast agents from interference by enzymes and enable prolonged systematic circulation in the living biological environment. The cancer targeting mechanism is achieved by conjugating the NPs to F3 peptides, which trace nucleolin overexpressed on the surface of cancer cells. Preliminary studies have shown that the NPs are capable of staining the PCa cells in vivo.

9708-17, Session 3
Optoacoustic endoscopy in curved scanning mode
Hailong He, Andreas Buehler, Vasilis Ntziachristos, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany)

Optoacoustic (photoacoustic) imaging is well suited for endoscopic procedures. Optoacoustic techniques in particular has been shown to resolve anatomical, functional and molecular features at depths that go beyond what is possible using optical imaging or optical microscopy. In this paper, we interrogate the merits of optoacoustic endoscopy implemented by translating a sound detector in linear or curved geometries. The linear and curved detection geometries are achieved by scanning an intravascular ultrasound transducer (IVUS) within a plastic guide shaped to line or a curve. This curved geometry offers larger effective acceptance angles, along the direction of image formation, which can improve image quality compared to rotational systems. Phantoms and in vivo mouse measurement show that the curved scanning endoscopy achieved better image quality than the linear scanning configuration. Overall, the presented concept could be used together with optical endoscopes to yield hybrid optical and optoacoustic imaging performance better than the one be achieved by optical imaging alone.

9708-18, Session 3
Characterizing intestinal strictures with acoustic resolution photoacoustic microscopy
Guan Xu, Univ. of Michigan Medical School (United States); Hao Lei, Univ. of Michigan (United States); Shengchun Liu, Laura A. Johnson, Peter D. R. Higgins, Michael D. Rice, Univ. of Michigan Medical School (United States); Jun Ni, Univ. of Michigan (United States); Xueling Wang, Univ. of Michigan Medical School (United States)

Crohn’s disease (CD) is an autoimmune disease of the intestinal tract affecting 700,000 people in the United States. The pathology of CD is characterized by obstructing intestinal strictures due to inflammation, fibrosis, or a combination of both. Inflammatory strictures are medically treated. Fibrotic strictures have to be removed surgically. The accurate assessment of the strictures is thereby critical for the management of CD. The standard procedure for characterizing an intestinal stricture is endoscopic biopsy in which small sample pieces are extracted for histopathology. Conclusive diagnoses are reached by observing the histochimical content and stratified microarchitecture within the samples. Most concurrent imaging modalities can only assess either of the histological features. This study proposes an endoscopic, acoustic resolution photoacoustic (PA) microscopy system for characterizing intestinal strictures. The PA microscopy system can assess the histological features of the strictures at ultrasonic resolution and optical sensitivity. The noninvasive diagnostic approach allows unlimited sampling locations. A tunable laser with output range of 680-1700 nm was used to cover the strong optical absorption of hemoglobin and collagen, correlated to inflammatory and fibrotic strictures, respectively. A ring illumination pattern was generated at the sample surfaces with optical intensity of 1 mJ per square centimeter. An US transducer with bandwidth of 20 MHz, central frequency of 50 MHz and focal length of 3 mm was used to acquire the PA signals. Imaging experiments on phantoms and animal intestinal tissues have validated that the proposed approach can differentiate inflammatory and fibrotic strictures. Human tissue study is planned.

9708-19, Session 3
Photoacoustic endoscopy probe using a coherent fibre-optic bundle and Fabry-Pérot ultrasound sensor
Rehman Ansari, Paul C. Beard, Edward Z. Zhang, Adrien E. Desjardins, Univ. College London (United Kingdom)

There is considerable interest in the development of photoacoustic endoscopy (PAE) probes for the clinical assessment of pathologies in the gastrointestinal (GI) tract, guiding minimally invasive laparoscopic surgeries and applications in foetal medicine. However, most previous PAE probes integrate mechanical scanners and piezoelectric transducers at the distal end which can be technically complex, expensive and pose challenges in achieving the necessary level of miniaturisation. We present two novel all-optical forward-viewing endoscopic probes operating in widefield tomography mode that have the potential to overcome these limitations. In one configuration, the probe comprises a transparent 40 Hz Fabry-Pérot ultrasound sensor deposited at the tip of a rigid, 3 mm diameter coherent fibre-optic bundle. In this way, the distal end of coherent fibre bundle acts as a 2D array of wideband ultrasound detectors. In another configuration, an optical relay is used between the distal end face of flexible fibre bundle and the Fabry-Pérot sensor to enlarge the lateral field of view to 6 mm x 6 mm. In both configurations, the pulsed excitation laser beam is full-field
Optoacoustic measurements of human placenta and umbilical cord blood oxygenation

Tatiana Nanovskaya, Mahmoud Ahmed, Irene Y. Petrov, Yuriy Y. Petrov, Svetlana Patrikeeva, Gary D. Hankins, Donald S. Prough, Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Adequate oxygenation is essential for embryogenesis and normal fetal growth and development. Perturbations in intrauterine oxidative environment are associated with early pregnancy loss, preeclampsia, and intrauterine fetal growth restriction. Our long term goal is to adapt the existing optoacoustic (OA) technology for monitoring placental and umbilical cord blood oxygenation in real time. We report here on validation of an OA probe for monitoring of oxygenation in the umbilical cord blood and placental tissue using the ex vivo technique of placenta perfusion. The accuracy of circulating blood oxygenation monitored by transmission mode OP, in absence of placental lobule, was compared to that determined by CO-Oximeter. Gas mixtures of 95%N2 + 5%CO2 and 95%O2 + 5%CO2 were alternated to decrease or increase blood oxygenation, respectively. The data obtained revealed that the value for oxygenation of the circulating blood determined by OA was in agreement with those determined by the CO-Oximeter. The oxygen saturation of placental tissue perfused with cord blood was determined by reflection mode OA. The values for oxygen saturation in the outflow tubing (fetal vein) determined by CO-Oximeter correlated well with oxygen saturation in placental tissue monitored by OA. Accordingly, OA measurements of placental oxygenation could represent the oxygen status of blood going to the fetus. Therefore, data sitdemonstrate that OP was used successfully to determine placental and umbilical blood oxygenation in real time.

9708-23, Session 4

Targeted therapy of animal eyes by laser generated focused ultrasound

Taehwa Lee, Wei Luo, Hakan Demirci, L. Jay Guo, Univ. of Michigan (United States)
Cavitation therapy based on high-amplitude focused ultrasound (e.g., Histotripsy) has shown great promise in clinical trials. The technique realizes localized treatments of tissues and diseased cells by controlling cavitation zones, which can be even smaller than its acoustic spot sizes. Also, the short pressure pulse used in the technique can minimize the unwanted heat accumulation, which the conventional piezoelectric transducers suffer from due to low operating frequencies and relatively long acoustic pulses. However, this modality requires bulky system composed of array of piezoelectric elements and electric amplifiers in order to obtain high pressure amplitude. Moreover, especially when treating an area much smaller than the acoustic spot size, this approach may be vulnerable to nucleation sites within the focal volume, which can potentially induce cavitation and thus enlarge the total treatment area.

Here, we show targeted cell-level therapy by using laser generated ultrasound. By employing a concave lens coated by a carbon nanotube (CNT)-polymer composite, high-amplitude acoustic pressure pulse was obtained at a tight focal spot (<100 um). The small focal spot, comparable to cavitation zone, lead to controlled cavitation treatment. Such feature can be exploited for treating intraocular tumors but without harming other parts of the eye (e.g. healthy retina and choroid) and therefore preserve the vision of the patients. We demonstrate that the localized disruption effects can be used for cell-level surgery to remove cells and to kill cells. Some experimental examples are shown using animal eyeballs.

9708-24, Session 4
Photoacoustic imaging of angiogenesis in subdermal islet transplant sites
Wei Shi, Rena Pawlick, Andrew Pepper, Boris Gala Lopez, Antonio Bruni, Min Choi, Roger J. Zemp, James Shapiro, Univ. of Alberta (Canada)

Type I Diabetes is an autoimmune disease affecting 5 to 10 percent of cases of diabetes worldwide and typically requires vigilant blood glucose management and insulin injections. Islet transplantation has emerged as a viable means of nearly curing patients if the transplant is successful and not rejected. Major surgery has previously been required for such techniques yet it has recently been discovered that subdermal transplantation may be an effective alternative transplant site and would have advantages of easy access and potential for simple monitoring. Key to future success will be the ability to monitor islet viability and the transplant microenvironment.

To prepare the subdermal microenvironment for maximum islet viability, implantable catheter systems are being developed to facilitate angiogenesis in the area where the islets will be transplanted, however, researchers are lacking techniques to monitor such angiogenesis. In our study we use photoacoustic and micro-ultrasound imaging to track the angiogenesis in longitudinal and non-longitudinal studies. We imaged mice with 1, 2, 3 and 4 weeks post-catheter implant on both side of abdomen using a FujiFilm VisualSonics Vevo-LZR system with a linear transducer mounted perpendicular to a linear motor to realize 3D imaging. Images before removing implant were taken as well to indicate the implant position once the implant was removed. Multi-wavelength excitation is used for further oxygenation studies. Quantitative analysis on vessel densities shows the gradually growth of vasculature in the implant position, indicating vessel growth successfully induced by implant. Our study demonstrates the ability to track angiogenesis around catheter sites prior to islet transplantation. The photoacoustic methodology has clinical translation potential owing to the longitudinal monitoring opportunities afforded by the non-invasive and safe imaging technique.
(HbT) and the haemoglobin oxygen saturation (sO2) were obtained. Bone formation was assessed using 7CT after 4 and 8 weeks of the surgery. High frequency linear-array based co-registered PA/US imaging has been found promising in terms of non-invasiveness, sensitivity, adaptability, high spatial and temporal resolution at sufficient depths for the analysis and qualitative assessment of the reparative ability of differentiated MSCs in a rat critical size bone repair defect model.

9708-27, Session 4
Characterizing intraocular tumors with physio-chemical photoacoustics
Guang Xu, Hakan Demirci, Zeynep Gursel, Xueding Wang, Univ. of Michigan Medical School (United States)

Intraocular tumors are life-threatening conditions. Long-term mortality from uveal melanoma, which accounts for 80% of primary intraocular tumors, could be as high as 25% depending on the size, ciliary body involvement and extraocular extension. The treatments of intraocular tumors include eye-sparing approaches such as radiotherapy and thermotherapy, and the more aggressive enucleation. The accurate diagnosis of intraocular tumors is thereby critical in the management and follow-up of the patients. The diagnosis of intraocular tumors is usually based on clinical examination with acoustic backscattering based ultrasonography. By analyzing the high frequency fluctuations within the ultrasound (US) signals, microarchitecture information inside the tumor can be characterized. However, US cannot interrogate the histochemical components formulating the microarchitecture. One representative example is the inability of US imaging (and other contemporary imaging modalities as well) in differentiating nevoid and melanoma cells as the two types of cells possesses similar acoustic backscattering properties. Combining optical and US imaging, photoacoustic (PA) measurements encode both the microarchitecture and histochemical component information in biological tissue. This study attempts to characterize ocular tumors by analyzing the high frequency signal components in the multispectral PA images. Ex vivo human eye globes with melanoma and blastoma tumors were scanned using less than 6 mJ per square centimeters laser energy with tunable range of 600-1700 nm. A PA-US parallel imaging system with US probes CL15-7 and L22-14 were used to acquire the high frequency PA signals in real time. Preliminary results show that the proposed method can identify melanoma against blastoma tumors.

9708-28, Session 4
In vivo microwave-based thermoacoustic tomography in rats
Li Lin, Yong Zhou, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Microwave-based thermoacoustic tomography (TAT), based on the measurement of ultrasonic waves induced by microwave pulses, can reveal tissue dielectric properties that may be closely related to the physiological and pathological status of the tissues. Using microwaves as the excitation source improved imaging depth because of their deep penetration into biological tissues.

We demonstrate, for the first time, in vivo microwave-based thermoacoustic imaging in rats. The transducer is rotated around the rat in a full circle, providing a full two-dimensional view. Instead of a flat ultrasonic transducer, we used a virtual line detector based on a cylindrically focused transducer. A 3 GHz microwave source with 0.6 μs pulse width and an electromagnetically shielded transducer with 2.25 MHz central frequency provided clear cross-sectional images of the rat’s body. The high imaging contrast, based on the tissue’s rate of absorption, and the ultrasonically defined spatial resolution combine to reveal the spine, kidney, muscle, and other deeply seated anatomical features in the rat’s abdominal cavity. This non-invasive and non-ionizing imaging modality achieved an imaging depth beyond 6 cm in the rat’s tissue. Cancer diagnosis based on information about tissue properties from microwave band TAT can potentially be more accurate than has previously been achievable.

9708-101, Session PSun
In vivo photoacoustic imaging in rabbit tumor models
Yeh-Chan Ahn, Pukyong National Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Jung-Eun Park, Yu-Gyeong Chae, Pukyong National Univ. (Korea, Republic of); Chul-Ho Oak, Eun-Kee Park, Jee-Yeong Jeong, Kosin Univ. (Korea, Republic of); Van Phuc Phuc, Hyun Wook Kang, Junghwan Oh, Pukyong National Univ. (Korea, Republic of); Sung Won Kim, Kosin Univ. (Korea, Republic of)

Photoacoustic tomography (PAT) is able to image microvasculature using endogenous and exogenous molecular contrasts. Angiogenesis is a process to provide tumor with nutrient and oxygen by making premature blood vessels around tumor. Indocyanine green (ICG) has been utilized for imaging tumor because the premature blood vessels have lots of ICG leakage toward the tumor. While there have been many PAT researches, in vivo tumor imaging has been limited mostly to melanoma or breast cancer.

In this study, we developed rabbit tumor models at thyroid, conjunctiva, and tunica vaginalis and obtained their photoacoustic images with/without ICG. We emphasized the importance of the developed models in studying a highly aggressive anaplastic thyroid cancer, an association between HIV infection and conjunctival tumor, and the asbestos-related mesothelioma. A photoacoustic system was developed with nanosecond Nd:YAG pulsed laser, OPO, axicon lens/condenser for Bessel beam illumination, and single-element ultrasound transducer. The photoacoustic imaging was also compared with Doppler ultrasound and histopathology.

9708-102, Session PSun
Photoacoustic detection of blood in dental pulp by using short-time Fourier transform
Azusa Yamada, Tohoku Univ. (Japan); Satoko Kakino, Tokyo Medical and Dental Univ. (Japan); Yuji Matsuura, Tohoku Univ. (Japan)

Diagnosis of dental pulp vitality is important in clinical practice and a non-invasive diagnosis method that is alternative to conventional dental probe or electrical stimulation is desired. Here we report experimental results for detection of blood in dental pulp by using photoacoustic analysis which can make further progress in diagnosis of dental pulp viability. In our experiment a 1064-nm microchip laser with pulse energy of 2 mJ and pulse width of 1.4 nsec is used as a light source. We used near-infrared light for excitation of photoacoustic wave because it has low absorption and scattering in dental hard tissues. To detect photoacoustic signals from the inside of tooth, a polymer-based, acoustic probe is attached on the tooth surface. Firstly it was shown that the photoacoustic signals from the teeth containing hemoglobin solution in the pulp cavity provide vibration in high frequency region and vibration intensity is dependent on the concentration of hemoglobin. We also examined the time-frequency distribution of the photoacoustic signals by applying a short-time Fourier transform. As a result, high frequency signals were detected at a delay time that was correspondent to the distance between the surface of the tooth and the dental pulp.
Fast and compact optical-resolution photoacoustic microscopy using a waterproof 2-axis MEMS scanner, and a step forward to clinical applications

Jin Young Kim, Changho Lee, Geunbae Lim, Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Optical-resolution photoacoustic microscopy (OR-PAM) is a novel microscopic tool to provide in vivo optically sensitive images in biomedical research. Conventional OR-PAM systems are typically slow and/or bulky because of the linear scanning stages with stepping motors. For practical purposes, however, the fast imaging speed and small footprint are crucial. To address these issues, we have developed a real-time compact OR-PAM system equipped with a waterproof 2-axis MEMS scanner. The OR-PAM system is composed of key components such as an ultrasonic transducer with a bandwidth of 50 MHz, an opto-acoustic beam combiner, and the MEMS scanner. These are all inside a small water tank, which has a size of only 30 × 30 mm along x, y, and z axes, respectively. A pulsed laser with a repetition rate of 50 kHz is confocally aligned with the photoacoustic (PA) waves in the beam combiner to maximize SNRs. The fast scanning ability of the MEMS scanner is able to fully utilize the A-scan speed of 50 kHz. For instance, B-scan and C-scan imaging speeds are 125 and 0.625 Hz, respectively when an acquired PA maximum amplitude projection image has 200 × 200 pixels along x and y axes, respectively. The measured lateral resolution of 3.6 µm and axial resolution of 27 µm are sufficient to resolve the small capillaries. Finally, we have successfully obtained the in vivo PA images of iris microvascular structures in mice. This real-time and compact OR-PAM system is optimized to examine the small animals and clinical studies.

Imaging of matrix metalloproteinases activity by using a photoacoustic microscopy system

Esra Aytac-Kipergil, Nasire Uluc, Aytac Demirkiran, Bogazici Univ. (Turkey); Hakan Erkol, Univ. of California, Irvine (United States); Mehmet Burcin Unlu, Bogazici Univ. (Turkey)

In cancer research, the evaluation of angiogenic and proangiogenic therapies as well as early detection of the disease is crucial, hence non-invasive imaging techniques monitoring molecular processes associated with the tumor angiogenesis are required. Expression of matrix metalloproteinases (MMP), particularly MMP-2 and MMP-9, is needed for angiogenesis and has been found to be upregulated in every type of human cancer, thus MMP can serve as a specific biological target for imaging of angiogenesis. However, the effect of MMPs in tumor angiogenesis is still unknown for most cancers. Photoacoustic microscopy system (PAM) with high resolution and sensitivity can be used for assessing the physical location and the time frame of MMP enzymatic activity. The goal of this study is to monitor MMP activity by photoacoustic imaging and to understand the role of the MMP in tumor growth. We developed a photoacoustic microscopy system (PAM) with unique supercontinuum (600-1100 nm) all fiber laser for the purpose. MMPSense-680 probe that provides signal only after the activation by MMP-2 is used to distinguish MMP-2-positive (HT-1080, human fibrosarcoma) and MMP-2-negative (BT20, adenocarcinoma) cell lines. The presence of MMP-2 is investigated with PAM by means of activation-dependent absorption changes. The study sheds light on understanding the role of the MMP in tumor growth and its correlation to the stage of cancer and its metastatic properties.

Combined label-free optical and optoacoustic imaging of model organisms at mesoscopy and microscopy resolutions

Dominik Soliman, Helmholtz Zentrum München GmbH (Germany); George J. Tsirelakakis, Foundation for Research and Technology-Hellas (Greece); Murad Omar, Helmholtz Zentrum München GmbH (Germany); Vasilis Ntziachristos, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany)

Contemporary optical microscopy methods have played a crucial role in terms of obtaining cellular and sub-cellular information from biological samples. However, due to ballistic light excitation, the maximum achieved imaging depth of conventional microscopy does usually not exceed a few hundred micrometers in tissue. For many biological applications, such as monitoring development, it is, however, necessary to image cellular-, tissue- and whole-organism level features and processes, as well as their inter-scale correlations, simultaneously. To achieve this, an appropriate imaging methodology should ideally combine mesoscopic and microscopic multi-contrast imaging capabilities, which is usually not offered by single modalities. We present a 5-modal multi-scale imaging system, combining optoacoustic mesoscopy and microscopy, two-photon, as well as second- and third-harmonic generation microscopy. All integrated modalities impart label-free imaging, thus avoiding photobleaching. The multi-photon and optoacoustic microscopy sub-systems are demonstrated to achieve lateral resolutions of 1.8µm and 0.8µm, respectively and an axial resolution of 5.8µm. On the other hand, optoacoustic mesoscopy allows for scanning 3 orders of magnitude larger volumes than microcopy, up to 3mm depth at 30µm lateral and 5.8µm axial resolution. We showcase the system’s unique zoom-in capability by performing multi-scale label-free imaging of two model organisms ex-vivo, a mouse ear and a zebrafish larva. The results confirm the broad resolution and depth penetration range offered by the developed system. Furthermore, the integration of several microscopy modalities provides highly complementary contrast over a wide range of biological structures. In this manner, a deeper understanding of complex properties at different scales is strongly enhanced.

Photoacoustic microscopy based on polydimethylsiloxane thin film Fabry-Perot optical interferometer

Soongho Park, Jonghyun Eom, Jun Geun Shin, Sungahn Rim, Byeong Ha Lee, Gwangju Institute of Science and Technology (Korea, Republic of)

We present a 5-modal multi-scale imaging system, combining optoacoustic mesoscopy and microscopy, two-photon, as well as second- and third-harmonic generation microscopy. All integrated modalities impart label-free imaging, thus avoiding photobleaching. The multi-photon and optoacoustic microscopy sub-systems are demonstrated to achieve lateral resolutions of 1.8µm and 0.8µm, respectively and an axial resolution of 5.8µm. On the other hand, optoacoustic mesoscopy allows for scanning 3 orders of magnitude larger volumes than microcopy, up to 3mm depth at 30µm lateral and 5.8µm axial resolution. We showcase the system’s unique zoom-in capability by performing multi-scale label-free imaging of two model organisms ex-vivo, a mouse ear and a zebrafish larva. The results confirm the broad resolution and depth penetration range offered by the developed system. Furthermore, the integration of several microscopy modalities provides highly complementary contrast over a wide range of biological structures. In this manner, a deeper understanding of complex properties at different scales is strongly enhanced.

Combined label-free optical and optoacoustic imaging of model organisms at mesoscopy and microscopy resolutions

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the sample is calculated. In order to evaluate the system performance, human hairs located in a water tank are used as the sample. This proposed imaging method can be used in various applications for the detection and measurement of acoustic waves.

9708-107, Session PSun

**Bessel beam Grueneisen relaxation photoacoustic microscopy with extended depth of field**

Junhui Shi, Lidai Wang, Lihong V. Wang, Washington Univ. in St. Louis (United States); Cedric Noordam, Univ. Twente (Netherlands)

In conventional optical resolution photoacoustic microscopy (OR-PAM), the short focal depth of the typically used Gaussian beam limits the volumetric imaging speed. A Bessel beam, which is essentially diffraction-free, provides a long focal depth, but its side-lobes deteriorate image quality if the Bessel beam is directly employed in OR-PAM. In this paper, to suppress the side-lobe artifacts in photoacoustic imaging, we present a nonlinear approach based on the Grueneisen relaxation effect. This method uses a two-step measurement scheme with Bessel beams. In the first step, one probe laser pulse generates a photoacoustic signal; in the second step, a heating laser pulse is applied before the second probe laser pulse is fired. Due to the nonlinear Grueneisen effect, the difference between the two signals from the two probe laser pulse excitations is proportional to the square of the optical fluence of the light beams. Therefore, the side-lobe signals from the Bessel beam are significantly attenuated in the differential photoacoustic image. Nonlinear Bessel-beam PAM effectively extends the focal depth of OR-PAM and speeds up volumetric imaging. We imaged a carbon fiber and red blood cell samples, experimentally demonstrating a 1-mm focal depth with a 7-μm lateral resolution.

9708-108, Session PSun

**A broadband PVDF-based hydrophone with integrated readout circuit for intravascular photoacoustic imaging**

Verya Daeichin, Erasmus MC (Netherlands); Chao Chen, Delft Univ. of Technology (Netherlands); Qing Ding, Technische Univ. Delft (Netherlands); Min Wu, Robert Beurskens, Geert Springeling, Erasmus MC (Netherlands); Emilie Noothout, Martin Verweij, K. W. A. van Dongen, Johan G. Bosch, Technische Univ. Delft (Netherlands); Antonius F. W. van der Steen, Erasmus MC (Netherlands) and Delft Univ. of Technology (Netherlands); Nico de Jong, Erasmus MC (Netherlands) and Technische Univ. Delft (Netherlands); Gijs van Soest, Erasmus MC (Netherlands)

Intravascular photoacoustic (IVPA) imaging can visualize the coronary atherosclerotic plaque composition on the basis of the optical absorption contrast. Most of the photoacoustic (PA) energy of human coronary plaque lipids was found to lie in the frequency band between 2 MHz and 15 MHz requiring a very broad band transducer, especially if a combination with intravascular ultrasound is desired. We have developed a broadband low-frequency polyvinylidene difluoride (PVDF) transducer (0.6 mm ? 0.6 mm, 52 ?m thick) with integrated electronics to match the low capacitance of such a small PVDF element (< 5 PF) with the high capacitive load of the long cable (> 100 PF). The new readout circuit provides an output voltage with a sensitivity of about 3.8 μV/Pa at 2.25 MHz. Its response is flat within 10 dB in the range from 2 MHz to 15 MHz. The root mean square (rms) output noise level is 259 μV over the entire bandwidth (1 MHz to 20 MHz), resulting in a minimum detectable pressure of 30 Pa at 2.25 MHz. Ultimately, such a small and sensitive detector can be incorporated inside an IVPA catheter with diameter of 1 mm. For optimal flexibility of the catheter only one coaxial cable is used to power the IC and to read out the received signal from the IC. Such a catheter can significantly improve the sensitivity of IVPA imaging of intraplaque lipids in humans and dramatically decrease the required energy of the laser pulse.

9708-109, Session PSun

**Optoacoustic response from carbon nanotubes embedded in a soft tissue-like phantom by using high-power diode-laser assemblies**

Luca Leggio, Daniel C. Gallego, Sandeep Babu GAWALI, Ehsan Dadrassnia, Univ. Carlos III de Madrid (Spain); Marek Osiński, The Univ. of New Mexico (United States) and Univ. Carlos III de Madrid (Spain); Guillermo Carpentero del Barrio, Horacio Lamela, Univ. Carlos III de Madrid (Spain)

The optoacoustic (OA) techniques for biomedical applications have inspired a growing interest in the scientific community during the last twenty years. Numerous studies have experimentally investigated the laser-excited OA tomography to detect chromophores within turbid tissues with different optical properties. Likewise, the contrast agents, such as nanoparticles and molecular chromophores with strong optical absorption in the near-infrared (NIR) spectral range, have been widely investigated to diagnose the presence of disease symptoms. Solid-state lasers and diode lasers have been both used for OA experiments, depending on the scope of the investigation. Although the solid-state lasers have the advantage to offer very high peak power compared to the diode lasers, they are bulky and costly. The preferred solution is to use assemblies of high-power diode lasers to efficiently combine their emitted optical power in an OA experiment.

In this work, we investigate the OA response and the optical absorption of carbon nanotubes embedded in a tissue-like phantom by using assemblies of high-power diode lasers operating in the NIR range. In order to achieve a better signal-to-noise ratio of OA signals, an optimization of the optical coupling between the diode laser beams and the optical fiber has to be considered. The optical beams are collimated with aspheric lenses and then coupled in an optical fiber bundle to achieve a good coupling efficiency. Afterwards, the OA signals and absorption coefficients of the carbon nanotubes are evaluated.

9708-110, Session PSun

**Planar waveguide light transmission modality for backward-mode photoacoustic tomography**

Mason W. Schellenberg, Paul J. D. Whiteside, Heather K. Hunt, Univ. of Missouri (United States)

Prior research in photoacoustic tomography has consistently demonstrated its ability to image structures near the surface of tissue with a high degree of optical contrast. However, despite significant advancements in the field, there has been little to no development of clinical applications for photoacoustic tomography, primarily due to the requirement for backward-mode operation, i.e., it must detect the photoacoustic signal on the same side of the tissue as the incident laser light. This results in the use standard ultrasonic transducer occluding the path of the incident laser beam. Therefore, developing a technique to deliver light into the tissue, while incorporating commonly available ultrasonic detection equipment without occluding the beam propagation or modifying the equipment in any way, would provide a significant benefit to the field, and potentially improve its clinical applicability. Here, we propose a new method to accomplish this
aim, using planar optical waveguides that employ the optical tunneling phenomenon to transmit light directly into tissue (pig skin) through physical contact with the sample. A commercially available, 15MHz, unfocused ultrasonic transducer was positioned on the rear face of the waveguide and was used to detect photoacoustic signals generated within the tissue as the signals propagated perpendicularly through the waveguide substrate. Unlike alternative solutions to the occlusion problem, this modality does not necessitate the use of custom manufactured transducers, expensive dichroics, or additional laser systems, and thereby represents a viable approach for the easy implementation of photoacoustic tomography in a clinical setting.

9708-113, Session PSun
Resonance effect of the laser generated ultrasound due to the reflection at the soft boundary
Wei Luo, Taehwa Lee, Univ. of Michigan (United States); Qiuyun Fu, Huazhong Univ. of Science and Technology (China); L. Jay Guo, Univ. of Michigan (United States)

The laser generated ultrasound technique are now attracting more and more attention for their wide bandwidth, high frequency and non contact characteristics. The optoacoustic conversion layer played a key role in this technique. The generated wave is a mono polar wave composed of wide frequencies. For some application such as the ultrasound imaging, signals with single frequency are more preferable. In that case, the resonance effect of the laser generated ultrasound signals were found due to the reflection at the optoacoustic conversion layer/air soft boundary.

When the laser shine on the optoacoustic conversion layer, the layer absorb the light energy and generate the longitudinal acoustic wave propagating with opposite directions. The upper propagating wave meets the soft layer/air boundary and turns back with a pi phase shift. The reflected wave and the generated wave will interfere with each other. The interference will be determined by the phase difference of the two waves, which is composed of the pi phase shift and the propagation phase shift. The conversion layer thickness is one important factor. If the layer thickness can fulfill the 2 pi propagation phase delay, a resonance will happen to the combined waves of which the amplitude will greatly enhanced. The frequency dependence of this resonance effect were investigated in both theoretical and experimental ways. The signals received at higher frequencies are more sensitive to the resonance effect than the low frequency ones.

This phenomenon could be utilized in the laser generated ultrasound technology to get a stronger narrow band signal.

9708-114, Session PSun
Optimization of the image reconstruction procedure in multi-focal photoacoustic computed tomography
Hongying Wan, Depeng Wang, Univ. at Buffalo (United States); Jing Meng, College of Information Science and Engineering (China); Liang Song, Shenzhen Institute of Advanced Technology (China); Leslie Ying, Jun Xia, Univ. at Buffalo (United States)

Photoacoustic-computed microscopy (PACM) is an emerging technology that provides wide-field high-resolution images of tissue optical absorption. PACM differs from conventional photoacoustic microscopy (PAM) imaging techniques in a way that thousands of optical foci are generated simultaneously, using a two-dimensional microlens array, and raster-scanning these optical foci provides wide-field images. A major limitation of PACM is the slow imaging speed caused by the use of high power pulsed lasers and large amount of acoustic detectors. In this study, we addressed this problem through compressed sensing and image inpainting. Compressed sensing can be used because the optical foci naturally form a sparse condition in the image domain. In addition to conventional L1 norm based compressed sensing, we incorporate the optical foci information as a partially known support to further shorten the iteration time and improve the image quality. To minimize the scanning time, we use an inpainting technique to sparsely scan the imaging field. Combining these two approaches, we improved the imaging speed by eight times in a simulation study, while still preserving the fine structures of the object.
Optoacoustic processing algorithms for intravascular imaging using optical interferometric ultrasonic sensors

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The optoacoustic (OA) effect consists ultrasound generation due to the absorption of electromagnetic radiation of certain wavelength. It offers visualization of optical contrast in tissues, within several millimetres to centimetres, with resolutions that are typical of ultrasound imaging. In many clinical applications, high frequency ultrasounds can only be used in combination with intravascular techniques due to the high acoustic attenuation in organic tissue at tens of megahertz. Optoacoustic imaging (OAI) has the goal of calculating the distribution of the optical absorption coefficient in tissues and requires computer-based reconstruction algorithms. The exact time-domain reconstruction formula produces images with excellent resolution but poor sensitivity, and an approximate implementation using a wavelet family resembling the theoretical N-shaped OA signal can be used to sharpen object boundaries while simultaneously preserving high contrast of the reconstructed objects.

In this work, we present the ultrasonic probe of an optoacoustic intravascular endoscope based on an interferometric optical ultrasonic sensor, being an important aspect the catheter design, particularly the optimization of the optical and acoustic parameters. To facilitate this process, we develop a model to simulate the ultrasonic pressure field, generated by optical absorption in a physical phantom using an intrinsic ultrasonic optic sensor. We also compare the results provided by these different approximations of OAI with real OA signals collected from the physical phantom design using the ultrasonic optic sensor. Our analysis demonstrates that the image of back-projected wavelet-transformed and simultaneously integrated OA pressure signals possesses the highest contrast and adequate resolution for this OA Intravascular imaging.

In vivo switchable optical- and acoustic-resolution photoacoustic microscopy

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Photoacoustic microscopy (PAM) provides a high-resolution and a deep penetration depth by utilizing high optical sensitivity and low scattering of ultrasound. The hybrid PAM system can be classified into two categories: optical-resolution photoacoustic microscopy (OR-PAM) and acoustic-resolution photoacoustic microscopy (AR-PAM). OR-PAM provides a very high lateral resolution with a strong optical focus, but the penetration depth is limited to one optical transport mean free path. AR-PAM provides a deeper penetration depth by using diffused light in biological tissues. The resolution of AR-PAM is determined by the ultrasonic parameters. In this study, we have tested a switchable OR-PAM and AR-PAM systems in vivo, manufactured by Micro Photoacoustics INC. In the combined system, two modes can be switchable by changing a collimator lens and optical fiber. The lateral resolution of the OR-PAM was measured by using a resolution test target and the full width at half-maximum (FWHM) of the edge spread function (ESF) was 4.79 μm. To calculate the lateral resolution of the AR-PAM, a 6-μm-diameter carbon fiber was used and the FWHM of the line spread function (LSF) was 45 μm. We have successfully demonstrated the multi-scale imaging capability of the switchable OR-/AR-PAM system by visualizing microvascular networks in mice ears, brains, legs, skins, and eyes.

Photoacoustic imaging probe to expand imaging range

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Photoacoustic imaging has attracted much attention to get B-scan images in medical diagnosis. Most of developed PAI probes are illuminated with light slightly oblique to the ultrasound beam to the side of the ultrasound detector. When the light and ultrasound paths are sloped at a predetermined angle, a dead zone may appear immediately below the probe. Tissue in the dead zone cannot be imaged using this structure. Therefore, a material, which has a similar acoustic impedance of human breast tissue such as a chicken breast or water, is required to achieve a clean image in the shallow depth region. For convenience in medical diagnosis, the PAI probe should be improved to minimize the dead zone, which is the undetectable region, and expand the imaging range. In order to eliminate the dead zone and to expand the imaging range, we here propose a probe structure aligning an illuminated axis of light with an axis of ultrasound using a beam combiner. It combines the light path and the ultrasound path using two slide glasses. Also The line-focus beam to obtain a high fluence for a B-scan is made by using a single optical fiber and three cylindrical lenses. Using our proposed photoacoustic imaging probe, we achieve an ultrasound imaging of a lymph node of nude mouse without loss of images right below our probe structure or additional medium between our probe and mouse skin.

Noncontact photoacoustic imaging by using a modified optical-fiber Michelson interferometer

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We demonstrate a noncontact photoacoustic imaging (PAI) system in which an optical interferometer is used for ultrasound detection. The system is based on a modified optical-fiber Michelson interferometer that measures the surface displacement caused by photoacoustic pressure. A synchronization method is utilized to keep the system at its high sensitive region in order to reduce the influence of ambient vibrations on the detection. The system is experimentally verified by imaging of a scattering phantom embodied with hairs and the blood vessels in a chick embryo. The experimental results indicate that the proposed system can be used for noncontact PAI with high resolution and high bandwidth.

High frame rate photoacoustic imaging using multiple wave-length LED array light source

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We have successfully imaged photoacoustic differences of light absorber between two images acquired by different wave-length LED array light source. Compared to photoacoustic imaging system using conventional solid-state
laser light source, LED light source can be driven at higher frequency pulses, so we were able to get the subtraction image at higher frame rate that calculated from two images which were captured at each wave-length LED light pulse timing.

We developed LED array light source which is composed to have two different wave-length chips, so each wave-length light pulse can be controlled and emitted freely. Thus LED array light source can be composed as multiple selectable wave-length more than two, and a various combination of subtraction image may become available at high frame rate.

9708-120, Session PSun

High energy laser pulse coupling in a multimode fiber for photoacoustic tomography

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In traditional photoacoustic tomography, external illumination is used to excite acoustic waves. However, with the assistance of fiber-transmitted light, multidirectional illumination or internal illumination can be achieved which brings more flexibility to the imaging system. This change is necessary for in vivo imaging on patient, because light can be guided and delivered to the imaging location with convenience. While, laser pulses delivered by fiber are energy-limited by the fiber’s core size and damage threshold. To maximize the amplitude of the photoacoustic waves and their penetration, it is necessary to improve the fiber coupling energy and efficiency. Currently, a multimode fiber with a core size of 1.5mm diameter can transmit at most 30mJ per pulse. The use of fiber bundles with core sizes of a few millimeters can even output 70mJ per pulse. Despite of coupling comparatively less energy, single fibers are more flexible and smaller in size than fiber bundles, which make them ideal for internal illumination especially in narrow spaces inside human body. To improve the coupling performance of single fibers, we use a cross-cylindrical lenses array to homogenize the incident beam before a coupling lens. Simulation in Zemax shows that this approach flattens the beam profile on the fiber front surface, decreasing the risk of fiber damage. Experimental results carried out with fibers of 1mm and 1.5mm diameter cores show that both types of fiber can output more than 50mJ per pulse at 700nm. The measured coupling efficiency as the wavelength is changed from 675nm to 900nm is maintained above 70% and even reached 90%. This improvement will benefit photoacoustic tomography applications using internal illumination.

9708-122, Session PSun

Numerical and experimental analysis of high frequency acoustic microscopy and infrared reflectance system design for early detection of melanoma

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Melanoma is a very malicious type of cancer as it metastasizes early and hence its late diagnosis leads to death. Consequently, early diagnosis of melanoma and its removal is considered the most effective way of treatment. We present a design of a high frequency acoustic microscopy and infrared reflectance system for the early detection of melanoma. Specifically, the identification of morphological changes related to carcinogenesis is required. In this work, we simulate of the propagation of the ultrasonic waves of the order of 100 MHz as well as of electromagnetic waves of the order of 100 THz in melanoma structures targeting to the estimation and optimization of the basic characteristics of the systems. The simulation results of the acoustic microscopy subsystem aim to provide information such as the geometry of the transducer, the center frequency of operation, the focal length where the power transmittance is optimum and the spot size in focal length. As far as the infrared is concerned the optimal frequency range and the spot illumination size of the external probe is provided. This information is next used to assemble a properly designed system which is applied to melanoma phantoms as well as real skin lesions. Finally, the measurement data are visualized to reveal the information of the experimented structures, proving noteworthy accuracy.

9708-121, Session PSun

Photoacoustic radiation force on a microbubble

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Microbubbles can move through the vasculature of tumor due to their micron size. Because of this property, they have been proposed as carrier vehicles for drugs and genes and are also used as a contrast agent for photoacoustic imaging. In this study, the photoacoustic radiation force on a microbubble is theoretically investigated. Particularly, the effect of laser parameters on the radiation force is analyzed. For this purpose, a new and comprehensive analytical solution to the photoacoustic wave equation based on the Fourier transform is obtained for various absorbers. Then, an expression of the radiation force including explicit laser parameters, pulse duration, and beam width of the laser is obtained. Besides, the primary radiation force acting on a microbubble is calculated. It is shown that laser parameters and the position of the microbubble relative to a photoacoustic source have a considerable effect on the primary radiation force. Here, we report that an adjustable radiation force can be introduced on microbubbles, taking the tunability of pulse duration and repetition frequency of laser into account. High spatial control of applied force is ensured on account of smaller focal spots achievable by focused optics. In this regard, conventional piezoelectric acoustic source application could be surpassed. Additionally, the radiation force can be increased by making source wavelength with the absorption peak of absorber concurrent. Manipulation of -1.45 mJ per pulse by photoacoustic radiation force opens a cache of opportunities for applications including transportation of contrast agents, drugs and genes to the location of interest.

9708-123, Session PSun

High frame rate photoacoustic imaging using clinical ultrasound system

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Photoacoustic tomography (PAT) is a potential hybrid imaging modality which is gaining attention in the field of medical imaging. Typically a Q-switched Nd:YAG laser is used to excite the tissue and generate photoacoustic signals. But, they are not suitable for clinical applications owing to their high cost, large size. Also, their low pulse repetition rate (PRR) of few tens of hertz prevents them from being used in real-time PAT. So, there is a growing need for an imaging system capable of real-time imaging for various clinical applications. In this work, we are using a nanosecond pulsed laser diode as an excitation source and a clinical ultrasound system to obtain the photoacoustic imaging. The excitation laser is ~803 nm in wavelength with energy of ~1.45 mJ per pulse. So far, the reported frame rate for photoacoustic imaging is only a few hundred Hertz. We have demonstrated up to 7 KHz photoacoustic imaging (B-mode) and measured the flow rate of fast moving object. Phantom experiments were performed to test the fast imaging capability and...
measure the flow rate of ink solution inside a tube. This fast photoacoustic imaging can be used for various clinical applications including cardiac-related problems, where the blood flow rate is quite high, or other dynamic studies.

9708-124, Session PSun

A practical optical-resolution photoacoustic microscopy prototype using a 300 mW visible laser diode

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Optical-resolution photoacoustic microscopy (OR-PAM) is an emerging technique for microvascular imaging at high spatial resolution and contrast. As of yet, most OR-PAM systems use bulky and expensive solid-state lasers for photoacoustic excitation, which make the systems large, costly, and impracticable, complicating their clinical integration. However, there is an ever-growing hunger to develop compact and affordable OR-PAM systems for standard clinical applications. In this work, we present a practical visible laser-diode-based OR-PAM prototype for vasculature imaging, which has the desirable properties of being portable, low-cost, and label-free. The prototype employs a 300 mW pulsed laser diode in a 3.8 mm diameter package, emitting 172 ns pulses at 405 ± 5 nm wavelength and a pulse energy of 52 nJ. An aspheric objective with a NA of 0.60 is used to achieve microscopic optical illumination. The laser diode excitation has a compact size of 1.8 ? 1.2 cm assembled with the collimating lens, and the customized driver circuit also has a compact size of 7 ? 4.8 cm. After low-pass filtering, the photoacoustic signal has a SNR up to 38 dB with averaging several pulses. The lateral resolution has been tested to be 0.95 µm on a 7 µm carbon fiber, and the surface flaws on carbon fibers are clearly visualized. The subcutaneous microvasculature on a mouse back was imaged ex vivo without exogenous contrast agent, which demonstrates the potentiality of the proposed prototype for in vivo imaging skin chromophores, such as hemoglobin, melanin, and so on. Our ultimate aim is to provide a practical and affordable OR-PAM system as a routine instrument for clinical applications.

9708-125, Session PSun

Laser-scanning optical-resolution photoacoustic microscopy using a virtual point detector concept

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Recently, laser-scanning optical-resolution photoacoustic microscopy (LSOR-PAM) has been proposed to increase the 3D imaging speed; however, its use of a needle hydrophone or an unfocused ultrasound transducer for obtaining large field-of-view (FOV) compromises the signal-to-noise ratio (SNR). LSOR-PAM with an unfocused transducer also suffers the spatial impulse response (SIR) effect – the non-uniform piezoelectric response of the transducer to the photoacoustic sources in the FOV. The SIR effect also deteriorates the axial resolution and hinders the use of the high frequency broadband transducer required for spectroscopic applications. To solve these problems, we propose a virtual point detector concept for LSOR-PAM to improve the SNR and mitigate the SIR effect while retaining the imaging speed and minimizing the loss in FOV. The focal point of a focused transducer is viewed as a virtual point detector which can be placed as close to the sample as possible to act like a real one even though the LSOR-PAM is with an optical and acoustic beam combiner. Experimental results showed that there was a trade off between the SNR and FOV determined by the distance between the virtual point detector and sample, which can be well tailored for different applications. The higher SNR (> 6 dB) than that of LSOR-PAM using an unfocused transducer could be obtained. Moreover, the suppression of the SIR effect was proven by the correlation among each A-line. Comparison between the transducers with different center frequencies and in vivo micro-vascular imaging of a mouse ear was also drawn.

9708-126, Session PSun

Simultaneous photoacoustic and optical attenuation imaging of single cells using photoacoustic microscopy

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A new technique for simultaneously creating photoacoustic images and images based on the optical attenuation in single cells has been developed. This technique was used to examine stained and unstained leukocytes and erythrocytes in human blood smears. A photoacoustic microscope outfitted with a 1000MHz transducer and a pulsed 532nm laser was used to image individual cells with micrometer resolution. The transducer and 20X optical objective used for laser focusing were aligned coaxially on opposing sides of the sample. Absorption of laser photons by the sample yielded traditional photoacoustic signals, while photons which passed through the sample fell incident directly upon the ultrasound transducer and generated a photoacoustic signal within it. Both signals, which were separated in time, were recorded by the system in a single a-line. Co-registered photoacoustic images and optical attenuation (OA) images were created by raster scanning and time-gating the acquired signals.

In stained leukocytes, cell nuclei were clearly visible in both photoacoustic and OA images. Cellular regions exhibiting strong photoacoustic signals corresponded to high optical attenuation in the OA images. In unstained blood smears, endogenous absorption at 532nm in leukocytes is too low for the generation of photoacoustic signals; however, OA images demonstrated nuclear delineation not observable even in optical microscopy images. Since the OA signal is generated within the transducer, images were also created without the use of ultrasound coupling fluids. This imaging method has applications in probing optical absorption and attenuation parameters at the sub-cellular level, and examining samples which cannot be immersed in liquid.

9708-127, Session PSun

Fast integrated intravascular photoacoustic/ultrasound catheter

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Photoacoustic (PA) imaging is a medical imaging technique with a high ultrasound (US) resolution and strong optical contrast. In this study, we have successfully developed an integrated intravascular photoacoustic/ultrasound catheter (IV-PAUS) with a diameter of 1.2 mm to image lipid rich atherosclerosis. An Nd:YAG pulsed laser with an excitation wavelength of 1064 nm was utilized, and 20,000 A-line PA images were acquired without signal averaging. The IV-PAUS offers 5-mm depth penetration in tissue mimicking phantoms. An axial and lateral resolutions of PA images measured were 96 and 242 µm, measured by imaging a 6 µm carbon fiber. We successfully obtained the 3D co-registered PA/US images of metal stents. Finally, we successfully obtained the 3D co-registered PA/US ex vivo images of rabbit arteries which contained lipid-rich plaques inside. After ex vivo imaging, our results were validated through histology.
Evaluation of intracellular delivery efficiency of gold nanoparticles by integrated photoacoustic microscopy and confocal fluorescence microscopy

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The dependence of photoacoustic signal from cells after being incubated with gold nanoparticles (AuNPs) on density of cell targeting ligands on the surface of AuNPs is not fully understood. We investigated such dependence by using RGD peptide-conjugated AuNPs and prostate carcinoma DU145 cells. DU145 cells were prepared via overnight incubation in culture medium containing equal concentration of RGD-peptide conjugated AuNPs only differing in the density of RGD immobilized onto AuNPs. Integrated photoacoustic microscopy (PAM) and confocal fluorescence microscopy (CFM) reveal that signal first reach the maximum with density increase of RGD peptide bound onto AuNPs and then decrease when further increasing the surface density of RGD peptide.

An ultra compact laser diode source for integration in a hand held point-of-care photoacoustic scanner

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Photoacoustics has repeatedly demonstrated its high potential for early detection of different diseases such as skin cancer, cardiovascular diseases or rheumatology. One of the hurdles for its introduction into the clinic, or even in clinical pilot studies and larger trials, is the bulkiness and price of the laser source. This paper describes how recent progress in both laser diode technology and its associated driver electronics led to a compact handheld ultrasound scanner with built-in photoacoustic functionality. This is a key for the introduction of the photoacoustic technology in the clinic and future point-of-care systems.

The developed laser source includes a laser diode and a pulse generator that can deliver ultra-short pulses (100 ns or less). The unprecedented pulse energies of up to 4mJ were up to now only achievable with Nd:YAG lasers. Beam shaping optics provide a homogenous spot on the skin with a size of 25 x 4 mm or allow coupling into a fiber. This innovative laser source provides up to 4 different wavelengths in the near infrared which can be operated independently. The choice of wavelengths allows optimization of the photoacoustic imaging contrast for different types of cells or tissues, or to do differential (absolute) measurements. The electro optical efficiency and compactness enables integration of the laser source plus driver in a handheld probe. This paper highlights the development of the diode laser and driver technology and its radical integration with a medical ultrasound scanner, leading to a first prototype for clinical pilot studies.

Advanced laser system for 3D optoacoustic tomography of the breast

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We describe the ongoing development and performance of a high-pulse-energy wavelength-cycling laser system for three-dimensional optoacoustic tomography. Joule-level energies are desired for achieving the required penetration depths while maintaining safe fluence levels, below 20 mJ/cm². Wavelength cycling provides a pulse sequence which repeatedly alternates between two wavelengths (756 and 797 nm) that provide differential imaging. This improves the co-registration of captured differential images and the quantification of blood oxygen saturation. New design features have been developed for and incorporated into a commercial prototype laser system, to improve efficacy and ease of use in the clinic. We describe the integration of the laser system with a clinical prototype laser optoacoustic ultrasonic imaging system (LOUSA-3D). We present images of well-characterized phantoms resembling breast tumors and vessels, and mimicking breast optoacoustic properties. These include functional imaging maps of blood oxygen saturation. We also evaluate the effect of laser parameters on image contrast, resolution, and image acquisition time. We discuss future laser developments that will provide further benefit for clinical imaging applications.

Novel fibre lasers as excitation sources for photoacoustic tomography and microscopy

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Photoacoustic signals are typically generated using Q-switched Nd:YAG pumped OPO or dye systems, as they can provide the necessary mJ pulses for operating in widefield tomography mode and the high quality beam and uJ pulses required for optical resolution photoacoustic microscopy (OR-PAM). These excitation sources have played an essential role in the development of photoacoustic imaging in the laboratory. However for clinical applications they present several drawbacks. To achieve mJ pulse energies for tomography mode, the sources are often bulky, require external water cooling and provide low pulse repetition frequencies (PRF<100Hz) thus limiting image frame rate. For OR-PAM applications, their limited PRF (10's of kHz) is also a significant drawback to achieving high imaging frame rates.

Fibre lasers offer a viable alternative, which can overcome these limitations and offer greater flexibility in their temporal output characteristics (e.g., pulse shaping and duration). Until now however, fibre lasers have found limited use for photoacoustic tomography (PAT) due to the relatively low pulse energy (<1mJ) provided by commercial systems. These low pulse energies are a consequence of small core diameter (<25um) fibres being used to achieve a high beam quality. For PAT high beam quality is not a requirement and therefore fibre lasers with larger core diameters (>100um) can be used, enabling significantly higher pulse energies (~20mJ) to be achieved. Commercial fibre lasers operating at PRFs of the order of a few hundred kHz have previously been used for OR-PAM. However, with careful design PRFs exceeding 1MHz is possible enabling significantly higher frame rates to be achieved.

Two custom fibre lasers have been developed. One is dedicated to PAT and uses a custom drawn large core diameter fibre (200um) to provide high pulse energies (20mJ) and variable PRFs (100Hz-1kHz) and pulse durations (10-200ns). It is also compact (of comparable dimensions to a desktop PC) and does not require external water cooling. Tomographic images of the microvasculature of the palm of a hand were obtained, and the pulse shaping capability of the laser for the purpose of optimising SNR.
is demonstrated. The second laser is dedicated to OR-PAM applications and provides a high quality beam (M2<1.2), pulse energies >100 nJ with a PRF of 1 MHz and 532 nm emission wavelength. The high PRF of this laser was exploited to acquire OR-PAM images of the microvasculature of a mouse ear at high imaging frame rates (e.g. >5 fps at 400x400 points). The capability of generating pulses at irregular time intervals was also investigated, to compensate for the non-linear velocity of the galvanometer mirrors when continuously scanning. The compact size and enhanced functionality of these novel fibre lasers will aid the translation of photoacoustic imaging to practical application in medicine and biology.

9708-32, Session 5

**An optical resolution photoacoustic microscopy system with unique supercontinuum fiber laser and detection of several cancer cells**

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The specific absorption characteristics of distinct type of cancer cells make their detection possible by photoacoustic imaging systems in vitro and in vivo. Here, we report a photoacoustic microscopy system with a custom developed supercontinuum fiber laser in order to study several cancer cell lines. The laser is unique in that all the important parameters are adjustable: pulse duration (1-3 ns), energy (up to 10 μJ), and repetition rate (50 kHz - 3 MHz) independently from each other. The broadband of supercontinuum, from 500 to 1100 nm, enables selection of wavelength that corresponds to the utmost absorption coefficient in the absorption spectra of chromophores in cells. The lateral resolution of the system is experimentally quantified as 2.19 μm by imaging a United States Air Force 1951 test target. In this study, photoacoustic signals produced by monolayers of cell lines MCF7 (breast cancer) and MeWo (melanoma) are presented in vitro. The melanoma cells produce detectable photoacoustic signal due to the fact that they contain optically absorbing melanin particles. When compared to MeWo, MCF7 cells produce negligible photoacoustic signal, thus these cells are stained with trypan blue. Pulse energy of the laser is adjusted for optimizing signal to noise ratio. In addition to this, image of the melanoma cells are acquired with the Optical Resolution Photoacoustic Microscopy (OR-PAM) system. The system with compact, robust and all-fiber laser with adjustable properties has the capability to widen preclinical and clinical applications.

9708-33, Session 5

**Limited view multi-source quantitative photoacoustic tomography with a circular transducer of finite-dimension**

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Quantitative photoacoustic tomography (QPAT) is a hybrid imaging modality that simultaneously reconstructs absorption and scattering coefficient with multi-source or multi-wavelength setting. In contrast with PAT, QPAT eliminates the artifacts due to photon transfer in depth and characterizes the intrinsic biological attributes. However, data acquisition for QPAT is time consuming because for each optical source (or wavelength), a 360-degree acoustic measurement on the boundary of imaging region is required. In this work, we investigate a novel limited-view multi-source (LVMS) QPAT scheme and reconstruction algorithm based on coupled optoacoustic model. A unique setting that binds optical source and acoustic transducer together is presented. Under each illumination, only a limited-view measurement is acquired, which is incomplete for PAT reconstruction but sufficient for direct QPAT reconstruction; then the source and detector rotate to next data acquisition position synchronously. In terms of reconstruction, a sparsity-regularized formulation based on tensor framelet is adopted here and optimized through Alternative direction method of multipliers with LBFGS solver, during which the adjoint method is used for rapid computation of numerical gradient of objective function. However, the aperture effect i.e. anisotropic angular sensitivity of a finite-dimension transducer would cause temporal distortion of receiving acoustic signal and further resolution reduction. Therefore, we numerically integrate ideal PAT system spatial impulse response (SIR) with the angular response from circular transducer to improve the modeling accuracy. In summary, the proposed LVMS-QPAT has the potential to shorten the data acquisition time, enhance system SNR and improve image resolution.

9708-34, Session 5

**Thin metal film-polymer composite for efficient photoacoustic generation**

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Photoacoustic (PA) conversion of metal film absorbers is known to be inefficient because of their low thermal expansion and high light reflectance, as compared to polymeric materials containing light absorbing fillers. Specifically, the PA signal for metal films is typically an order of magnitude lower than for PDMS-based composites consisting of carbon materials such as carbon blacks, carbon nanotubes, and carbon fibers. However, the carbon-PDMS composites have several disadvantages, e.g., difficulty in controlling film thickness, aggregation of the carbon fillers, and poor patternability.

To overcome these issues and achieve comparable PA amplitudes, a polymer-metal film composite was developed consisting of a thin metal absorber and adjacent transparent polymer layers. The proposed structure shows efficient PA conversion. The measured PA amplitude of the metal film composite is an order of magnitude higher than that of metal-only samples, and comparable to those of the carbon-PDMS composites. The enhanced PA conversion is accomplished by using metal film of a few tens of nanometers, which greatly facilitates heat transfer from the metal film to the surrounding polymers. Moreover, integrating the metal film composite with a photonic cavity can compensate light absorption loss of the thinner metal film. Theoretical and experimental analysis is conducted for understanding the mechanism behind such improvement.

This strategy could be implemented for spatial PA signal patterns, especially for deep tissue PA imaging of implants or image-guiding tools. Furthermore, this approach also provides a guideline for designing photoacoustic transmitters and contrast agents.

9708-35, Session 5

**Multimodal system for non-contact photoacoustic imaging, optical coherence tomography, and mid-infrared photoacoustic spectroscopy**

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We present a multimodal optical setup, allowing non-contact photoacoustic imaging (PAI), optical coherence tomography (OCT), and mid-infrared photoacoustic spectroscopy (PAS). OCT is a fast and non-contact imaging method that allows acquisition of depth-resolved images of subsurface features in turbid media. The method is sensitive to changes in the specimen’s refractive index, thereby offering complementary information to photoacoustic signals, which are induced by light absorption. A multimodal setup for OCT and PAI/PAS should ideally not rely on any physical contact.
to a specimen. Thus, commonly used transducers for photoacoustic signal detection, which require acoustic coupling to the specimen, should be avoided. In this work photoacoustic signals are acquired by measuring the surface displacement of a specimen using a fiber-optic Mach-Zehnder interferometer. Photoacoustic signals are excited with a Nd:YAG laser for PAI and a tunable quantum cascade laser (QCL) for PAS. The interferometer for non-contact PAI/PAS detection and the OCT system are realized in the same fiber-optic network; light from the PAI/PAS detection laser and the OCT source are multiplexed into one fiber and the same objective is used for both imaging modalities. Light reflected from specimens is demultiplexed and guided to the respective imaging systems. To allow fast non-contact PAI and OCT imaging the detection spot is scanned across the specimens’ surface using a galvanometer scanner. As the same fiber-network and optical components are used for photoacoustic and OCT imaging the obtained images are co-registered intrinsically. Imaging is demonstrated on tissue mimicking and biological samples; spectral information is obtained for hemoglobin.

9708-36, Session 6
Photoacoustic spectrum analysis of tissue microstructure using an optical micro-ring resonator
Qiaochu Li, Univ. of Michigan (United States)

Photoacoustic spectrum analysis (PASA) has been demonstrated to have the ability of objectively evaluating the histological micro-structures in biological tissues. However, the capability of PASA technique in characterizing the micron-size features and changes is limited by receiving bandwidth of the ultrasound detector. In this work, we have for the first time investigated the feasibility and performance of PASA powered by an optical micro-ring resonator based ultrasonic detector. The unique advantages of micro-ring over conventional PZT transducers include its super broad and very flat detection band which greatly benefits characterization of tissue micro-features by PASA. The first experiment was conducted on phantoms containing different sizes of optical absorbers (i.e. block microspheres with diameters of 3, 6, 10, 20 and 45 μm). The photoacoustic spectral parameter slope of each phantom was quantified, and the results from a micro-ring and a needle hydrophone were compared. Benefited from its broad detection bandwidth, the micro-ring successfully distinguished the phantoms containing different sizes of optical absorbers, while the needle hydrophone failed to sense the difference when the sizes of the optical absorbers were smaller than 20 μm in diameter. To understand the result better, measurements have also been conducted on single particles of different sizes. The results show that the micro-ring could detect the spectral peak of the photoacoustic signal generated from the single particle as small as 3 μm. To explore potential applications of the PASA technique, we have also conducted experiment on the red blood cells (RBCs) with different morphology. The results demonstrated that the micro-ring based PASA technique has the ability to get higher resolution in characterizing micron-scale morphological features of biological tissues.

9708-37, Session 6
A compact polymer optical fibre ultrasound detector
Christian F. B. Broadway, Univ. Carlos III de Madrid (Spain); Andreas Pospori, Michal G. Zubel, Aston Univ. (United Kingdom); Daniel C. Gallego, Univ. Carlos III de Madrid (Spain); Getinet T. Woyessa, DTU Fotonik (Denmark); David J. Webb, Kate Şuğden, Aston Univ. (United Kingdom); Ole Bang, DTU Fotonik (Denmark); Guillermo Carpiñtero del Barrio, Horacio Lamela, Univ. Carlos III de Madrid (Spain)

Polymer optical fibre (POF) is a relatively new and novel technology that presents an innovative approach for endoscopic applications. Currently, piezo electric transducers are the typical detectors of choice, albeit possessing a limited bandwidth due to their resonant nature and a sensitivity that decreases proportionally to their size. Optical fibres provide immunity from electromagnetic interference and POF in particular boasts more suitable physical characteristics than silica optical fibre. The most important of these are lower acoustic impedance, a reduced Young’s Modulus and a higher acoustic sensitivity than single-mode silica fibre at both 1MHz and 10MHz. POF therefore offers an interesting alternative to existing technology.

Intrinsic fibre structures such as Bragg gratings and Fabry-Perot cavities may be inscribed into the fibre core using UV lasers. These gratings are a modulation of the refractive index of the fibre core and provide the advantages of high reflectivity, customisable bandwidth and point detection. A Fabry-Perot cavity allows sensing between two gratings that have the same Bragg wavelength.

We present a compact ultrasonic detector based upon a POF Fabry-Perot cavity. We design and verify this detector by modelling Bragg gratings and Fabry-Perot cavities. We resolve issues with ultrasonic POF detection that our group has previously identified and assess the uniformity of the detected signal voltage at different points around the fibre circumference. Finally, we demonstrate a proof of concept for endoscopic ultrasonic detection including POF connectorisation that demonstrates lower losses than previously shown.

9708-38, Session 6
Photoacoustic and ultrasound imaging with a gas-coupled laser acoustic line detector
Jami L. Johnson, Kasper van Wijk, The Univ. of Auckland (New Zealand); James N. Caron, Quarktet (United States) and Research Support Instruments (United States); Miriam Timmerman, Univ. Twente (Netherlands)

Conventional contacting transducers are highly sensitive and readily available for ultrasonic and photoacoustic imaging. On the other hand, optical detection can be advantageous when a small sensor footprint, large bandwidth and no contact are essential. However, most optical methods utilizing interferometry or Doppler vibrometry rely on the reflection of light from the object. We present a non-contact detection method for photoacoustic and ultrasound imaging – termed Gas-Coupled Laser Acoustic Detection (GCLAD) – that does not involve surface reflectivity. GCLAD measures the displacement along a line in the air parallel to the object. Information about point displacements along the line is lost with this method, but resolution is increased over point-based techniques when used as an integrating line detector. We will present the underlying physics of GCLAD and describe the methods for obtaining a uniform, unbiased response along the probe beam. The theory for quantifying surface displacement from GCLAD waveforms will be discussed, as well as validation of our results by comparison with a commercial vibrometer. Finally, we will present experimental results using GCLAD as a line detector for photoacoustic and laser-ultrasound imaging.

9708-39, Session 6
All-optical highly sensitive broadband ultrasound sensor without any deformable parts for photoacoustic imaging
Wolfgang Rohringer, Stefan Preisser, Medizinische Univ. Wien (Austria) and XARION Laser Acoustics GmbH (Austria); Mengyang Liu, Zhe Chen, Boris Hermann, Harald Sattmann, Medizinische Univ. Wien (Austria); Nicole
For photoacoustic imaging, mainly piezoelectric transducers and recently developed miniaturized optical sensors are used today. The frequency response of these sensors is defined by the mechanical properties of deformable structures. Such mechanically deformable structures include piezo crystals, deformable Fabry-Perot cavities and micro-ring resonators.

To enable fully astatic sensing of ultrasound waves in a broad frequency range at high sensitivity, we developed a novel optical ultrasound sensor. It consists of a rigid, fiber-coupled Fabry-Perot etalon with a central opening. The sensing principle relies exclusively on the detection of pressure-induced refractive index changes in the liquid itself, located between the etalon mirrors. The resonance-free nature of the detection method enables linear broad-bandwidth detection while maintaining highest sensitivity. The optical transparency of the filling liquid permits excitation light to pass the central opening with only minimal losses, thus allowing reflection as well as transmission mode imaging.

To demonstrate the potential of this technology for both reflection and transmission mode imaging, we present application results of ex-vivo stained plant cells and in-vivo embryonic imaging. Our results show that the sensor combines the outstanding sensitivity of best-in-class commercially available piezoelectric focused transducers with the flexibility provided by optical detection, while maintaining a large full field of view of ~2 mm by 2 mm. Transparent in axial direction, this sensor enables highly sensitive fast-scanning reflection-mode OR-PAM as well as easy integration with other imaging modalities.

9708-40, Session 6

Air-coupled ultrasound optical detector based on optofluidic ring resonator

Kyu Hyun Kim, Wei Luo, Cheng Zhang, L. Jay Guo, Xudong Fan, Univ. of Michigan (United States)

We develop an air-coupled ultrasound detector based on an optofluidic ring resonator (OFRR) suspended on a U-shaped holder. The OFRR is a glass capillary with an outer diameter of approximately 100 µm and a wall thickness in the order of 1-10 µm. The circular cross section of the OFRR supports the high-Q whispering gallery mode (WGM) that circulates along the circumference. Incoming ultrasound wave results in a small refractive index change of the glass wall and geometrical change in the OFRR shape, both of which in turn lead to a spectral shift in the WGM that can be sensitively detected owing to WGM with high Q-factors (>10⁶). Due to the suspension nature of the OFRR, the ultrasound detection can be carried out in air, which is advantageous in comparison with other ultrasound detection that requires water immersion. Moreover, thanks to its axial symmetry, the OFRR detection is nearly omni-directional, which provides a large acceptance angle for the incoming ultrasound waves. Finally, the sensitivity can be tuned and optimized by changing the wall thickness and the materials that flow through the capillary. Besides the optical ultrasound detection, we also demonstrate optomechanical ultrasound detection, in which optomechanical vibration up to 10 GHz is first excited within the OFRR that is subsequently modulated by the ultrasound wave. Our work will lead to the development of a new type of air-coupled ultrasound detector that can be used for photo-acoustic imaging, non-invasive ultrasound detection of external objects, and ultrasound detection/characterization of internal objects (such as particles and liquids) flowing inside the capillary.
The acoustic waves generated in the sample propagate towards the CW probe beam and cause transient changes in the coupling medium refractive index, which result in the probe beam deflection. A balanced 2D array of photodiodes detects the probe beam deflections as a differential signal. The magnitude of a deflection is directly proportional to the amplitude of the acoustic pressure wave. Because of the unimpeded working distance, high numerical aperture objective lenses can be employed in this microscope (in this study a water-immersion lens of NA=1 was used) to achieve diffraction-limited lateral resolution of 0.5 µm at 532 nm, while optically scanning the excitation beam using galvanometer-mounted mirrors. The sensitivity of the current probe beam deflection sensor exposed as noise equivalent pressure (NEP) was measured as 12 Pa, which is comparable with NEP of the optical micro-ring resonator and commercial piezoelectric ultrasonic transducers. Due to the use of a low repetition rate laser (20 Hz) in the current setup, image scanning is slow; however, in a practical application micron-size detail was successfully resolved in histological sections of cardiac muscle harvested from a rhesus macaque. The all-optical OA microscope offers several benefits over current piezoelectric detector based system, such as increased lateral and axial resolution, higher sensitivity, robustness, and potentially more flexible implementation in multimodality imaging instruments.

9708-43, Session 7

Analysis of photoacoustic response from plasmonic nanostructures irradiated by ultrafast laser in water

Ali Hatef, Behafarid Darvish, Nipissing Univ. (Canada); Adrien Dagallier, École Polytechnique de Montréal (Canada); Christos Boutopoulos, Michel Meunier, École Polytechnique de Montréal (Canada)

Gold and silver plasmonic nanoparticles (NPs) are widely used as a contrast agent for photoacoustic (PA) imaging, taking advantage of the strong optical absorption cross-section of these particles due to their localized surface-plasmon resonance. Inspired by recent developments in ultra-high frequency wide-bandwidth transducers, we propose utilizing off-resonance ultrashort laser sources with a pulse width in the femtosecond (fs) and picosecond (ps) range to increase the efficiency of PA imaging. Also, from the fact that the laser pulse duration is shorter than the heat diffusion time of the materials, we expect practically no collateral damage of the laser irradiated biological tissues. Our preliminary studies show that irradiating the NPs with an ultrashort-pulsed laser has the potential to achieve substantially higher efficiency at generating the PA signal. Enhanced ion interaction and releases a pressure wave in the surrounding medium. However, in this process, it is crucial to precisely control the heat energy absorption in the NPs to avoid their fragmentation. In this talk we present a model to simulate the PA signals from collapsed vessels using a fractal model. The fractal model uses bifurcated-cylinder bases to represent vasculature trees. Normal vessels have circular absorption cross-sections. To simulate bleeding, micro-vessels with diameter <30 µm were assumed to have 2D surface-plasmon resonance around the vicinity of the NPs. Plasma relaxes through electron-ion interaction and releases a pressure wave in the surrounding medium. However, in this process, it is crucial to precisely control the heat energy absorption in the NPs to avoid their fragmentation. In this talk we present a model to simulate the PA signals from collapsed vessels using a fractal model. The fractal model uses bifurcated-cylinder bases to represent vasculature trees. Normal vessels have circular absorption cross-sections. To simulate bleeding, micro-vessels with diameter <30 µm were assumed to have 2D surface-plasmon resonance around the vicinity of the NPs. Plasma relaxes through electron-ion interaction and releases a pressure wave in the surrounding medium. However, in this process, it is crucial to precisely control the heat energy absorption in the NPs to avoid their fragmentation. In this talk we present a model to simulate the PA signals from collapsed vessels using a fractal model.

9708-46, Session 7

Pulsed photoacoustic flow imaging of whole blood with low-frequency detection

Pim J. van den Berg, Khalid Daoudi, Wiendelt Steenbergen, Univ. Twente (Netherlands)

The biomedical imaging of blood flow is a growing field of research. For instance, flow imaging can be used for the estimation of the wall shear rate.
and the characterization of angiogenesis. Existing modalities like pulse-echo ultrasound and optical coherence tomography are used extensively for this purpose. However, they suffer from tissue background clutter that interferes with the flow estimation. Photoacoustic flow imaging (PFI) does not have this issue, and can take advantage of a good absorption contrast between vascularity and the surrounding tissue.

While more elaborate PFI techniques have been demonstrated, PFI using only a pulsed laser (pulsed PFI) has a two major benefits: reduced complexity and favorable imaging speed. Pulsed PFI relies on tracking the photoacoustic (PA) response from red blood cells (RBCs). The method has only been shown with high-frequency (±30 MHz) ultrasonic detection and green (e.g. 532 nm) light. This combination was typically chosen to maximize the SNR, since RBCs are small and dense, and therefore have a high-frequency PA response. However, this limits the imaging depth significantly.

We will show that neither high-frequency ultrasonic detection nor green light is an absolute necessity, taking advantage of the broadband nature of PA response of RBCs in vessels. Careful optimization of the signal processing is required however. Flow imaging on whole blood will be demonstrated using ~15 MHz ultrasonic detection combined with a near infrared laser. This will help open up a new path to deep PA flow imaging.

9708-47, Session 7

**Microvascular pressure estimation using compression and vessel-tracking photoacoustic microscopy**

Min Choi, Roger J. Zemp, Univ. of Alberta (Canada)

Microvascular pressure is a crucial parameter for driving perfusion in tissues but to date there are no non-invasive techniques for measuring it in deep tissues. Small deep vessels including those with low flow velocities are difficult to visualize using traditional radiological imaging methods including ultrasound and Doppler ultrasound, however, photoacoustic imaging offers significant promise for visualizing these small vessels. We propose a method to estimate microvascular pressure by compressing tissues and imaging the point at which these vessels collapse. At this point the external pressure overcomes the internal pressure. To validate the technique, we use 200 µm cellulose capillary tubes flowing blood at variable flow-rates set by a syringe pump. The tubes are embedded in chicken tissue. We apply external pressure and measure the force applied using a simple scale. We record the point at which the vessels collapse and confirm the collapse-point by observing flow stoppage. The measurements were taken using VEVO LAZR system with 40 MHz transducer from Fujifilm Visualsonics Inc., which allows both ultrasound and photoacoustic imaging to be done in real-time. With flow rates from 0.10 ml/min to 0.20 ml/min, the force applied to close the tube increased in quadratic way. The closure is noticed by the pinching of the photoacoustic signal then followed by subsequent weakening. We also apply the technique in human subjects and process movies of ultrasound-photoacoustic images to estimate the collapse point for each applied pressure to create maps of microvascular relative pressure distributions. This new technique may offer considerable promise for mapping microvascular pressures and perfusion in a non-invasive manner and hence has substantial clinical translational potential for evaluating peripheral vascular disease, ulceration and wound healing, and for characterizing tumor perfusion, among other applications.

9708-49, Session 7

**Measurement of changes in blood oxygenation using multispectral optoacoustic tomography (MSOT) allows assessment of tumor development**

Michal R. Tomaszewski, Univ. of Cambridge (United Kingdom) and Cancer Research UK (United Kingdom) and EPSRC-CRUK Cancer Imaging Ctr. in Cambridge and Manchester (United Kingdom); Isabel Quiro’s-Gonzalez, Cancer Research UK (United Kingdom); James Joseph, Univ. of Cambridge (United Kingdom) and Cancer Research UK (United Kingdom); Sarah E. Bohndiek, Univ. of Cambridge (United Kingdom) and Cancer Research UK (United Kingdom) and CRUK & EPSRC Cancer Imaging Ctr. in Cambridge & Manchester (United Kingdom)

The ability to evaluate tumor oxygenation in the clinic could indicate prognosis and enable treatment monitoring, since oxygen deficient cancer cells are more resistant to chemotherapy and radiotherapy. MSOT is a hybrid technique combining the high contrast of optical imaging with the spatial resolution and penetration depth similar to ultrasound. We aimed to demonstrate that MSOT imaging can be used to monitor changes in tumor oxygen delivery and consumption, challenging to measure otherwise, using blood oxygenation measurements.

We performed MSOT on nude mice (n=15) bearing subcutaneous xenograft tumors using the inVision MSOT 256 (IThera Medical). The mice were maintained under inhalation anesthesia during imaging and inspired oxygen content was modified between 21% and 100%. The measurements from early (week 1), medium (week 4) and late (week 7) stages of tumor development were compared. Tumors were then excised for histopathological staining with H&E, CD31 and pimonidazole. Within the tumor region, we measured a 105% increase in averaged hemoglobin saturation (from 19(2)% to 40(3)%) in response to changing the breathing gas from air to oxygen. After the change back to air, no significant difference to initial signal level was found (p=0.98). Exponential fitting showed kinetics of the signal increase differed significantly between early and medium stage tumors (p=0.02) and between medium and late stage (p=0.04).
Blood oxygenation response to gas challenge changes significantly with tumor development. MSOT can be used successfully to monitor these changes. Future work will explore the change in kinetics of the oxygenation variation due to cancer treatment response.

9708-50, Session 8

Bayesian parameter estimation in spectral quantitative photoacoustic tomography

Aki Pulkkinen, Univ. of Eastern Finland (Finland); Ben T. Cox, Simon R. Arridge, Univ. College London (United Kingdom); Jari P. Kaipio, Univ. of Eastern Finland (Finland) and The Univ. of Auckland (New Zealand); Tanja Tarvainen, Univ. of Eastern Finland (Finland)

Photoacoustic tomography (PAT) is an imaging technique combining strong contrast of optical imaging to high spatial resolution of ultrasound imaging. These strengths are achieved via photoacoustic effect, where the spatial absorption of light pulse is converted into measurable propagating ultrasound wave. The method is seen as a potential tool for small animal imaging, pre-clinical investigations, study of blood vessels and vasculature, as well as for cancer imaging.

The goal in PAT is to form image of the absorbed optical energy density field via acoustic inverse problem approaches from the measured ultrasound data. Quantitative PAT (QPAT) proceeds from these images and forms quantitative estimates of the optical properties of the target. This optical inverse problem of QPAT (SQPAT) utilizes the PAT data, formed at multiple optical wavelengths, simultaneously with optical parameter models of tissue, to form quantitative estimates of the parameters of interest.

In this work, the inverse problem of SQPAT is investigated. Light propagation is modelled using the diffusion equation. Optical absorption is described with chromophore concentration weighted sum of known chromophore absorption spectra. Scattering is described by Mie scattering theory with an exponential power law. To alleviate the issue, spectral QPAT (SPQAT) utilizes the PAT data, formed at multiple optical wavelengths, simultaneously with optical parameter models of tissue, to form quantitative estimates of the parameters of interest.

In our approach of combining PA and AO, the experiment involved two consecutive PA measurements, exciting the medium at two points, and an AO measurement in transmission mode. During the AO measurement the consecutive PA measurements, exciting the medium at two points, and the spatially varying unknown parameters of interest are the chromophore concentrations, the Mie scattering parameters (power law factor and the exponent), and Grüneisen parameter. The inverse problem is approached with a Bayesian method. It is numerically demonstrated, that estimation of all parameters of interest is possible with the approach.

9708-51, Session 8

Nanoparticle-enhanced spectral photoacoustic tomography: effect of oxygen saturation and tissue heterogeneity

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Molecular imaging for breast cancer detection, infectious disease diagnostics and preclinical animal research may be achievable through combined use of targeted exogenous agents – such as nanoparticles – and spectral Photoacoustic Tomography (PAT). However, tissue heterogeneity can alter fluence distributions and acoustic propagation, corrupting measured PAT absorption spectra and complicating in vivo nanoparticle detection and quantitation. Highly absorptive vascular structures represent a common confounding factor, and variations in vessel hemoglobin saturation (SO2) may alter spectral content of signals from adjacent/deeper regions. To evaluate the impact of this effect on PAT nanoparticle detectability, we constructed heterogeneous phantoms with well-characterized channel-inclusion geometries and biologically relevant optical and acoustic properties. Phantoms contained an overlying vascular network containing whole bovine blood, and a deeper target network containing blood doped with varying concentrations of gold nanorods with absorption peaks at 700 and 780 nm. Both overlying and target network SO2 was tuned using sodium dithionite. Phantoms were imaged from 700 to 900 nm using a custom PAT system comprised of a tunable pulsed laser and a research-grade ultrasound system. Nanoparticle and hemoglobin concentrations were estimated from multi-spectral image datasets using linear spectral unmixing techniques. Nanoparticle concentration estimation accuracy, contrast-to-noise ratio, and sensitivity (i.e., detection limit) were quantified as functions of target and background SO2. Phantom results were compared with both spectrophotometry and PAT data from water-immersed cuvettes containing blood and nanoparticle solutions. Results suggested that nanoparticle selection for a given PAT application should take into account expected tissue vascular density and oxygenation state to achieve optimal performance.

9708-52, Session 8

Combined photoacoustic and acousto-optic tomography setup for quantitative spectroscopy

Altas Hussain, Jacob W. Staley, Erwin Hondebrink, Khalid Daoudi, Wiendelt Steenbergen, Univ. Twente (Netherlands)

Biomedical photoacoustics (PA) enables high resolution imaging of absorbing objects in turbid media like biological tissue. A photoacoustic signal is proportional to the product of the optical absorption coefficient and the local optical fluence; quantitative PA measurement therefore requires the exact knowledge of the local optical fluence. In past we demonstrated that the combination of PA and acousto-optics (AO) can be employed to correct PA signals for variations in fluence, which yields the signals that are proportional to optical absorption only. Further, we showed that the fluence corrected PA signals using the combination of PA and AO at two wavelengths can be used to correctly estimate the absolute blood oxygen saturation in optically inhomogeneous tissue phantoms.

In our approach of combining PA and AO, the experiment involved two consecutive PA measurements, exciting the medium at two points, and an AO measurement in transmission mode. During the AO measurement the illumination and light detection points must coincide with the two excitation points of the PA experiment. The resulted experimental geometry of this methodology has limited scope in real life applications. Here we present a more versatile method to combine PA with AO for fluence correction of PA signals. This method allows multipoint simultaneous illumination of the sample in any possible configuration, which is more suitable in tomographic settings. Further, we built a combined PA and AO tomography setup for small animal imaging, where AO provides the measure of local fluence which facilitates fluence compensated PA images. We experimentally show the concept of quantitative photoacoustic spectroscopy in tomographic settings in phantoms and ex-vivo samples.

9708-53, Session 8

Photoacoustic spectroscopy using linear frequency modulation chirp

Bahman Lashkari, Sung soo (Sean) Choi, Edem Doivol, Andreas Mandelis, Univ. of Toronto (Canada)

In this study, we present some examples of waveform engineering applications in frequency-domain photoacousticics (FD-PA). Linear frequency modulation (LFM) has been employed in many different fields such as radar, sonar, ultrasound and photoacoustics to perform temporal encoding of the transmitted signal. Encoding the transmission and matched filtering in receive mode tends to increase the signal-to-noise ratio (SNR) while maintaining resolution. An example of using LFM for photoacoustic spectroscopy is simultaneous probing/imaging with multiple wavelengths. Use of mismatched coded waveforms enables encoding the signal sources
and, therefore, facilitates simultaneous probing and imaging. This method enables high frame rate functional imaging with reduced motion artifacts. It is shown that multispectral PA spectroscopy is possible with more than two simultaneous laser excitations. The suitable design of coded waveforms is discussed which facilitates uniform SNR and resolution for different images. Furthermore, it is shown that phase of the cross-correlation of PA signal modulated with a linear chirp can yield the absolute absorption coefficient of chromophores. This method is not affected by fluorescence attenuation or variation due to the absorption and scattering of the overlayer material, thereby providing a calibration-free approach for quantitative PA imaging.

9708-54, Session 8
Photoacoustic physio-chemical analysis of liver conditions in human subjects
Guan Xu, Chao Tian, Shanshan Wan, Theodore H. Welling, Anna S. F. Lok, Jonathan M. Rubin, Xueding Wang, Univ. of Michigan Medical School (United States)

Non-alcoholic fatty liver disease (NAFLD) is a common liver disease affecting 30% of the population in the United States. Biopsy is the gold standard for diagnosing NAFLD. Liver histology assesses the amount of fat, and determines type and extent of cell injury, inflammation and fibrosis. However, liver biopsy is invasive and is limited by sampling error. Current radiological diagnostic modalities can evaluate the ‘physical’ morphology in liver by quantifying the backscattered US signals, but cannot interrogate the ‘histochemical’ components forming these backscatterers. For example, ultrasound (US) imaging can detect the presence of fat but cannot differentiate steatosis alone from steatohepatitis. Our previous study of photoacoustic physio-chemical analysis (PAPCA) has demonstrated that this method can characterize the histological changes in livers during the progression of NAFLD in animal models. In this study, we will further validate PAPCA with human livers. Ex vivo human liver samples with steatosis, fibrosis and cirrhosis will be scanned using optical illumination at wavelengths of 680-1700 nm and compared to histology results. In vivo study on human subjects with confirmed steatosis is planned using our PA-ultrasound (US) parallel imaging system based on Verasonics US imaging platform with an L7-4 probe. 10 mJ per square centimeters per pulse optical energy at 755 nm will be delivered to the skin surface, which is under the safety limit of American National Standard Institute. Preliminary study with ex vivo human tissue has demonstrated the potential of the proposed approach in differentiating human liver conditions.

9708-55, Session 8
Lifetime-resolved photoacoustic (LPA) spectroscopy for monitoring oxygen change and photodynamic therapy (PDT)
Janggun Jo, Chang Heon Lee, Raoul Kopelman, Xueding Wang, Univ. of Michigan (United States)

The Methylene Blue loaded Polycrylamide Nanoparticles (MB-PAA NPs) are used for oxygen sensing and Photodynamic therapy (PDT), a promising therapeutic modality employed for various tumors, with distinct advantages of delivery of biomedical agents and protection from other bio-molecules overcoming inherent limitations of molecular dyes. Lifetime-resolved photoacoustic spectroscopy using quenched-phosphorescence method is applied with MB-PAA NPs so as to sense oxygen, while the same light source is used for PDT. The dye is excited by absorbing 650 nm wavelength light from a pump laser to reach triplet state. The probe laser at 810 nm wavelength is used to excite the first triplet state at certain delayed time to measure the dye lifetime which indicates oxygen concentration. The 9L cells (106 cells/ml) incubated with MB-PAA NP solution are used for monitoring oxygen level change during PDT in situ test. The oxygen level and PDT efficacy are confirmed with a commercial oximeter, and fluorescence microscope imaging and flow cytometry results. This technique with the MB-PAA NPs allows us to demonstrate a potential non-invasive theranostic operation, by monitoring oxygen depletion during PDT in situ, without the addition of secondary probes. Here, we demonstrate this theranostic operation, in vitro, performing PDT while monitoring oxygen depletion. We also show the correlation between O2 depletion and cell death.

9708-130, Session PMon
Comparison of spectral fitting and spectral differential as unmixing methods in multispectral photoacoustic imaging to visualize enzymatically activatable photoacoustic probe
Takeshi Hirasawa, Shinpei Okawa, National Defense Medical College (Japan); Ryu J. Iwatake, Mako Kamiya, Yasuteru Urano, The Univ. of Tokyo (Japan); Miya Ishihara, National Defense Medical College (Japan)

In photoacoustic molecular imaging, multispectral photoacoustic (MS-PA) imaging has been used to image target probe signals in the presence of strong background signals produced from intrinsic optical absorbers. Spectral fitting method (SFM) images to reference spectra of the probe and background, respectively. Because hemoglobin, a dominant intrinsic optical absorber, was usually used as a substitute for unknown background absorbers, background signals produced from other optical absorbers cause discrimination error. We
propose spectral differential method (SDM) that discriminates probe signals which have spectral peak at the absorption maximum of the probe using derivative spectra of MS-PA images. SDM suppresses background signals produced from various optical absorbers without use of reference spectra. Clarities of probe signals discriminated from MS-PA images by using SFM and SDM were compared. The MS-PA images were acquired from subcutaneous tumors of mice injected with rhodamine and silicon-rhodamine probes whose absorption maxima wavelengths are 500, 530, and 650 nm, respectively. Furthermore, we performed MS-PA imaging using activatable probes which show their original colors only in the presence of -glutamyltranspeptidase, an enzyme associated with cancer. The probes have been successfully used for rapid fluorescence imaging of cancer. As results, SDM clearly discriminated probes except for the probe which have same peak wavelength with hemoglobin. In particular, SDM successfully discriminated the probe with peak wavelength of 500 nm while presence of various background optical absorbers other than hemoglobin. Our MS-PA imaging methods afforded successful imaging of tumors in mice administered with activatable photoacoustic probes.

9708-131, Session PMon

Effects of the optical properties of gold nanoparticles on photoacoustic signals

Shinpei Okawa, Takeshi Hirasawa, National Defense Medical College (Japan); Ryota Sato, Kyoto Univ. (Japan); Toshihiro Kushibiki, Miya Ishihara, National Defense Medical College (Japan); Toshiharu Teranishi, Kyoto Univ. (Japan)

Various shapes and sizes of the gold nanoparticles are used in the photoacoustic (PA) imaging. Some gold nanoparticles (AuNP) improve the quality of the PA images as a contrast agent. So there can be some characters of the AuNPs which generate the PA signals effectively. In this study, the effects of the optical properties of the AuNP on the PA signals were investigated with the numerical simulations and some experiments using various spherical and polyhedral AuNPs. The mechanisms caused the temporal profile of the PA signal from the AuNPs were discussed. In the numerical simulations, the Monte Carlo (MC) simulation was used to calculate the distribution of the fluence rate which determined the temporal profiles of the PA pressure wave. The absorption and scattering cross sections calculated by the discrete dipole approximation method were used for the MC simulation as the optical properties of the AuNPs. It was demonstrated that the PA signals were characterized not only by the absorption cross sections and but also by the scattering cross section. The scattering medium containing the AuNPs enhanced the amplitude of the PA signals. The PA signals from the AuNP suspensions were measured by use of a P(VDF-TrFE) piezoelectric film and a Q-switched Nd:YAG laser operated at 532 nm in the experiments. The results of the experiments were consistent with the numerical simulations.

9708-132, Session PMon

Photoacoustic imaging and surface-enhanced Raman spectroscopy using dual modal contrast agents

Sungjo Park, Seunghyun lee, Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of); Myeonggeun Cha, Dae-Hong Jeong, Seoul National Univ. (Korea, Republic of)

Recently, photoacoustic tomography (PAT) have been a remarkable non-invasive imaging modality, which provides a strong optical absorption contrast, high ultrasonic resolution, and great penetration depth. Thus, the PAT has been widely used as an in vivo preclinical imaging tool. Surface-enhanced raman spectroscopy (SERS) is another attractive sensing technology in biological research because it provide highly sensitive chemical analyses and multiplexed detection. By performing dual-modal imaging of SERS and PAT, high resolution structural PAT imaging and high sensitive SERS sensing can be achieved. At the same time, developing a dual modal contrast agent for this purpose is equally important. To perform both PAT and SERS, we synthesized PEGylated Ag-nanosheets. The Ag-nanosheets generate the strong PA signals due to their strong optical absorption properties and the sensitive SERS signals due to the surface plasmon resonance effect. Then, multiplexed Raman chemicals were synthesized to enhance the sensitivity. We have photoacoustically imaged the sentinel lymph nodes of small animals after intradermal injection of the multiplexed agents. Further, the chemical composition of each agent were distinguished through SERS.

9708-133, Session PMon

Magnetic nanoparticles for thermal lysis and application in cancer treatment

Sumana Das, Brahmanandam Javvaji, Sarath Chandra Veerla, D. Roy Mahapatra, Indian Institute of Science (India)

In this paper we report on the molecular dynamics simulation of magnetic heating of nanoparticles for application in cancer therapy. Synthesized magnetic nanoparticles (MNP) functionalized using 1-ethyl-3-(3-dimethylamino propyl) (EDC) and N-hydroxy succinamide (NHS) are considered in experiment, where a stable active ester forms and binds with tumor specific anti-antigen, which further helps to target specific tumor cell. Target cell suspensions are subjected to low-medium RF magnetic field. Magnetic heating causes thermal lysis of tumor cell. Heating characteristics of MNP with optimum frequency and time duration, with and without cell suspension are analyzed. Magnetic dipole vibration and temperature are estimated which can be used in a coarse-grained continuum finite element model of tissue/cellular system. In order to evaluate the simulation-guided outcome, the viability of tumor cell is studied using trypan blue and MTT assay. Microscopic study is performed using EBr/Acrindine orange staining. Detailed understanding from simulation on the size of MNP, excitation frequency and magnetic field strength on MNP heating is correlated to the experimental observation. Also, the effective electric charge accumulation on the MNP surface from the dipole rotations are determined. Electrically assisted breakdown of cells as complimentary mechanism besides thermo-lysis is also considered, which could be useful in certain types of MNPs. Temperature is estimated as an ensemble average of velocity of the MNP atoms surrounded by water molecules. Interestingly, such MNPs can be designed to have IR/NIR signatures, which could also be useful in image-guided specific targeting of thermo-lysis.

9708-134, Session PMon

Depth discrimination in acousto-optic cerebral blood flow measurement simulation

Adi Tsalach, Zeev Schiffer, Eliahu Ratner, Ilan Breskin, Reuven Zeitak, Revital Shechter, Michal Balberg, Ornim Medical Ltd. (Israel)

Monitoring cerebral blood flow (CBF) is crucial, as inadequate perfusion, even for relatively short periods of time, may lead to brain damage or even death. Thus, significant research efforts are directed at developing reliable monitoring tools that will enable continuous, bedside, simple and cost-effective monitoring of CBF. All existing NIRS based monitoring methods, such as Laser Doppler or DCS, tend to underestimate CBF in adults, due to the indefinite effect of extra-cerebral tissues on the obtained signal. If those are to find place in day to day clinical practice, the contribution of extra-cerebral tissues must be eliminated and data from the depth (brain) should
Improvement and evaluation of a low-cost laser diode photoacoustic microscopy system for ovarian tissue imaging

Mohsen Erfanzadeh, Hassan S. Salehi, Patrick D. Kumavor, Quing Zhu, Univ. of Connecticut (United States)

Photoacoustic microscopy (PAM) is capable of imaging tumor angiogenesis. Clinical applications of PAM are limited due to the use of expensive and bulky pulsed laser sources. High power pulsed laser diodes (PLD) can be suitable substitute light sources for PAM. Multiple active elements in high power PLDs and intrinsic anisotropy of the PLD beam challenge maintaining low-loss focusing of light for ovarian tissue PAM imaging. Here, a laser diode optical resolution photoacoustic microscopy (LD-OR-PAM) system that utilizes a 905 nm, 650 W output peak power PLD and a low-loss optical setup is evaluated for imaging ovarian tissues. An aspheric lens and two cylindrical lenses collimate the light and correct for the continued extension of the beam in two perpendicular directions. Light is focused on the tissue by an aspheric lens with a numerical aperture (NA) of 0.7. When using similar PLDs, this optical system introduces 6.8 dB less loss compared to a previously reported optical system that incorporated an aspheric lens for collimation and a 0.7 NA microscope objective for focusing. The lateral resolution is measured to be 40 µm using edge spread function estimation. Images of black human hairs and polyethylene tubes filled with rat blood are presented. The photoacoustic signal to noise ratios (SNR) of the aforementioned phantoms were 27.6 dB and 32.7 dB, respectively. Images of mouse ear ex vivo and porcine ovary ex vivo are presented. The photoacoustic SNR between biological samples was above 20 dB. The initial results indicate the great potential of this compact and low-cost system for imaging and characterization of ovarian cancer.

Acoustic characterization of a highly sensitive broadband all-optical ultrasound sensor without any deformable parts

Stefan Preisser, Wolfgang Rohringer, Medizinische Univ. Wien (Austria) and XARION Laser Acoustics GmbH (Austria); Mengyang Liu, Zhe Chen, Boris Herrmann, Christian Kollmann, Harald Sattmann, Behrooz Zabihian, Medizinische Univ. Wien (Austria); Stefan Zetter, Balthasar Fischer, XARION Laser Acoustics GmbH (Austria); Wolfgang Drexler, Medizinische Univ. Wien (Austria)

In photoacoustic imaging, many applications have the need of highly sensitive ultrasound sensors. This is to allow the detection of small ultrasound amplitudes as generated by optically absorbing molecules in photoacoustic microscopy or by absorbing structures deep within tissue in photoacoustic tomography. In addition to the detectability, higher scanning rates (at lower pulse energy and less averaging) are feasible if a sensor with a small noise equivalent pressure (NEP) is used. The most prominent sensor types to satisfy those needs are piezo-based transducers, where a mechanical deformation of the crystal due to the strain induced by ultrasonic waves generates a voltage. While these sensors are widely used for high sensitivity applications, there is a major trade-off between sensitivity and bandwidth.

Higher energy supercontinuum source suitable for photoacoustic microscopy

Magalie M. Bondu, NKT Photonics A/S (Denmark) and Univ. of Kent (United Kingdom); Christopher D. Brooks, Peter M. Moselund, Lasse Leick, NKT Photonics A/S
9708-139, Session PMon

Optimization of light delivery for an intravascular photoacoustic array using Monte Carlo simulations

Robin F. Castelino, Solomon Grant, Univ. of Toronto (Canada) and Sunnybrook Research Institute (Canada); F. Stuart Foster, Univ. of Toronto (Canada) and Sunnybrook Health Sciences Ctr. (Canada)

An optical delivery system for a side-viewing photoacoustic intravascular array device to image atherosclerosis was designed and optimized. A model was developed, using FREDD Optical Engineering Software (Photon Engineering, Tucson AZ), comprised of a catheter, consisting of a 32 element acoustic array (d=2mm) with an optical fiber (d=0.6mm) running through the center and a reflector to direct the light as to co-align it with the acoustic elements. The catheter was enclosed within a vessel (d=4mm). The optical source was modelled as a Gaussian beam (NA=0.17, beam width=0.7mm) and simulated using a Monte Carlo scheme such that the initial position and direction of each ray was independent and randomly selected from a Gaussian distribution over the beam aperture and divergence angle, respectively. The reflector was modelled as a perfectly reflective mirror embedded within a clear (non-absorbing, non-scattering) medium (n=1.5). A conic mirror was initially investigated. Repeated ray traces were performed, varying the height and distance from the light source of the mirror to maximize the delivery of light with the midpoint of the acoustic array in the longitudinal direction. Subsequently, mirror shapes with parabolic and circular curvature were also investigated for completeness. Due to computational constraints, a coarse optimization was first performed with repeated ray traces of 50,000 rays, while the height, degree of curvature, and distance from the light source of the mirror were permuted. Reflector configurations which showed good results under this coarse sampling were further investigated with a finer sampling and a greater number of rays (500,000). The reflector configuration which maximized the irradiance at the intima of the vessel was deemed optimal.

9708-140, Session PMon

Label-free quantitative evaluation of atherosclerotic lipid by spectral-coded intravascular photoacoustic imaging

Sihua Yang, South China Normal Univ. (China)

IVUS and OCT is being used to characterize atherosclerotic plaque. However, their application is limited by the acoustic and optic characters of biological tissue, respectively. Here, we studied the utility of spectral-coded intravascular photoacoustic (SCIPA) imaging, an emerging hybrid label-free technique that combines the merits of both optical imaging and ultrasonic imaging, for quantitative evaluation of lipid in atherosclerotic lesion. Cholesterol-fed New Zealand white rabbit served as the experimental model of atherosclerosis. Whole atherosclerotic aortas were in vitro examined by the SCIPA imaging system with a pulsed laser and an endoscopic ultrasonic detector. Morphologic information of atherosclerotic lesion was provided in the SCIPA images with high axial resolution (~50 ?m) and contrast. Subsequently, concentration map of lipid was identified from SCIPA images using the optical fingerprint character of lipid. There was an excellent agreement between the SCIPA images and the histopathology. SCIPA imaging based atherosclerotic plaque evaluation is accurate. The results provide great promise for evaluating atherosclerotic plaque with simultaneous morphologic and functional information in clinical trials and in prospective studies.

9708-141, Session PMon

Acoustic resolution photoacoustic Doppler velocimetry: the heterogeneity requirement

Joanna Brunker, Paul C. Beard, Univ. College London (United Kingdom)

Acoustic resolution photoacoustic Doppler velocimetry provides a major opportunity to overcome the limitations of existing blood flow measuring methods. By enabling measurements with high spatial resolution several millimetres deep in tissue, it could yield insights into microvasculature blood flow, which plays critical roles in many diseases. Although previous work has demonstrated proof-of-concept in solid phantoms, it has proved challenging to make measurements in blood, thus preventing translation to in vivo applications. A common explanation for this difficulty is the requirement for sufficient heterogeneity in the flow medium to track changes in the photoacoustic signals generated. However, by undertaking a rigorous study using carefully controlled blood-mimicking fluid phantoms, we found that this problem is more subtle than previously thought. Conventionally, it is thought that the perceived heterogeneity is a simple function of the detector bandwidth, but we have identified a second influence due to the detector that effectively causes bandlimiting and reduces heterogeneity in the same way. This effect is a spatial averaging that occurs over the detector field-of-view and can result in a suspension being perceived as homogeneous even when the detector bandwidth is infinite. Evidence for this is seen in both numerical simulations and experimental data, where the frequency content of the photoacoustic signals is progressively downshifted with increasing absorber concentration. The two-fold bandlimiting effect has profound implications for acoustic resolution photoacoustic Doppler velocimetry in whole blood and will direct future work towards realising the potential of this technique for making deep tissue blood flow measurements in the microvasculature.
EXPERIMENTAL EVALUATION OF CMUT AND PZT TRANSDUCERS IN RECEIVE ONLY MODE FOR PHOTOACOUSTIC IMAGING

Omri Warshavski, Cyril Meynier, Nicolas Sénégon, Pascal Chatain, An Nguyen Dinh, Vernon S.A. (France)

Capacitive Micro machined Ultrasound Transducers (cMUT) are an alternative and promising developing technology that complement conventional ultrasound transducer technologies. The unique characteristics of cMUT, such as broadband response, design flexibility and natural packaging capabilities with integrated circuit technology make them highly suitable for optoacoustic signal detection and Photoacoustic imaging. Commercial piezoelectric transducers that are commonly used in Photoacoustic or Photoacoustic systems are by design dedicated to perform both for transmit and receive operations.

The goal of the characterization is to establish reliable requirements to support the development of two-dimensional CMUT array for real time, high frame rate three-dimensional Photoacoustic Imaging systems.

DUAL MODALITY PHOTOACOUSTIC AND OPTICAL COHERENCE TOMOGRAPHY IMAGING WITH ANGIOGRAPHIC EXTENSION

Behrooz Zabihian, Laurin Ginner, Daniel J. Fechting, Zhe Chen, Boris Hermann, Mengyang Liu, Rainer Andreas Leitgeb, Wolfgang Drexler, Medizinische Univ. Wien (Austria)

In the past few years, the complementarity of photoacoustic tomography (PAT) and optical coherence tomography (OCT) has been demonstrated in both ex vivo preclinical and in vivo clinical studies. While PAT resolves the vasculature in deep tissue beyond the optical mean free path, OCT can provide co-registered tissue information in the first two millimeters, providing a valuable structural background. However, though permitting deep tissue vasculature information in a fairly large volume, PAT is limited in resolution due to the sensor’s frequency response and the scanning pattern. The requirement of a powerful excitation source further keeps PAT from a real-time imaging method volumetrically. To tackle these issues, we report a dual modality PAT/OCT system with OCT angiographic imaging extension. A swept source OCT (SS-OCT) sub-system is combined optically with an optical detection PAT sub-system which uses a Fabry-Perot polymer film sensor. With the superb low phase noise of our Insight swept source (Bismuth Silicon Oxide: Bi12SiO20) photorefractive crystal. Due to the photorefractive grating generated in the crystal, the reference beam gets diffracted in the direction of the signal beam. By interfering both beams on a photodiode, one can measure the phase shifts via amplitude modulations of the laser intensity and thus the ultrasonic displacements on the sample surface. Using the PZT vibration, we optimized this interferometer by changing the signal and reference beam intensities ratio and their incident angle. An external electric field is applied to the photorefractive crystal to enhance the signal to noise ratio (SNR).

This non-contact PAM was first tested to measure the displacement by applying sinusoidal voltages on the piezochips (PZO) in the order of nanometers with different frequencies. By surface vibrations, the reflected beam gets modulated and interferes with the reference beam in a BSO crystal and demonstrate an all-optical-component photoacoustic microscopic (PAM) technique in study of breast cancer detection. This work presents an experimental comparative analysis of the performance characteristics of individual elements from various cMUT and PZT 1D transducer arrays for superficial imaging. The benchmarking is focused on receive sensitivity, frequency response, angular response and noise equivalent pressure (SNR). We used a dedicate Analog Front End which enables high degree of adjustability and optimization of the receive chain parameters for different transducer types. The elementary RF signal analysis is followed by a K-Wave based simulation that links influence of tested transducer receive performances to the final image quality.

This setup was finally utilized to investigate the risk level of breast cancer among different cell lines. The difference was observed among different types of cancer cell lines with different risk levels, such as nonaggressive cancerous cells (MCF-7), and aggressive cancerous cells (MDA-MB-231) as well as normal cell line (Fibroblast).

OPTICALLY INDUCED MICROBUBBLES AROUND GOLD NANORODS: THE INFLUENCE OF PARTICLE PARAMETERS AND ENVIRONMENT ON CAVITATION THRESHOLD

Lucia Cavigili, Fulvio Ratto, Marella de Angelis, Sonia Centi, Sarah Lai, Alberto Cini, Istituto di Fisica Applicata “Nello Carrara” (Italy); Claudia Borri, Istituto di Fisica Applicata “Nello Carrara” (Italy) and Univ. degli Studi di Firenze (Italy); Stefano Colagrande, Univ. degli Studi di Firenze (Italy); Roberto Pini, Istituto di Fisica Applicata “Nello Carrara” (Italy)

Photoacoustic imaging and microsurgery are attracting interest as innovative approaches to manage several pathological conditions such as cancer. In these applications, the absorption of short light pulses, in the nanosecond duration regime, triggers a cascade of photothermal and thermoelastic processes that result into the emission of ultrasounds and even the generation of vapor microbubbles. The emission of ultrasounds may be exploited to reconstruct images that combine high penetration depth and optical contrast. The generation of vapor microbubbles may be used to destroy individual cells, with the advantage, when compared with traditional optical hyperthermia, of a more limited heat diffusion. The use of optical contrast agents that exhibit high optical absorbance and specificity for a certain biological target, such as a tumor, enhances the potential of photoacoustic imaging and microsurgery.

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In this context, plasmonic particles such as gold nanorods (GNRs) have emerged as an ideal option, since absorb light in the near infrared window of greatest transmittance through biological tissues, are inert in the body and convenient for modification with functional moieties. GNRs are widely investigated as contrast agents for photoacoustic imaging, but less it is known about the process of bubbles generation. In particular their photostability is in conflict with the trigger of the cavitation regime. Here we study the influence of GNRs parameters, such as size, coating and environment, on the cavitation threshold. We expect these results will provide useful indications to the development of GNRs as contrast agents for innovative theranostics techniques based on light and ultrasound interaction.

9708-146, Session PMon

Dynamics of double-pulse photoacoustic excitation

Maxim N. Cherkashin, Carsten Brenner, Lena Göring, Benjamin Döpke, Nils C. Gerhardt, Martin R. Hofmann, Ruhr-Univ. Bochum (Germany)

In contrast to the well-established and widely used theory of photoacoustic signal generation by single delta-like pulses, the field of multiple pulses excitation is not yet studied well. The beneficial effect of an alternation of the excitation sequence has been shown previously in coding experiments, as well as both aiding resolution and adding a new contrast mechanism for photoacoustic microscopy via local change of the Grüneisen parameter and as photoacoustic resonance spectroscopy. This technique is highly promising, as it allows using a new contrast mechanism for tissue differentiation based on its mechanical properties. The exploitation of these possibilities requires clarification of the transducer influence. This study aims to identify the strength of this influence for the region of short inter-pulse delays below one microsecond, which has not been studied well.

We investigate the photoacoustic responses of a phantom sample to double-pulse excitation with varying inter-pulse delays, measured with different ultrasound transducers. Both focused and flat surface single element transducers are used in the study. The central frequencies are chosen in the low-frequency band as they are most widely used in clinical ultrasound. One higher frequency transducer is taken for comparison.

9708-147, Session PMon

All fiber sensor array for ultrasound sensing

Haniel Gabai, Idan Steinberg, Avishay Eyal, Tel Aviv Univ. (Israel)

Ultrasound sensing has a key role in the field of biomedical ultrasonography. Currently, detection of ultrasound is dominated by piezoelectric sensors. However, such sensors suffer from several inherent limitations: they are prone to electromagnetic interference (EMI) opaque and rigid. Moreover, arrays of piezoelectric elements require costly parallel detection circuitry. Optical fiber sensors (OFS), however, are immune to EMI, flexible and can be operated at extreme conditions. Moreover, OFS compatibility with photoacoustics can be used in situations where traditional piezoelectric probes are limited.

Although very attractive for ultrasound sensing, multiplexing several fiber based ultrasound sensors remains a challenging and costly task. In this work, we demonstrate cost-effective and straightforward approach for multiplexing multiple OFS for ultrasound sensing. The design is based on a recently developed system in which all sensing elements are connected to a single interrogator and to a single digitizing circuit. Moreover, it facilitates optical phase drift compensation and enables the sensor array to be interrogated at very high repetition rates (kHz) making it ideal for applications where imaging of dynamic processes is desired.

To experimentally test our method, we have constructed a 6 sensors array. The array was exposed to an ultrasound excitation, generated by a sequence of low driving (2.5V-10V vs. 100V-400V in similar works) 1MHz, 50μs long, tone bursts. Using the appropriate analysis we were able to successfully reconstruct the tone burst sequence from all sensors (each with bandwidth of 320kHz) and measure the array’s geometry from the obtained signals.

9708-148, Session PMon

Photoacoustic imaging using lock-in amplification and tunable-repetition-rate pulsed fiber lasers

Wei Shi, Roger J. Zemp, Univ. of Alberta (Canada)

Most photoacoustic imaging systems rely on broadband amplifiers to record time-domains photoacoustic signals. Other groups have also used modulated continuous-wave lasers as an excitation source for frequency-domain imaging. Frequency-domain imaging offers the potential of lock-in amplification which has sensitivities as low as nV in even in noise orders of magnitude higher than the signal. However, modulated CW sources do not satisfy thermal and stress confinement conditions required for optimal photoacoustic signal strength. We report for the first time to our knowledge a photoacoustic methodology using pulsed fiber lasers with tunable repetition rates and lock-in amplification. The repetition-rates can be matched to the transducer passband. We demonstrate phantom measurements comparing the signal-to-noise of our lock-in technique with broadband time-domain measurements. Instead of 15 dB SNR acquired by using a broadband ultrasound pulse-receiver and a 1 MHz ultrasound transducer, we achieved 48 dB SNR by using lock-in amplification with the same low excitation laser pulse energy setting. Take into account ~180 times averaging during lock-in amplification, and also ~250 Hz equivalent noise bandwidth of lock-in amplification over broadband amplification, our lock-in technique was shown to exhibit signal-to-noise ratios several orders of magnitude more sensitive than the broadband detection techniques per unit frequency. Phantom studies on imaging depth also demonstrated photoacoustic imaging using lock-in amplification and tunable-repetition-rate pulsed fiber lasers was able to achieve similar imaging resolution at ~10 times deeper imaging depth compared to photoacoustic imaging using traditional broadband time-domain measurements. The high sensitive lock-in amplification technique has shown its potential to greatly extend the depth of photoacoustic imaging.

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9708-149, Session PMon

Photoacoustic tomography of inflammatory arthritis based on a FDA-approved macrophage-targeted contrast agent clofazimine

Chao Tian, Rahul K. Keswani, Gustavo Rosania, Xueming Wang, Univ. of Michigan (United States)

Arthritis is a leading cause of disability among adults worldwide. Photoacoustic tomography (PAT), which combines optical absorption contrast and ultrasonic resolution, provides a unique opportunity to overcome the limitations of purely optical imaging modalities, and is poised to exert a profound impact on the diagnosis and treatment of joint disorders. However, although label-free PAT based on endogenous contrasts of different tissue types shows advantages over ultrasound imaging, the specificity and sensitivity of PAT to different anatomical and functional changes is still limited. Therefore, the development of PAT contrast agents could lead to better visualization of the pathophysiology of inflammatory arthritis, leading to improvements in its diagnosis and therapy. Moreover,
PAT contrast agents provide unique opportunities for direct visualization of specific cellular or molecular targets and the quantification of changes to the relevant markers in physiologically authentic environments. Here, we re-engineered and formulated a small molecule functional contrast agent Clofazimine (CFZ) for PAT of human arthritis. CFZ is a FDA-approved, strongly pigmented, macrophage-targeted, rimenophenazine antibiotic. Originally discovered in the 1950s, it is included in the WHO-recommended list of essential medications and is clinically useful due to its potent anti-inflammatory activity. Photoacoustic spectroscopy measurement and photoactivity study of CFZ show that the new photoacoustic contrast agent has strong absorbance in the visible band and is more photostable comparing to Indocyanine Green (ICG), a commonly used optical contrast agent. Dual-modality photoacoustic and ultrasound imaging of gel phantoms and human cadaver joints reveal that CFZ has strong photoacoustic signals and holds promise to be used as a new contrast agent for functional imaging of inflammatory arthritis.

9708-150, Session PMon

**Numerical phantom for 3D optoacoustic breast imaging**

Yang Lou, Mark A. Anastasio, Catherine Appleton, Kenji Mitsuhashi, Washington Univ. in St. Louis (United States); Alexander A. Oravsky, TomoWave Labs, Inc. (United States)

Optoacoustic computed tomography (OAT) is an emerging medical imaging modality for breast cancer detection and diagnosis. Developing an optimal OAT breast imaging system requires balancing multiple designing constraints that can be expensive and time-consuming. Therefore, computer simulation studies are often conducted to facilitate this task. However, most existing computer simulation studies of OAT breast imaging employ simple phantoms that oversimplify the complex anatomical structures in breasts, thus limiting the value of these studies in guiding real world system design. In this work, we propose a method to generate realistic numerical breast phantoms for OAT research. The phantom includes five types of major structures with shapes and dimensions determined from clinical Magnetic Resonance Angiography (MRA) images: a skin layer, fat/glandular tissues that homogeneously fill the inside of the breast phantom as a background, and major vessel branches that are segmented from the MRA images. The phantom also includes capillary vessels that are simulated by use of a random vessel generator based on the Murray’s law, and tumors that are represented by denser vessel nests generated using the same vessel generator. By assigning realistic optical and acoustic parameters to different tissues, we established both optical and acoustic breast phantoms, which can be exported into standard data formats for cross-platform usage. Computer-simulation studies are conducted to demonstrate the use of the developed phantom in mimicking realistic OAT breast imaging and in the real world system design and optimization. In addition, the proposed method can generate an ensemble of breast phantoms that will facilitate image quality studies grounded in statistical decision theory.

9708-152, Session PMon

**In vivo photoacoustic flowmetry in the optical diffusive regime based on saline injection**

Yong Zhou, Joemini Poudel, Guo Li, Lihong V. Wang, Washington Univ. in St. Louis (United States)

So far, because of the small photoacoustic (PA) signal changes due to flowing red blood cells, no proposed acoustic resolution photoacoustic tomography (AR-PAT) method has succeeded in measuring blood flow velocity in vivo. Here we present a saline-injection-based method to increase the PA signal changes. By injecting saline into the blood stream, the PA signals have sharp changes at the saline-blood interfaces 7 blood’s PA signal is strong, while saline’s signal is negligibly low. Thus, by monitoring the time course of the PA signals from the interface, the flow velocity in the blood stream can be quantified. We first demonstrated our method in phantom experiments, where a root-mean-squared error of prediction of 0.29 mm/s was achieved. By injecting saline into a mouse tail vein covered with 1 mm chicken tissue, we showed that the flow velocity in the tail vein could be measured at depths, which is especially pertinent to monitoring blood flow velocity in patients undergoing intravenous infusion. Considering the difficulty that ultrasound encounters in measuring slow blood flow and the oxygen saturation of hemoglobin (sO2), PA-based deep flow and sO2 measurement open a new window for quantifying metabolic rate of oxygen (MRO2) in humans. The MRO2 measurement has potentially significant applications, such as noninvasive tumor screening and blood disorder diagnosis.

9708-153, Session PMon

**Accuracy and feasibility of quantitative photoacoustic tomography inversion schemes: from simulation to experiment**

Martina B. Fonseca, Univ. College London (United Kingdom); Bajramp Zeqiri, National Physical Lab. (United Kingdom); Paul C. Beard, Benjamin T. Cox, Univ. College London (United Kingdom)

The ability to accurately quantify chromophore concentration in high resolution, 3D, photoacoustic tomography (pPAT) would have a major impact on pre-clinical imaging, and be a valuable tool for clinical studies of photobiomodulation and pathology. Nevertheless, most in vivo studies performing quantification have used overly-simplified strategies of limited accuracy and little validation. Recent years have seen significant advances in the theoretical understanding of qPAT and in the development of model-based inversion strategies. These mostly concentrate on the need to account for
Unmixing chromophores in human skin with a 3D multispectral optoacoustic mesoscopy system

Mathias Schwarz, Juan Aguirre, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany); Dominik Soliman, Helmholtz Zentrum München GmbH (Germany) and Technische Universität München (Germany); Andreas Buehler, Vasilis Ntziachristos, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany)

The absorption of visible light by human skin is governed by a number of natural chromophores: Eumelanin, phaeomelanin, oxyhemoglobin, and deoxyhemoglobin are the major absorbers in the visible range in cutaneous tissue. Label-free quantification of these tissue chromophores is an important step of optoacoustic (photoacoustic) imaging towards clinical application, since it provides relevant information in diseases. In tumor cells, for instance, there are metabolic changes (Warburg effect) compared to healthy cells, leading to changes in oxygenation in the environment of tumors. In malignant melanoma changes in the absorption spectrum have been observed compared to the spectrum of nonmalignant nevi. So far, optoacoustic imaging has been applied to human skin mostly in single-wavelength mode, providing anatomical information but no functional information. In this work, we excited the tissue by a tunable laser source in the spectral range from 413-680 nm with a repetition rate of 50 Hz. The laser was operated in wavelength-sweep mode emitting consecutive pulses at various wavelengths that allowed for automatic co-registration of the multispectral datasets. The multispectral raster-scan optoacoustic mesoscopy (RSOM) system provides a lateral resolution of <60 µm independent of wavelength. Based on the known absorption spectra of melanin, oxyhemoglobin, and deoxyhemoglobin, volumetric absorption maps of all three absorbers were calculated from the multispectral dataset. We determined blood oxygenation in human skin and compare our findings to the literature. In this work, we show, to the best of our knowledge, the first functional volumetric optoacoustic images of human skin in vivo on a mesoscopic scale.

Spectral correction of OA signals based on multiple irradiation sensing: theoretical considerations

H. Güngör Akarcay, K. Gerrit Held, Jaroslav Ricka, Martin Frenz, Michael Jaeger, Univ. Bern (Switzerland)

As part of our research on quantitative optoacoustic OA imaging - e.g., the determination of blood oxygen saturation levels in well-localized vessels/arteries - we focus on the spectral correction of the measured OA signals. These are initially distorted with respect to the actual blood spectra, due to the wavelength-dependent attenuation of the light fluence inside tissues. The distortion can be experimentally corrected on a broad spectral range, based solely on OA imaging using multiple-irradiation sensing. We model tissue as a strongly scattering, homogeneous background, optically characterized by its spectrally varying effective attenuation coefficient $\alpha_{eff}(\lambda)$. In this background, a discrete number of blood vessels, with an excess absorption with respect to said background, are sparsely distributed. In the experiment, the OA signals generated by the vessels, which serve as “intrinsic fluorescence detectors”, are recorded as a function of the light propagation distance. The recorded signals are fitted to an appropriate light diffusion model, whose main parameter is $\alpha_{eff}(\lambda)$. Our preliminary studies underlined the main limitations of the widely used diffusion model for a homogeneous, semi-infinite medium. We conducted further theoretical work to develop an empirical model, whose computational efficiency is suitable for real-time clinical applications and which enables retrieving $\alpha_{eff}(\lambda)$ from
OA measurements in more realistic tissue samples with arbitrarily irregular geometries, where the role of boundaries is influential. Likewise, the effect of the excess absorption in the presence of several vessels which disturb the background fluence was quantified and accounted for. Here, we present numerical experiments (Monte Carlo simulations performed with our general purpose in-house jaMCP3 software) and phantom studies. We discuss how the accuracy of the \( \text{eff}(\cdot) \) estimation affects the spectral correction of the OA signals.

9708-183, Session PMon

Study of resting-state functional connectivity in the mouse brain using photothermal microscopy

Ali Hariri, Nicholas Bely, Chen Chen, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

The increasing use of mouse models for human brain disease studies, coupled with the fact that existing high-resolution functional imaging modalities cannot be easily applied to mice, presents an emerging need for a new functional imaging modality. Utilizing both mechanical and optical scanning in the photoacoustic microscopy, we imaged spontaneous cerebral hemodynamic fluctuations and their associated functional connections in the mouse brain. The images were acquired noninvasively with a fast frame rate, a large field of view, and a high spatial resolution. Correlation was investigated inter-hemispherically between bilaterally homologous regions, as well as intra-hemispherically within the same functional regions. The functional connectivity in different regions are studied. The resultant map can then be used in the study of brain disorders such as stroke, Alzheimer’s, schizophrenia, multiple sclerosis, autism, and epilepsy. Our experiments show that photoacoustic microscopy is able to detect connectivity between different functional regions, promising a powerful functional imaging modality for future brain research.

9708-57, Session 9

Dual-wavelength optical-resolution photoacoustic microscopy for cells with gold nanoparticle bioconjugates in threedimensional cultures

Po-Yi Lee, Wei-Wen Liu, Shu-Ching Chen, Pai-Chi Li, National Taiwan Univ. (Taiwan)

Three-dimensional (3D) in vitro models bridge the gap between typical two-dimensional cultures and in vivo conditions. However, conventional optical imaging methods such as confocal microscopy and two-photon microscopy cannot accurately depict cellular processing in 3D models due to limited penetration of photons. We developed a dual-spectral optical-resolution photoacoustic microscopy (OR-PAM), which provides sufficient penetration depth and spatial resolution, for studying CD8+ cytotoxic T lymphocytes (CTLS) trafficking in an in vitro 3D tumor microenvironment. CTLS play a cardinal role in host defense against tumor. Efficient trafficking of CTLS to the tumor microenvironment is a critical step for cancer immunotherapy. For the proposed system, gold nanoparticles (AuNPs) have been a popular choice for contrast agent for photoacoustics due to their high optical absorption. Targeted cells conjugated with AuNPs can be identified by photoacoustic irradiation based on their distinct absorption spectra determined by their size and shape. Moreover, we use an x-y galvanometer scanner to obtain high scanning rate. Using the proposed system, lateral and axial resolutions were measured at 1.2 \( \mu \)m and 5 \( \mu \)m, respectively. We successfully showed that dual-wavelength OR-PAM can map both the distribution of CTLS with gold nanospheres at a visible wavelength of 523 nm and the 3D structure of tumor spheres with gold nanorods at a near-infrared wavelength of 815 nm in an in vitro 3D microenvironment. Our OR-PAM provides better biological relevant information in cellular interaction and is highly potential for preclinical screening of anti-cancer drugs that can expedite the tumor cell targeting of CTLs.

9708-58, Session 9

Cost-effective design of a concurrent photoacoustic-ultrasound microscope using single laser pulses

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A method for concurrent photoacoustic (PA) and ultrasound (US) imaging with single laser pulses was previously demonstrated. A light-absorbing multilayer film that can generate a US pulse based on the thermoelastic effect is used. With such a film, the generated US can be adjusted so that it does not overlap with the spectrum of the PA signal generated by the light transmitting through the layer. Thus, the US signal and the PA signal can be separated. In our study, we continue with the same concurrent imaging approach and propose a cost-effective and portable design. The design consists of a pulsed laser diode (805nm, SPL_LL90_3, Osram, Germany) with the repetition rate up to 20 kHz and energy of 300 nJ/pulse. A multilayer film is employed to generate narrow band US signals under laser excitation for US imaging. A focused transducer is used for reception of both US backscattered signals and the PA signals. The signals are digitized at 80 MSamples/s with a pulser/receiver (US-Key, Lecouer, France). With simple spectral filtering, the PA signals and the US signals can be separated. With optical resolution, the system has a theoretical lateral resolution of 1.6 \( \mu \)m in PA imaging and 40 \( \mu \)m in US imaging. One of the applications of the proposed microscope is for tumor biology, where angiogenesis is an essential topic for understanding tumor growth and tumor metastasis. We will demonstrate performance of the proposed system by imaging vasculature networks.

9708-59, Session 9

Photoacoustic imaging with multiple speckle illumination: towards deep tissue imaging below acoustic resolution

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Photoacoustic imaging recently emerged as a powerful technique to image optical absorption at a few millimeters depth inside biological tissue. Resolution is however limited by acoustic diffraction. High-frequency acoustic detectors have been engineered to increase this resolution, but due to stronger attenuation of these frequencies in tissue, resolution cannot be indefinitely increased at arbitrary depth. Here we propose a fluctuation-based method to resolve optically absorbing structures below the acoustic diffraction limit. This method draws inspiration from a super-resolution fluorescence imaging technique called Super-resolution Optical Fluctuation Imaging (SOFI). Using stochastically blinking fluorophores, Dertinger and colleagues showed that high order fluctuation images could resolve sub-diffraction objects [1]. It has been indeed shown that the variance of successive fluorescence images involves the squared point-spread-function (PSF) of the system, which is sharper than the PSF itself. We recently showed that fluctuation of the photoacoustic signal could be
induced using dynamic speckle illumination. We showed that this could solve visibility problems of photoacoustic imaging due to limited-view detection and finite bandwidth [2].

Here we report on the use of multiple speckle illumination to perform super-resolution photoacoustic imaging.

The variance of photoacoustic images measured under different speckle illuminations is shown to resolve absorbers below the acoustic diffraction limit. A deconvolution approach was found to be crucial to fully highlight the resolution enhancement. This is to the best of our knowledge the first super resolution photoacoustic imaging method. This work paves the way for sub-acoustic resolution photoacoustic imaging at depth in biological tissue.

References:
[1] Dertinger et al. PNAS, 106(52), 22287-22292, 2009

9708-60, Session 9

Multi-acoustic lens design methodology for a low cost C-scan photoacoustic imaging camera

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We have designed and implemented an acoustic lens that fits into a photoacoustic imaging camera to focus the photoacoustic signals from absorbers in an object plane simultaneously on to an image plane. We use a multi-element ultrasound transducer array to capture the focused photoacoustic signals. Acoustic lens eliminates the need for expensive data acquisition hardware systems, faster compared to electronic focusing and enables real-time image reconstruction. Using this photoacoustic lens camera, we have imaged more than 150 ex-vivo human prostate, kidney and thyroid specimens with a millimeter resolution for cancer detection. The acoustic lens was fabricated using liquid photopolymer resins with rapid prototyping technology. Using the water referenced refractive index of these resins; we designed the acoustic lens with “Code V” optical lens software. We have compared a single acoustic lens system with multi-acoustic lens and evaluated the image quality of the two systems using simulations and experiments. An advanced acoustics toolbox in MATLAB was used for simulating a two-dimensional gridded model that incorporates realistic photoacoustic signal generation, acoustic wave propagation with medium property defined. We have calculated the point spread function from the simulations and experiments to demonstrate the image quality. Our goal was to incorporate the best acoustic lens system into a one inch diameter transrectal prostate probe for in-vivo testing. Using a multi-acoustic lens system over a single lens, significant improvements were quantified, though there were some differences between simulations and experimental point spread function that are under investigation.

9708-61, Session 9

Reflection-artifact-free photoacoustic imaging using PAFUSion (photoacoustic-guided focused ultrasound)

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Reflection artifacts are a main challenge to photoacoustic (PA) imaging of acoustically inhomogeneous tissue. High light fluence beneath the ultrasound (US) probe generates strong PA transients from superficial optical absorbers (skin, blood vessels), which propagate into the tissue and echo back from acoustically thicker structures (tendon, bone). This generates artifacts, reducing the contrast and thus the imaging depth. This problem is prominent in epi-style imaging geometry where light irradiation is from the same side where US signals are acquired. Considering the broad interest in developing reflection-mode handheld systems both for pre-clinical and clinical applications, it is vital to develop methods for reducing these reflection artifacts.

We already proposed the idea of PAFUSion to identify reflection artifacts in PA imaging. This technique mimics the inward travelling wave-field from PA sources by applying focused US pulses. Reflection artifacts caused by these PA sources are thus obtained without the outward travelling direct PA signal and can therefore be identified and subtracted from the PA image.

In this work, we will demonstrate reflection artifact correction in addition to identification, towards achieving artifact-free PA images in real-time. In view of clinical applications where a high framerate is required, we implemented a synthetic aperture approach where US is synthetically focused to any desired point in the imaging plane, requiring a minimum number of signal acquisitions. Phantom experiments and initial in vivo measurements will be presented to demonstrate reflection artifact reduction using our method. PAFUSion thus foresees good potential for improving real-time PA imaging of deep tissue.

9708-62, Session 9

Design and development of an endovaginal ultrasound and photoacoustic imaging system

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Gynecologic cancers such as cervical, ovarian, uterine, vulvar, and vaginal cancers are common cancers in females. In 2015, more than 98,000 women are expected to be diagnosed with gynecologic cancer which would lead to more than 30,000 deaths. Combined ultrasound (US) and photoacoustic (PA) imaging has demonstrated promising capabilities in cancer diagnosis. While US provides an excellent morphological image of tissue structures, PA can add complementary functional information such as blood oxygenation level. Besides, PA augmented with cellular and molecular targeted nano-sized contrast agents is capable of imaging cancer at the cellular and molecular levels. Therefore, an endovaginal US/PA imaging system can potentially become a suitable screening tool for various types of gynecologic cancers. In this study, we present design and development of an endovaginal US/PA probe, consisted of a commercially available transvaginal ultrasound transducer (ATL C9-5) and light delivery via a customized fiber optic bundle. The fiber bundle consisted of 19 large core diameter (1000-3m core diameter) multimode fibers and was used to uniformly deliver the light to the US imaging plane. The fibers orientations were optimized to provide the maximum energy delivery and to the elevation focus of the US transducer. A tunable, powerful, pulsed laser pulses (SpectraPhysics Quanta-Ray PRO 270) were coupled to the fiber bundle. A fully digital and programmable US scanner (Verasonics Vantage 128) was used to perform both US imaging and PA data acquisition. A MATLAB-based user interface was developed to process and provide overlaid US/PA images. The functionality of the developed probe was tested in a set of tissue mimicking phantoms, indicating the ability of the system to perform robust spectroscopic (680-840 nm) PA imaging at clinically relevant depths (more than 10 mm).
9708-63, Session 9

3D optoacoustic tomography system for molecular imaging of contrast agents in small animals
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We developed a new and improved Laser Optoacoustic Imaging System, LOIS-3D for preclinical research applications in small animal models. The advancements include (i) a new stabilized imaging module with a more homogeneous illumination of the mouse yielding a better spatial resolution (<0.2 mm) and (ii) a new low noise amplifier incorporated into the ultrasonic probe and providing the noise equivalent pressure of 1 Pa resulting in increased signal-to-noise ratio and the optical absorption sensitivity of about 0.03/cm. We also improved scan time and the image reconstruction times. This prototype has been commercialized for a number of biomedical research applications, such as imaging vascularization and measuring hemoglobin / oxyhemoglobin distribution in the organs as well as imaging exogenous or endogenous optoacoustic contrast agents. As examples, we present an in vivo experiments using mice with and without tumor injected with contrast agents based on nanoparticles of gold (long nanorods, GNR), melanin (MNP) and indocyanine green (ICG). LOIS-3D was capable of detecting -1-2 pmole of the ICG, -1-2 fmoles of MNP and -0.1-0.2 fmoles of GNR in tissues with relatively low blood content. With its high sensitivity and excellent spatial resolution LOIS-3D is an advanced alternative to fluorescence and bioluminescence based modalities for molecular imaging in live mice.

9708-64, Session 10

Developing a slip-ring based multi-transducer photoacoustic tomography system
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Slip ring has been widely used in X-ray computed tomography, which enables the continuously fast rotational scanning. In this study, we employed the slip ring to build a versatile multi-transducer scanning photoacoustic tomography system. This system has several advantages, first, with multi-transducers, this system performs like an “array” that can significantly reduce the imaging period; secondly, multiple transducers with different bandwidths or other characteristics can be mounted and detect PA signals simultaneously. The performance of this system was demonstrated by both phantom experiments and in vivo animal imaging. Our results demonstrated that the slip-ring-based PAT system has great potential for in vivo animal studies.

9708-65, Session 10

In vivo photoacoustic imaging of mouse brain voltage signals
Bin Rao, Ruiying Zhang, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Optical imaging of brain voltage signals is significantly limited in depth due to optical scattering and the absorptive property of brain tissue. Photoacoustic (PA) imaging promises to break this hard limit by utilizing both ballistic and diffused photons. To demonstrate the feasibility of PA, we used an in vivo mouse model. The brain cortex tissue was stained with dipicrylamine dye, electrically stimulated, and imaged with a customized dual-isosbestic-wavelength PA microscope (DIW-PAM). DIW-PAM separates voltage-induced PA signals from blood-induced PA signals and thereby allows recording the voltage response of mouse cortex tissue without interference from hemoglobin responses. The resting state PA voltage response signal exhibited a noise-like signal in the frequency domain. Upon 3 Hz electrical stimulation, the PA voltage response signal showed frequency peaks of 3.2 Hz and 6.3 Hz (Fig. 1). Although dipicrylamine dye is not fast enough for recording neuron action potentials, it served well for the purpose of this feasibility study. In conclusion, we successfully demonstrated in vivo photoacoustic imaging of mouse brain voltage signals for the first time. If a fast voltage-sensitive dye is available, using photoacoustic computed tomography (PACT) instead of PA microscopy could allow acquiring full-field PA action potential images at a speed limited only by the laser pulse repetition rate.

9708-66, Session 10

Solution-processed CNT-PDMS photoacoustic lens for long-range high-precision treatment
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The photoacoustic effect has been adopted to generate ultrasound with high-amplitude and high-frequency characteristics for transducer applications. A carbon nanotube (CNT)-polydimethylsiloxane (PDMS) composite thin-film transmitter is known as a highly efficient source exhibiting extraordinary energy conversion efficiency and generating a powerful pressure output over a broadband high-frequency range under pulsed laser excitation. Previously, the CNTs were all grown by using a high-temperature chemical vapor deposition (CVD) process. This often caused an issue of spatial non-uniformity of transmitters, resulting in a low yield, when they are formed over large area (e.g. > a few cm2) or on a curved structure. In order to overcome such issue, we developed a solution-based CNT-PDMS composite film and an photoacoustic lens, using chemically functionalized CNTs and a spin-coating process. An output pressure was measured for comparing the new transmitter with a 100-nm thick Cr film (reference) and an Ag nanoink-PDMS composite film. A pressure strength from the CNT-PDMS composite film was 21-fold more powerful than that of the Cr, and 8-fold than that of the nanoink composite. The current solution-based process can be useful to develop high-efficiency photoacoustic transmitters with large-area for example, a high-focal gain and long-range photoacoustic lens. Furthermore, we characterized various photoacoustic lenses with different focal depths (15-30 mm) and f-numbers in terms of laser energy versus pressure amplitude, focal spot width (<100 um), and cavitation threshold (an order of several mJ). These lenses can be used for long-range high-precision ultrasonic treatment.

9708-67, Session 10

Non-contact photoacoustic imaging by raster scanning a piezoelectric air-coupled transducer
Xosé Luis Deán-Ben, Helmholtz Zentrum München GmbH (Germany); Genny A. Pang, Technische Univ. München (Germany); Francisco Montero de Espinosa, Consejo Superior de Investigaciones Científicas (Spain); Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

Optoacoustic techniques rely on ultrasound transmission between optical absorbers within tissues and the measurement location. Much like in ultrasonography, commonly used piezoelectric transducers require either direct contact with the tissue or through a liquid coupling medium. The contact nature of this detection approach then represents a disadvantage of standard optoacoustic systems with respect to other imaging modalities.
(including optical techniques) in applications where non-contact imaging is needed, e.g. in open surgeries or when burns or other lesions are present in the skin. Herein, non-contact optoacoustic imaging using raster-scanning of a spherically-focused piezoelectric air-coupled ultrasound transducer is demonstrated. When employing laser fluence levels not exceeding the maximal permissible human exposure, it is shown possible to attain detectable signals from objects as small as 1 mm having absorption properties representative of blood at the near-infrared wavelengths with a relatively low number of averages. Optoacoustic images from vessel-mimicking tubes embedded in an agar phantom are further showcased. The initial results indicate that the air-coupled ultrasound detection approach can be potentially made suitable for non-contact biomedical imaging with optoacoustics.

9708-68, Session 10
A novel two-axis micromechanical scanning transducer for handheld 3D ultrasound and photoacoustic imaging
Chih-Hsien Huang, Jun Zou, Texas A&M Univ. (United States)

Ultrasound and photoacoustic imaging has become two useful non-destructive diagnostic techniques. To conduct 3D ultrasound and photoacoustic imaging, the time-variant ultrasound field at a 2D array of locations has to be properly recorded for image reconstruction. Although 2D ultrasound transducer arrays can be used to detect the incoming ultrasound signals, a large number (100s~1000s) of transducer elements and T/R channels are required. Alternatively, the ultrasound signals can also be received by mechanically scanning a single-element transducer over the imaging target. However, the use of 2-axis motor stages makes the entire imaging system complex and bulky. Second, the slow mechanical scanning frequency limits the date acquisition speed. In this paper, we report a new 2-axis micromechanical scanning transducer technique to enable fast and versatile 3D ultrasound and photoacoustic imaging. The 2-axis micromechanical scanning transducer consists of a miniaturized single-element transducer mounted onto a unique 2-axis water-immersible electromagnetic micro actuator. With a mechanical scanning frequency of 19.532 Hz and an ultrasound pulse repetition rate of 5 kHz, the scanning transducer was scanned along 60 concentric paths with 256 detections on each to simulate a 2D ultrasound array with 60x256 elements. Using the scanning transducer, 3D pulse-echo ultrasound imaging of multiple targets immersed in water as the imaging target was successfully conducted with only one T/R channel. The lateral resolution of the 3D ultrasound imaging was further improved with SAFT. The two-axis micromechanical scanning transducer can be further miniaturized to be fitted into a liquid-filled compact probe for handheld operations.

9708-69, Session 10
Wavelength-modulated differential photoacoustic (WM-DPA) imaging towards noninvasive diagnosis of cancer
Edem Dovlo, Bahman Lashkari, Andreas Mandelis, Univ. of Toronto (Canada)

This study explores wavelength-modulated differential photoacoustic (WM-DPA) imaging for non-invasive early cancer detection via sensitive characterization of functional information such as hemoglobin oxygenation (SO2) levels. Well-known benchmarks of tumor formation such as angiogenesis and hypoxia can be addressed this way. While most conventional photoacoustic imaging has almost entirely employed high-power pulsed lasers, frequency-domain photoacoustic radar (FD-PAR) has seen significant development as an alternative technique. It employs a continuous wave laser source intensity-modulated and driven by frequency-swept waveforms. WM-DPA imaging utilizes chirp modulated laser beams at two distinct wavelengths for which absorption differences between oxy- and deoxy-genated hemoglobin are minimum (zero at the isosbestic point, 805 nm) and maximum (680 nm) to simultaneously generate two signals detected using a standard commercial array transducer as well as a single-element transducer that scanned the sample. Signal processing is performed using Lab View and Matlab software developed in-house.

Minute changes in total hemoglobin concentration (THb) and oxygenation levels are detectable using this method since background absorption is suppressed due to the out of phase modulation of the laser sources while the difference between the two signals are amplified, allowing pre-malignant tumors to therefore be identifiable. By regulating the signal amplitude ratio and phase shift the system can be tuned to applications like cancer screening, SO2 quantification and hypoxia monitoring in stroke patients.

Experimental results presented demonstrate WM-DPA imaging of sheep blood phantoms in comparison to single-wavelength FD-PAR imaging. Future work includes the functional PA imaging of small animals in vivo.

9708-70, Session 10
Engineering a near-infrared dark chromoprotein as a probe for photoacoustic imaging
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An optimal genetically-encoded probe for photoacoustic (PA) imaging should exhibit high optical absorption, low fluorescence quantum yield, and an absorption maxima within the near-infrared (NIR) window. One promising candidate is a newly engineered chromoprotein (CP), designated dark small ultra-red fluorescent protein (dsmURFP), which is based on a cyanobacterial phycobiliprotein. To optimize dsmURFP characteristics for PA imaging, we have developed a directed evolution method to iteratively screen libraries of protein variants with three different screening systems. Firstly, we took inspiration from dark-acceptor (also known as dark-quencher)-based Förster resonance energy transfer (FRET) constructs, and used dsmURFP as a dark acceptor from a mCardinal fluorescent donor. The rationale for this design was that the higher the extinction coefficient of the dsmURFP, the more the emission of the donor would be quenched. In addition, more energy transferred to the dark acceptor would lead to more thermoelastic expansion and a stronger PA signal. Three rounds of evolution using this first strategy resulted in dsmURFP1.3 that quenched the emission of mCardinal -2 fold more efficiently than dsmURFP. Secondly, an absorption-based screening based on visual inspection of plates led to identification of the variant dsmURFP1.4, which exhibited a 2-fold higher absorbance and a 5 nm red shift. Thirdly, we developed a colony-based photoacoustic screening method. To demonstrate the utility of our optimized variants, we used photoacoustic imaging to visualize dsmURFP and its variants in phantom and in vivo experiments using chicken embryo models and murine bacterial bladder infection models.

9708-71, Session 11
Dual-wavelength photoacoustic imaging of a photoswitchable reporter protein
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Photoacoustic (PA) imaging has been shown to provide detailed 3-D images of genetically expressed reporters, such as fluorescent proteins and tyrosinase-induced melanin. Their unambiguous detection in vivo is a vital prerequisite for molecular imaging of biological processes at a cellular and molecular level. This typically requires multiwavelength imaging and spectral unmixing techniques, which can be computationally expensive. In addition, fluorescent proteins often exhibit fluence-dependent ground state depopulation and photobleaching which can adversely affect the specificity of unmixing methods. To overcome these problems, a phycochrome-based reporter protein and a dual-wavelength excitation method have been developed to obtain reporter-specific PA contrast. Phycochromes are non-fluorescent proteins that exhibit two isomeric states with different absorption spectra. Using dual-wavelength excitation pulses in the red and near-infrared wavelength region, these states can be switched, resulting in a modulation of the total absorption coefficient, and hence the PA signal amplitude. Since this is not observed in endogenous chromophores, signals acquired using simultaneous and separate pulses can be subtracted to provide a reporter-specific contrast mechanism and elimination of the tissue background. PA signals measured in protein solutions using separate and simultaneous excitation pulses at 670 nm and 755 nm (< 6 mJ/cm²) showed a different amplitude and time delay between the states. Photobleaching was not observed. To demonstrate suitability for in vivo applications, mammalian cells were transduced virally to express phycrome, and imaged in tissue phantoms and in mice in an initial preclinical study. The results show that this method has the potential to enable deep-tissue PA reporter gene imaging with high specificity.

9708-72, Session 11
Motion corrected photoacoustic difference imaging of fluorescent contrast agents in vivo
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In fluorophores, such as exogenous dyes and genetically expressed proteins, the excited state lifetime can be modulated using pump-probe excitation at wavelengths corresponding to the absorption and fluorescence spectra. Simultaneous pump-probe pulses induce stimulated emission (SE) which, in turn, modulates the thermalized energy, and hence the photoacoustic (PA) signal amplitude. For time-delayed pulses, by contrast, SE is suppressed. Since this is not observed in endogenous chromophores, the location of the fluorophore can be determined by subtracting images acquired using simultaneous and time-delayed pump-probe excitation. This simple experimental approach exploits a fluorophore-specific contrast mechanism, and has the potential to enable deep-tissue molecular imaging at fluences below the MPE. In this study, some of the challenges to its in vivo implementation are addressed. First, the PA signal amplitude generated in fluorophores in vivo is often much smaller than that in blood. Second, tissue motion can give rise to artifacts that correspond to endogenous chromophores in the difference image. This would not allow the unambiguous detection of fluorophores. A method to suppress motion artifacts based on fast switching between simultaneous and time-delayed pump-probe excitation was developed. This enables the acquisition of PA signals using the two excitation modes with minimal time delay (20 ms), thus minimizing the effects of tissue motion. The feasibility of this method is demonstrated by visualizing a fluorophore (Atto680) in tissue phantoms, which were repositioned during the image acquisition to mimic tissue motion. In addition, in vivo imaging of a fluorescent cell marker (Cy5.5) in a tumor model is shown.

9708-73, Session 11
Four dimensional optoacoustic imaging of perfusion in preclinical breast tumor model in vivo
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Imaging plays an increasingly important role in clinical management and preclinical studies of cancer. Application of optical molecular imaging technologies, in combination with highly specific contrast agent approaches, eminently contributed to understanding of functional and histological properties of tumors and anticancer therapies. Yet, optical imaging exhibits deterioration in spatial resolution and other performance metrics due to light scattering in deep living tissues. High resolution molecular imaging at the whole-organ or whole-body scale may therefore bring additional understanding of vascular networks, blood perfusion and microenvironment gradients of malignancies.

In this work, we constructed a volumetric multispectral optoacoustic tomography (vMSOT) scanner for cancer imaging in preclinical models and explored its capacity for real-time 3D intravital imaging of whole breast cancer allografts in mice. Intrinsic tissue properties, such as blood oxygenation gradients, along with the distribution of externally administered liposomes carrying clinically-approved indocyanine green dye (lipo-ICG) were visualized in order to study vascularization, probe penetration and extravasation kinetics in different regions of interest within solid tumors. The use of v-MSOT along with the application of volumetric image analysis and perfusion tracking tools for studies of pathophysiologial processes within microenvironment gradients of solid tumors demonstrated superior volumetric imaging system performance with sustained competitive resolution and imaging depth suitable for investigations in preclinical cancer models.

9708-74, Session 11
Photoacoustic imaging and photothermal therapy using biodegradable melanoidin
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Photo-induced energy transfer has been widely investigated for biomedical applications such as fluorescence imaging, photoacoustic tomography, photothermal and photodynamic therapy. Various optical absorbing nanoparticles such as plasmic and carbon based nanoparticles are applied in diagnostic and therapy applications. Unfortunately, their potential cytotoxicities and long-term safety are still bottlenecks for true clinical translation. In this work, we have successfully developed an optically active polymer of melanoidin as a new class of biodegradable contrast agents for photoacoustic imaging and photothermal therapy. Melanoidin is usually contained in food, showing a high light-to-heat conversion efficiency, biocompatibility and efficient renal clearance with a low synthetic cost.
Melanoidin was prepared by non-enzymatic polymerization of glucose and glycine at 37 oC and pH 7.4 to avoid the formation of cytotoxic and mutagenic compounds. Then, in vivo photoacoustic imaging of sentinel lymphatic node via local delivery and gastrointestinal tract via oral delivery was successfully carried out in rats and mice using melanoidin as a contrast agent. We also successfully performed the photo-ablation therapy in tumor model mice using melanoidin. The preliminary toxicity tests and time-resolved photoacoustic bladder monitoring results revealed that melanoidin was biodegradable and removed from the body through renal clearance after subcutaneous injection. These results demonstrate the feasibility of optically active melanoidin for further clinical applications.

9708-75, Session 11

Monitoring cancer treatment response using photoacoustic and ultrasound spectral analysis in combination with oxygenation and perfusion measurements

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At clinically-relevant depths, the frequency content of photoacoustic signals encodes information about the size, concentration and spatial distribution of non-resolvable blood vessels. This study evaluates whether photoacoustics can detect cancer therapy-induced vascular perturbations. Photoacoustic/ultrasound (PA/US) spectral analysis was combined with functional, PA-based oxygenation and power Doppler (PD) perfusion estimates to assess treatment response.

Co-registered, in vivo US/PA/PD imaging of mice bearing breast cancer tumors was performed pre-treatment and 30m/2h/5h/24h/7d post-treatment (VevoLAZR, Fujifilm VisualSonics). Hyperthermia treatment (1h, 43C) was performed after systemic injections of doxorubicin-loaded thermosensitive liposomes (TSL, n=13) or free doxorubicin (DOX, n=11). Response was classified according to 2h, PA-based oxygenation drop and endpoint (>90%), caliper-based volume reduction. At all time-points/wavelengths (750/850nm), the spectral-slope (SS) was computed from the normalized US/PA power spectra using depth-matched reference phantoms. The percent-vascularity (PV) was estimated for the animal with the largest oxygenation-drop at 2h.

TLS-treated responders decreased their PA-SS by 1.9x @750nm and 5.8x @850nm 30m post-treatment and remained constant for 24h; tumor oxygenation followed the same trend. Non-responding SS remained unchanged for 24h. The 750nm SS was 18.7x lower than 850nm suggesting the TSL is sensitive vessel oxygenation. Responder PV decreased 100% when the 30m oxygenation dropped 15% and increased 7x when the 7d oxygenation increased 20%. DOX-responders exhibited similar trends to TSL-responders although the 750nm PA-SS was 1.6x smaller and post-treatment PV was 50% higher. The US-SS remained unchanged until 7d post-treatment suggesting its sensitivity to tumor cell-death. These findings suggest that PA spectral analysis has potential in monitoring cancer treatment response.

9708-76, Session 11

Imaging the distribution of photoswitchable probes with temporally-unmixed multispectral optoacoustic tomodraphy

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Synthetic and genetically encoded chromo- and fluorophores have become indispensable tools for biomedical research enabling a myriad of applications in imaging modalities based on biomedical optics. The versatility offered by the optoacoustic (photoacoustic) contrast mechanism enables to detect signals from any substance absorbing light, and hence these probes can be used as opaccoacoustic contrast agents. While contrast versatility generally represents an advantage of optoacoustics, the strong background signal generated by light absorption in endogeneous chromophores hampers the optoacoustic capacity to detect a photo-absorbing agent of interest. Increasing the optoacoustic sensitivity is then determined by the capability to differentiate specific features of such agent. For example, multispectral optoacoustic tomography (MSOT) exploits illuminating the tissue at multiple optical wavelengths to spectrally resolve (unmix) the contribution of different chromophores. Herein, we present an alternative approach to enhance the sensitivity and specificity in the detection of optoacoustic contrast agents. This is achieved with photoswitchable probes that change optical absorption upon illumination with specific optical wavelengths. Thereby, temporally unmixed MSOT (tuMSOT) is based on photoswitching the compounds according to defined schedules to elicit specific time-varying optoacoustic signals, and then use temporal unmixing algorithms to locate the contrast agent based on their particular temporal profile. The photoswitching kinetics is further affected by light intensity, so that tuMSOT can be employed to estimate the light fluence distribution in a biological sample. The performance of the method is demonstrated herein with the reversibly switchable fluorescent protein Dronpa and its fast-switching fatigue resistant variant Dronpa-M159T.

9708-77, Session 11

Biodegradable polymer based theranostic agents for photoacoustic imaging and cancer therapy

Yan J. Wang, Eric M. Strohm, Michael C. Kolios, Ryerson Univ. (Canada)

In this study, multifunctional phase-change theranostic agents for photoacoustic, ultrasound and fluorescent imaging, and for therapeutic drug delivery were developed and tested. These agents consist of biodegradable Poly(lactide-co-glycolic acid) (PLGA) polymer, loaded with perfluorohexane (PFH) liquid and gold nanoparticles (GNP) in the core, lipophilic carbocyanines (DiD) fluorescent dye and Paclitaxel (PAC) in the shell. Their multifunctional capacity was investigated in an in vitro study. The PLGA-GNP particles were synthesized by a double emulsion technique. The average PLGA particle diameter was 600 nm with a 60 nm thick shell. The PLGA-GNP particles were synthesized by a double emulsion technique. The average PLGA particle diameter was 600 nm with a 60 nm thick shell, with gold nano-spheres 50 nm in diameter and a 20 nm silica coating layer. MCF7 breast cancer cells were incubated with PLGA-GNP-DiD for 24 hours. Fluorescent and photoacoustic images were recorded using a fluorescent/acoustic microscope using a 1000 MHz transducer and a 532 nm pulsed laser. For the drug delivery test, MCF7 cells were incubated with the PLGA-GNP-PAC and PLGA-GNP particles for 6, 12 and 24 hours. The effect of particle delivery and vaporization on cell viability was examined by irradiating the cells with a laser fluence of 100 mJ/cm2 using the MTT assay. The photoacoustic images of MCF7 cells containing PLGA-GNP-DiD were spatially coincident with the fluorescent images, and confirmed particle uptake. After exposure to the PLGA-GNP-PAC for 6, 12 and 24 hours, the cell survival rate was 43%, 38%, and 36% respectively compared with the control group, confirming drug delivery. Upon vaporization, cell viability decreased by 20%. The particles show potential as imaging agents and drug delivery vehicles.
Multispectral reconstruction methods for quantitative photoacoustic tomography

Emma Malone, Benjamin T. Cox, Simon R. Arridge, Univ. College London (United Kingdom)

Quantitative photoacoustic tomography (QPAT) aims to recover the optical absorption and scattering coefficients of biological tissues from photoacoustic (PA) images acquired at multiple wavelengths. While conventional PA images are not directly related to the underlying tissue morphology and functionality, QPAT can provide clinically valuable images of endogenous chromophores, such as oxy- or deoxy-hemoglobin, melanin, lipids and water. Exogenous contrast agents, enzymes, or proteins linked to the expression of a gene of interest, may also be imaged. We present a novel method for reconstructing QPAT images via a multispectral reconstruction-classification algorithm. This method exploits knowledge that optical parameters are determined by a limited number of classes to iteratively improve their estimate. In contrast with pre-existing chromophore reconstruction methods, this approach does not require accurate prior knowledge of the characteristic spectra of the absorbers, which is not always available. The classification algorithm increases the robustness to experimental error, and allows for the treatment of the case in which the absorption spectra of the chromophores are unknown or uncertain. Numerical experiments performed on anatomically realistic 3D phantom show that this approach allows for improved recovery of both the optical absorption and scattering with respect to reconstruction-only methods, and accurate classification of chromophores of clinical interest.

Sparsifying transformations of photoacoustic signals enabling compressed sensing algorithms

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Compressed Sensing allows performing much fewer measurements than advised by the Shannon sampling theory. This is surprising because it requires the solution of a system of equations with much fewer equations than unknowns. It is possible if one can assume sparsity of the solution, which means that only a few components of the solution are significantly different from zero. Then such a sparse solution can be found by minimizing the l1 norm. This concept has already been used several times for photoacoustic tomography and allows for high-quality imaging with few detectors, therefore leading to a significant acceleration.

Sparsity of the pressure wave as a function of space and time is not valid directly. Therefore, we propose the application of a sparsifying temporal transformation to the detected pressure signals, which allows obtaining theoretical recovery guarantees for our compressed sensing scheme. Such a sparsifying transform can be found because spatial and temporal evolution of the pressure wave is not independent, but connected by the wave equation. In Fourier space this is expressed by the dispersion relation, which states that the angular frequency is equal to the product of the wavenumber and the sound velocity. Image reconstruction methods like Fourier reconstruction for photoacoustic tomography or frequency domain synthetic aperture focusing technique (F-SAFT) for ultrasomics exploit this relation. We give an example of a sparsifying transform and apply our compressed sensing scheme to reconstruct images from simulated data as well as from measured data with fiber-optic detectors as integrating line detectors.

In vivo light fluence correction for determination of tissue absorption coefficient using multispectral optoacoustic tomography

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Optoacoustic Tomography is a fast developing imaging modality, combining the high resolution and penetration depth of ultrasound detection with the high contrast available from optical absorption in tissue. The spectral profile of the near infrared excitation light is modified by absorption and scattering as it propagates deep into biological tissue. The resulting images therefore provide only qualitative insight into the distribution of tissue chromophores. Knowledge of the spectral profile of excitation light across the mouse is needed for accurate determination of the absorption coefficient in vivo.

Under the conditions of constant Grueneisen parameter and accurate light fluence, a linear relationship should exist between the initial optoacoustic pressure amplitude and the tissue absorption coefficient. Using data from a commercial optoacoustic tomography system, we implemented an iterative optimization for fluence correction based on the delta-Eddington approximation to the Radiative Transfer Equation. We segmented images into phantom targets, or mouse organs, and used known scattering coefficients for initialization [1,2]. Performing the fluence correction in simple phantoms allowed the expected linear relationship between recorded and independently measured absorption coefficients to be retrieved. For in vivo data, the correction resulted in a wavelength-dependent enhancement of signal intensities in deep tissues. This improved our ability to visualize organs at depth (> 5mm) and modified the estimated oxygen saturation values by up to 10%. Future work will aim to perform the optimization with no data normalization.


Optoacoustic imaging quality enhancement based on geometrical super-resolution method

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Ideal optoacoustic image reconstruction implies dense spatial sampling of the tomographic data using point acoustic detectors. However, in practice, spatial resolution of the images is often limited by the suboptimal sampling of optoacoustic responses, e.g. when employing large-area focused ultrasound detectors. One standard approach to enhance image quality and spatial resolution comprises of advanced modeling and reconstruction algorithms which attempt to account for precise spatial and frequency characteristics of the detection elements and other experimental factors. Herein, we investigate an alternative geometrical super-resolution method that purely relies on images rendered with simple reconstruction algorithms. The method is based on integrating information from multiple optoacoustic images acquired at sub-diffraction steps into one high resolution image by means of an iterative registration algorithm. This approach does not relate to super-resolution methods aiming at breaking the diffraction limit of light and/ or ultrasound. Experimental validations performed in target phantoms and ex-vivo tissue samples confirm that the suggested approach renders
Taking advantage of acoustic inhomogeneities in photoacoustic measurements

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Photoacoustic offers promising perspectives in probing and imaging subsurface optically absorbing structures in biological tissues. The optical fluence absorbed is partly dissipated into heat accompanied with microsilatations that generate acoustic pressure waves, the intensity which is related to the amount of fluence absorbed. Hence the photoacoustic signal measured offers access, at least potentially, to a local monitoring of the absorption coefficient, in 3D if tomographic measurements are considered. However, due to both the diffusing and absorbing nature of the surrounding tissues, the major part of the fluence is deposited locally at the periphery of the tissue, generating an intense acoustic pressure wave that may hide relevant photoacoustic signals. Experimental strategies have been developed in order to measure exclusively the photoacoustic waves generated by the structure of interest (orthogonal illumination and detection). Temporal or more sophisticated filters (wavelets) can also be applied. However, the measurement of this primary acoustic wave carries a lot of information about the acoustically inhomogeneous nature of the medium. We propose a protocol that includes the processing of this primary intense acoustic wave, leading to the quantification of the surrounding medium sound speed, and, if appropriate to an acoustical parametric image of the absorption coefficient, in 3D if tomographic measurements are considered. The results show that DPARS, when evaluated using Contrast-to-Noise Ratio and Root-Mean-Square errors, outperforms the conventional Delay-and-Sum reconstruction method. Experimental results for PAT confirm that the DPARS provides images with higher resolution than DAS.
**9708-159, Session PTue**

**Photoacoustic image reconstruction utilizing ultrasound post-beamformed B-mode image**

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The beamforming requirement to reconstruct PA image is to have a synchronized channel data acquisition with laser firing. Unfortunately, most clinical ultrasound (US) systems don’t offer an interface to obtain synchronized channel data. To broaden the impact of clinical PA imaging, we propose a PA image reconstruction algorithm utilizing US B-mode image, which is defocused due to wrong delay function, but readily available from clinical scanners. US B-mode image involves a series of signal processing including beamforming, followed by envelope detection, and end with log compression. Our approach is simply trying to reverse the order of these steps and recover the original US post-beamformed RF data, in which a PA beamforming algorithm can be applied. Taking B-mode image as the input, we firstly recovered US post-beamformed RF data by applying log decompression and convoluting an acoustic impulse response to recover carrier frequency information. Then, the US post-beamformed RF data is utilized as pre-beamformed RF data for an adaptive PA beamforming algorithm, and the new delay function is applied by taking into account that the focus depth in US beamforming is at the half depth of PA case. The feasibility of the proposed method was validated experimentally using an acoustic point source. The point source was successfully beamforming from US B-mode image, and the full with at the half maximum of the point improved 3.97 times. Comparing this result to the ground-truth reconstruction using channel data, the FWHM was slightly degraded with 1.28 times caused by information loss during envelope detection.

**9708-160, Session PTue**

**Variational image reconstruction for dynamic high resolution photoacoustic tomography**

Felix Lucka, Marta M. Betcke, Nam Trung Huynh, Edward Z. Zhang, Paul C. Beard, Benjamin T. Cox, Simon R. Arridge, Univ. College London (United Kingdom)

Current photoacoustic tomography (PAT) systems offer either exquisite image quality or high frame rates but are not able to deliver high spatial and temporal resolution simultaneously. A particular example is the planar Fabry Perot (FP) photoacoustic scanner. This yields high-resolution 3D images but typically takes several minutes to scan the 2D sensor plane because the incident photoacoustic field is mapped sequentially, point-by-point. The key observation to overcoming this limitation is that, in many situations, the spatio-temporal complexity of the many absorbing tissue structures is rather low, and therefore data recorded in a conventional, regularly sampled, fashion is highly redundant in both space and time. It is possible, therefore, to speed up the data acquisition without a loss of image quality by exploiting this redundancy and measuring a subset of the data chosen in such a way as to maximise its non-redundancy.

However, a successful reconstruction from such compressed data is only possible if the image reconstruction algorithm incorporates additional knowledge about the absorbing tissue structures, e.g. that they are piecewise constant, or sparse in some basis. Conventional, uninform, PAT reconstruction approaches, such as time reversal, will fail to produce a good image using compressed data. We demonstrate using simulated and experimental data that variational image reconstruction approaches that use spatio-temporal sparsity constraints to model the absorbing tissue structures can recover high quality images even for heavily accelerated data acquisition. For instance, our results indicate that an order of magnitude reduction in data acquisition time, and possibly more, is achievable for the task of reliably recovering major image structures and boundaries in static images if total-variation is used as a spatial constraint. Furthermore, we also demonstrate that optical-flow models can be used as temporal constraints for simultaneous high-quality image reconstruction and motion estimation.

Finally, how to overcome the practical challenges involved in realizing these advantages, and thereby obtain high spatial and temporal resolution simultaneously, is considered and demonstrated with dynamic experimental in vivo data. These novel reconstruction strategies offer new opportunities to dramatically increase the acquisition speed of photoacoustic scanners that employ point-by-point sequential scanning as well as reducing the channel count of parallellised schemes that use detector arrays.

**9708-161, Session PTue**

**Automatic speed of sound correction with photoacoustic image reconstruction**

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Sound velocity measurement is of great importance to the application of biomedical especially in the research of acoustic detection and acoustic tomography. Using correct sound velocities in each medium other than a simple mean sound propagation speed, we can effectively enhance sound based imaging resolution. Photoacoustic tomography (PAT), is defined as cross-sectional or three-dimensional (3D) imaging of a material based on the photoacoustic effect and it is a developing, non-invasive imaging method in biomedical research. This paper proposes a method to concurrently calculate multiple acoustic speeds in different mediums.

Firstly, we get the size of infra-structure of the target by B-mode ultrasonic imaging method. Then we build the photoacoustic (PA) image of the same target with different acoustic speed in different medium. By repeatedly evaluate the quality of reconstruct PA image, we dynamically change the acoustic speeds in different medium to build a finest PA image. Thus, we take these speeds of sound as the correct acoustic propagation velocities in according mediums. Experiments show that our non-invasive method can yield correct speed of sound with less than 0.3% error which might benefit future research in biomedical science.

**9708-162, Session PTue**

**Freehand photoacoustic tomography for 3D angiography using local gradient information**

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Photoacoustic tomography (PAT) is capable of imaging optical absorption in depths beyond the diffusion limit. As blood is one of the main absorbers in tissue, an important application is the visualization of vascularisation, which can provide important clues for diagnosing diseases like cancer. While the state-of-the-art work in photoacoustic 3D angiography has focused on computed tomography systems involving complex setups, we propose...
Correction of light attenuation using segmentation prior in multispectral optoacoustic tomography

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In MSOT, the reconstructed images for each wavelength represent a map of the spatially varying optical energy being absorbed by the tissue. Assuming a uniform Gruneisen parameter in soft biological tissues, the absorbed energy is proportional to the product of the absorption coefficient and the light intensity at a given voxel. Obtaining accurate maps of concentration of individual chromophores implies on the one hand spectrally or temporally unmixing the distribution of the chromophore of interest from other substances contributing to absorption coefficient and on the other hand normalizing the optoacoustic images with the excitation light fluence distribution. Light fluence can be estimated from the optoacoustic images (Jetzfellner et. al.) but the latter need to be properly segmented so that optical properties can be accurately assigned to the different tissues. Here we propose a diffusion-theory-based light propagation model that uses segmented object’s boundaries as a method to extract quantified information from optoacoustic images. The interface between the scattering and non-scattering medium can be modelled e.g. using the Robin boundary condition, and the solution to the model is obtained numerically using a finite volume method (FVM) solution approach. As opposed to the earlier approaches that employed a geometrical approximation of the thresholded image to generate the FVM mesh, we herein use the precisely segmented boundary to provide a more accurate basis for generating the mesh. The performance of the segmentation of cross-sectional optoacoustic images, and its efficacy in optical fluence correction is demonstrated using phantom and small animal imaging experiments.

Differential phase photoacoustic imaging for enhanced lateral and axial resolution imaging

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The bandwidth limitation and aperture size of the transducer limits the resolution of a photoacoustic computed tomography system. If the separation between two sources is smaller than the point spread function width of the imaging system, they will appear as a single object at different wavelengths. It was shown previously in ultrasound motion imaging that phase difference between two consecutive frames can be used to detect lateral or axial motion with submicron resolution. We tested this method in the context of static PA imaging of two unresolved PA sources. We set up an experiment where we imaged a green and a yellow wire of 40 μm width with known relative absorption coefficients, separated by 3552μm. Imaging was performed at 650nm and 460nm. The PA signal is recorded by a single element flat 1MHz transducer (Panametrics 0.5” V303) in the plane of the wires, so the targets are axially spaced seen from the transducer. We reconstructed the signals originating from both unresolved sources and measured the separation between them to be 350 μm. Similar performance was obtained using an array transducer, viewing the wires from the top so they were laterally separated in the imaging plane. The signal at two different wavelengths was recorded using a commercial imaging system. The two-wavelength phase difference at every pair of channels provides an estimate of the distance between the two absorbers, determined to be 350 μm by the median of the two-channel estimates.
Linear ultrasonic transducer array-based photoacoustic tomography (PAT), due to its hand-held operation convenience and easy integration with existing ultrasound imaging platforms, has been widely used recently. The linear arrangement of the sensor elements, however, limits the viewing angle of the system and causes loss of sensitivity to features that are not parallel to the array surface. By employing rotational scanning, the full view angle in the focal plane can be covered. However, even with full-view in two dimensions (2D), the lack of coverage in three dimensions (3D) still causes nonphysical negative values in the reconstructed images when conventional back-projection methods are used. Previously, a multi-view Hilbert transformation method was introduced to produce unipolar full-view PAT images with rotational scan in 2D. However, while solving the limited-view and negative-value problem, this method degrades the in-plane resolution. Here, we present a reconstruction method based on multi-view image restoration that provides 2D isotropic and acoustic wavelength-limited resolution in the reconstructed full-view unipolar images. Our novel method approximates the PAT image formation as a 2D convolution and uses the experimentally measured point spread function to estimate a reconstructed image that minimizes the observation error. In a phantom, we were able to achieve a 31-m isotropic resolution in the focal plane with a 40 MHz transducer array, a twofold improvement over the multi-view Hilbert transformation-based method. We also imaged a zebrafish larva in vivo through a scattering medium and achieved better spatial resolution and image contrast than the multi-view envelope extraction methods provide.

9708-168, Session PTue
Compensation of acoustic heterogeneities in photoacoustic computed tomography via a temporal data truncation reconstruction method
Joemini Poudel, Thomas Paul Matthews, Lei Li, Mark A. Anastasio, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Photoacoustic computed tomography (PACT) is an emerging computed imaging modality that exploits optical contrast and ultrasonic detection principles to form images of the absorbed optical energy density within tissue. If the object possesses spatially variant acoustic properties that are unaccounted for by the reconstruction algorithm, the estimated image can contain distortions. While reconstruction algorithms have recently been developed for compensating for this effect, they generally require the object’s acoustic properties to be known a priori.

To circumvent the need for detailed information regarding an object’s acoustic properties, we have previously proposed a half-time reconstruction method for PACT. A half-time reconstruction method estimates the PACT image from a data set that has been temporally truncated to exclude the data components that have been strongly aberrated. However, because the degree of temporal truncation is the same for all measurements in the half-time approach, there remains an opportunity to improve upon it when the approximate sizes and locations of strongly heterogeneous structures such as gas voids or bones are known.

In this work, we investigate PACT reconstruction algorithms that are based on a variable temporal data truncation (VTDT) approach that represents a generalization of the half-time reconstruction approach. In the VTDT approach, the degree of temporal truncation for each measurement is determined by the distance between the corresponding transducer location and the nearest known bone or gas void location. Reconstructed images from both numerical phantoms as well as experimental PACT measured data are employed to demonstrate the feasibility and effectiveness of the approach.

9708-169, Session PTue
Application of signal detection theory to assess imaging depth in optoacoustic imaging of the breast
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Early studies have examined the sensitivity of optoacoustic (OA) imaging system for recording small tumors embedded at different depths using tissue-mimicking phantoms. However, those studies did not directly assess tumor detectability as a function of depth by use of signal detection theory. Moreover, previous studies employed simplified experimental phantoms that do not accurately represent anatomical structures in breast tissue. In this work, by applying signal detection theory, we propose a numerical framework to objectively assess tumor detection in OA breast imaging. We employ a numerical breast phantom that contains both slowly varying background and vessel structures as the background model, and superimpose a deterministic signal to emulate a tumor. We simulate the recorded pressure data of a single-element OA imaging system by use of a Monte Carlo photon propagation method and k-space method. Test statistics of a Hotelling observer are computed from a collection of object realizations. The area-under-the-curve (AUC) of the test statistics are calculated as a summary measure of the tumor detection performance of Hotelling observer. We systematically investigate tumor detectability as a function of imaging depth, under conditions varying in: (i) tumor pathological features including shape, size, and blood content, (ii) anatomical features including tissue background model, skin color, and (iii) imaging system characteristic including transducer element dimension. Our study quantifies, for the first time, how signal detection performance of a numerical observer will vary as a function of signal depth and imaging system characteristics. Such information will be invaluable for the systematic optimization of OA imagers.

9708-170, Session PTue
Compensation for the effect of air voids in photoacoustic computed tomography image reconstruction
Thomas P. Matthews, Lei Li, Lihong V. Wang, Mark A. Anastasio, Washington Univ. in St. Louis (United States)

Most image reconstruction methods in photoacoustic computed tomography (PACT) assume that the acoustic properties of the object and the surrounding medium are homogeneous. This can lead to strong artifacts in the reconstructed images when there are significant variations in sound speed or density. Air voids represent a particular challenge to image reconstruction methods due to the severity of the differences between the acoustic properties of air and water. In whole-body small animal imaging, the presence of air voids in the lungs, stomach, and gastrointestinal system can limit image quality over large regions of the object.

In this work, a systematic study of the impact of air voids on image quality in small animal PACT is presented. To mitigate image artifacts, advanced iterative image reconstruction methods based on the photoacoustic wave equation are proposed and implemented. We demonstrate that by exploiting the known location and acoustic properties of an air void, image distortions can be effectively removed and the resolution and contrast of the images improved. The sensitivity of the image reconstruction methods to noise and errors in the assumed air void geometries are also studied. Finally, a novel approach for mitigating the impact of air voids while reducing the computational burden of image reconstruction is identified. These results are verified by application to experimental data.
9708-171, Session PTue

**In vivo photoacoustic tomography of myoglobin oxygen saturation**

Li Lin, Junjie Yao, Lei Li, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Myoglobin is a primary oxygen-carrying protein expressed in skeletal muscle fibers and cardiac myocytes. It functions as an oxygen-storage unit, facilitating the diffusion of oxygen from the cell membrane to mitochondria. Myoglobin has been demonstrated, along with hemoglobin, as a main muscle chromophore in the visible and near-infrared spectral regions. Both myoglobin and hemoglobin have oxygen-bound and -unbound states that are spectrally distinct.

Photoacoustic tomography is a hybrid imaging technique provides ultrasonically defined spatial resolution at depths beyond the optical diffusion limit. We present a new method that uses photoacoustic computed tomography (PACT) to measure the distribution of myoglobin in tissue and the oxygen saturation of myoglobin (sO2-Mb). The PACT system was equipped with a ring-shaped ultrasonic transducer array and provided a fast imaging speed of 0.625 fps. At each of six wavelengths, we recorded the ratio of acoustic amplitudes measured at different SO2 states. By taking advantage of the dynamic variations in SO2, we validated our calibration-free quantification model both in phantoms and in vivo. From photoacoustic measurements of mice in different oxygenation states, we performed quantification of myoglobin distribution and the systemic SO2-Mb change in the backbone muscle in vivo.

Non-invasive in vivo measurements of SO2-Mb are potentially useful in medical and surgical procedures. The high spatial resolution of PACT might particularly benefit applications such as cardiac surgery and sports medicine.

9708-172, Session PTue

**Cuffing-based photoacoustic flowmetry in humans at depths in the diffusive regime**

Yong Zhou, Jinyang Liang, Lihong V. Wang, Washington Univ. in St. Louis (United States)

In this work, we present a cuffing-based method to measure blood flow speed in humans, a long standing challenge for photoacoustic tomography in the optical diffusive regime. This procedure has three steps. First, a window along the blood vessel of interest is imaged. Second, the blood flow upstream of the window is stopped by cuffing the blood vessel with a sphygmomanometer. A high pressure (220 mg Hg in our experiments) is maintained in the cuff for a short time (e.g., 10 seconds) until there is almost no blood left in the vessel in the imaging window. Finally, the sphygmomanometer is quickly released, and the blood flow speed is calculated by monitoring the blood wash-in process. In phantom experiments, we demonstrated that the minimum and maximum measurable flow speeds were 0.035 mm/s and 42 mm/s, respectively. In human experiments, flow speeds were measured in the different blood vessels: a radial artery in the right forearm, a radial artery in the index finger of the right hand, and a radial vein in the right forearm. Taking advantage of the handheld probe, our method can potentially be used to monitor blood flow speed in the clinic and at the bedside.

9708-173, Session PTue

**Non-traveling wave approach of the photoacoustic Z-scan measurements on biomaterials**

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Non-traveling wave approach of the photoacoustic Z-scan response was investigated. Experimentally the sample is scanned through the expanded-focused laser beam, delivery by an optical fiber or a lens, and the generated photoacoustic signal is recorded using a focused ultrasound transducer. Theoretically, assuming that the photoacoustic signal strength is directly proportional to the density of energy absorbed by the sample the non-traveling PAZ-scan pressure was obtained. This signal displays nonlinear behavior depicting the nonlinear optical absorption of the material. We test the model with experiments on rodamine 6G and mouse blood. The values of the nonlinear optical absorption coefficients obtained from experimental and theoretical approaches agreed well, demonstrating the potential use of this method in determining the optical absorption of biological and other types of organic materials.

9708-174, Session PTue

**All optical fiber combined imaging system of photoacoustic and optical coherence tomography**

Jonghyun Eom, Jun Geun Shin, Soongho Park, Byeong Ha Lee, Gwangju Institute of Science and Technology (Korea, Republic of)

We present an all optical fiber dual modal imaging system that combines non-contact photoacoustic tomography (NPAT) and optical coherence tomography (OCT) to simultaneously provide PA and OCT image. For the generation of PA signal, a pulsed light from a Nd:YAG laser illuminates a sample via a large core multimode optical fiber. The acoustic wave generated within the sample propagates to the surface of the sample. The vibration of the sample surface is optically captured with an all fiber Mach-Zehnder interferometer, equipped with a fiber laser operating at 1550 nm, which enables to get the photoacoustic signal without any physical contact with the sample. The fiber-based OCT operating at a center wavelength of 1310 nm allows combining with the fiber-based PAT system by sharing the same optical fiber probe. The lights from the fiber laser and the OCT source are guided into the probe through each port of an optical fiber coupler. The lights from the sample are guided to the respective imaging systems by the same coupler. Both NPAT and OCT signals are measured simultaneously without physical contact, and two and three dimensional imaging is implemented by mechanical scanning.

To demonstrate the feasibility of the proposed system, we have carried out a phantom experiment using a tissue mimicking phantom which contained a couple of empty and ink-filled tubes, respectively. The proposed imaging system consists of all fiber-optic configurations so it has the potential for minimally invasive diagnosis. The simultaneous dual modal system can improve the diagnosis and guide the treatment of many diseases.

9708-175, Session PTue

**Multiple-illumination photoacoustic-ultrasound tomography**

Quinn Barber, Roger J. Zemp, Univ. of Alberta (Canada)

Previously, we described the potential for multiple illumination photoacoustic tomography to provide quantitative reconstructions, however this work used only simulation data. We have developed a custom photoacoustic-ultrasound tomography system capable of multiple illuminations and parallel acquisition from a 256 element 5MHz transducer ring array with 8-cm diameter. The multiple illumination scheme uses a free-space light delivery geometry where a rotational stage scans a pulsed laser beam onto different incident locations around the sample. For each illumination location a photoacoustic image is reconstructed using a modified backprojection algorithm. Images from different source locations
We proposed and built a multi-wavelength, fiber-coupled, high-power, compact laser diode system for optoacoustic monitoring, imaging, and sensing. The system utilizes high peak power laser diode arrays operating in the near infrared spectral range and generating 130-ns pulses (to provide stress-confined irradiation conditions) at high pulse repetition rate (1 kHz). All diode arrays were coupled to fiber that is required for clinical applications of optoacoustic monitoring, imaging, and sensing. We invasively and continuously monitored in human subjects cerebral, central, and peripheral blood oxygenation by probing the superior sagittal sinus (a large central cerebral vein), central veins, and peripheral veins and arteries in the arm. The short duration and high energy of the pulses provided high signal-to-noise ratio of optoacoustic signals from the blood vessels, while the multiple wavelengths allowed for accurate measurement of oxygenation in these blood vessels. Moreover, the high pulse repetition rate and specially developed fast acquisition and processing software resulted in monitoring with lower motion artefacts compared to that typical with (OPO)-based, 10-20 Hz optoacoustic systems.

9708-176, Session PTue

Simulated microsurgery monitoring using intraoperative multimodal surgical microscopy

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We have developed an intraoperative multimodal surgical microscopy system that provides simultaneous real-time enlarged surface views and sub-surface anatomic information during surgeries by integrating spectral domain optical coherence tomography (OCT), optical-resolution photoacoustic microscopy (PAM), and conventional surgical microscopy. The superluminescence light emitting diode (central wavelength: 880 nm, bandwidth: 50 nm) was used as an OCT light source, and the near-infrared pulsed laser (wavelength: 1064 nm, repetition rate: 20 kHz) was used as a PA excitation source. By sharing the same optical path, both OCT and PAM images were simultaneously acquired. Additionally, the custom made needle type transducer received the generated PA signals enabling convenient surgical operation without using a water bath. Using a simple augmented device, the OCT and PAM images were projected on the view plane of the surgical microscope. To quantify the performance of our system, we measured spatial resolutions of our system (OCT: lateral resolution, 25 µm and axial resolution, 8 µm; PAM: lateral resolution, 40 µm and axial resolution 70 µm). Then, three microsurgery simulation and analysis were processed: (1) ex vivo needle tracking and monitoring injection of carbon particles in biological tissues, (2) in vivo needle tracking and monitoring injection of carbon particles in tumor bearing mice, and (3) in vivo guiding of melanoma removal in melanoma bearing mice. The results indicate that this triple modal system is useful for intraoperative purposes, and can potentially be a vital tool in microsurgeries.

9708-177, Session PTue

Optoacoustic monitoring of cerebral, central, and peripheral blood oxygenation with multi-wavelength, fiber-coupled, high-power, compact laser diode-based system

Yuriy Y. Petrov, Irene Y. Petrov, Donald S. Prough, Andrey Petrov, Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

are combined to form an improved deep-tissue image using our previously developed iterative algorithms. We complement the photoacoustic imaging data with unique ultrasound imaging data. Most previous ultrasound tomography (UST) methods have used migration algorithms, iterative ray-based analysis, wave-equation modeling, or frequency-based algorithms that all demand large amounts of data and computational power. We propose a new UST method that offers isotropic resolution, provides scattering contrast, as well as the potential for measuring ultrasound scattering anisotropy and decoupling density and compressibility contributions. The imaging system is driven by a Verasonics scan engine and programmed for both ultrasound and photoacoustic imaging modes. Resolution has been measured to be 150 µm for ultrasound and 200 µm for photoacoustic images. S-Sequence and virtual-source techniques are implemented to increase SNR. Imaging capabilities are demonstrated on phantoms with custom-tailored ultrasound-scattering and optical properties, as well as in murine models.

9708-178, Session PTue

Transmission (forward) mode, transcranial, noninvasive optoacoustic measurements for brain monitoring, imaging, and sensing

Irene Y. Petrov, Yuriy Y. Petrov, Donald S. Prough, C. Joan Richardson, Rafael A. Fonseca, The Univ. of Texas Medical Branch (United States); Claudia S. Robertson, Vasantha Asokan, Adaeeze Agbor, Baylor College of Medicine (United States); Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

We proposed to use transmission (forward) mode for cerebral, noninvasive, transcranial optoacoustic monitoring, imaging, and sensing in humans (both adults and neonates). In the transmission mode, the irradiation of the tissue of interest and detection of optoacoustic signals from it are performed from opposite hemispheres (preferably, at 1800 or in the range from about 1200 to 2400), while in the reflection (backward) mode the irradiation of the tissue of interest and detection of optoacoustic signals from it are performed from the same hemisphere. Recently, we developed new, transmission-mode optoacoustic probes for patients with traumatic brain injury (TBI) and for neonatal patients. The transmission mode probes have two major parts: a fiber-optic delivery system and an acoustic transducer. To obtain optoacoustic signals in the transmission mode, in this study we placed the transducer on the forehead, while light was delivered to the opposite side of the head. Using a medical grade, multi-wavelength, OPO-based optoacoustic system tunable in the near infrared spectral range (680-950 nm), we recorded optoacoustic signals generated in the posterior part of the head of adults and neonates. Typically, the optoacoustic signals had two distinct peaks: the first peak from the intracranial space and the second peak from the scalp. The first peak generated by cerebral blood was used to measure cerebral blood oxygenation. Moreover, the transmission mode measurements provided detection of intracranial hematomas in TBI patients. The obtained results suggest that the transmission mode can be used for optoacoustic brain imaging, tomography, and mapping in humans.

9708-179, Session PTue

Intravascular imaging of ex-vivo atherosclerotic rabbit vessels using ultrasound and frequency domain photoacoustics

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Aortic atherosclerotic plaque detection using a multiwavelength handheld photoacoustic imaging system

Susumu Hirano, Takeshi Namita, Kengo Kondo, Makoto Yamakawa, Tsuyoshi Shiina, Kyoto Univ. (Japan)

Aortic atherosclerotic plaque rupture can cause cerebral and cardiac infarction. Detecting lipid-rich vulnerable plaque early is important to prevent plaque rupture. However, evaluating such plaque using conventional modalities is difficult. Photoacoustic imaging can visualize tissue characteristics. Some evaluations of lipid-rich plaque have used intravascular photoacoustic imaging systems. To reduce invasiveness and ease handling, we developed a handheld photoacoustic imaging system. The possibility of detecting lipid-rich plaque was evaluated in phantom experiments.

A plaque was modeled by injecting beef fat into a bovine aortic wall. The bovine aorta was filled with saline or equine blood. Laser light was guided to the model phantom surface by an optical fiber bundle close to the linear ultrasound probe. The photoacoustic signal distribution was measured as photoacoustic images.

The photoacoustic images, at wavelengths where light absorbance of lipid is high, show strong photoacoustic signals from the boundary of fat. Although the peak wavelengths of photoacoustic spectra subtly differ from the absorption spectra of the lipid in both cases, the spectral shapes are similar. At wavelengths of 1240-1300 nm, the similarity between photoacoustic spectra and the absorption spectrum was evaluated by calculating the correlation coefficient in photoacoustic images. The results show high correlation (more than 0.9) at the boundary between the fat and the vessel wall. These analyses demonstrate detection of lipid-rich plaque even if highly absorbing object, e.g., blood, is in proximity to the lipid. Some optimization, e.g., light intensity, to improve SNR and experiments in vivo must be conducted in further studies.

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Ability of combined NIRS-IVUS imaging to detect lipid core plaques and estimate cap thickness in human autopsy coronary arteries

Stephanie Grainger, Jimmy Su, Cherry A. Greiner, InfraReDx, Inc. (United States); Matthew D. Saybolt, Robert L. Wilensky, Hospital of the Univ. of Pennsylvania (United States); Joel S. Raichlen, AstraZeneca Pharmaceuticals LP (United States); Sean P. Madden, James E. Muller, InfraReDx, Inc. (United States)

The ability to determine plaque cap thickness during catheterization is of utmost clinical importance for plaque vulnerability assessment. While methods to compositionally assess cap integrity are in development, a method utilizing currently available tools to measure cap thickness is highly desirable. NIRS-IVUS is a dual imaging method clinically utilized that may provide cap thickness information to the skilled reader; however, this is as yet unproven.

Ten autopsy hearts (n=15 arterial segments) were scanned with the multimodality NIRS-IVUS TVC catheter (InfraRedex, Inc.) to identify lipid core plaques (LCPs). Skilled readers made predictions of cap thickness over regions of chemogram LCP, using NIRS-IVUS. Artery segments were perfusion fixed and cut into 2 mm serial blocks. Thin sections stained with Movat's pentachrome were analyzed for cap thickness at LCP regions. Block level predictions were compared to histology, as classified by a blinded pathologist.

Within 15 arterial segments, 117 blocks were found by NIRS to contain LCP. Utilizing NIRS-IVUS, blocks were divided into 4 categories: thin capped fibroatheromas, thick capped fibroatheromas, PIT/lipid pool (no defined cap), and calcified/unable to determine cap thickness. Sensitivities/specificities for thin caps, thick caps, and PIT/lipid pools were 0.54/0.99, 0.68/0.88, and 0.80/0.97, respectively. The overall accuracy rate 70.1% (chi-squared p=0.075, 22 blocks unable to predict). In the absence of calcium, NIRS-IVUS imaging provided predictions of cap thickness over LCP with moderate accuracy. The ability of this multimodality imaging method to identify vulnerable coronary plaques requires further assessment in an outcomes study in patients undergoing NIRS-IVUS imaging.

Comparison between transrectal photoacoustic, Doppler and magnetic resonance imaging for prostate cancer detection

Miya Ishihara, Akio Horiguchi, Hiroshi Shinmoto, Hitoshi Tsuda, National Defense Medical College (Japan); Kaku Irisawa, Takatsugu Wada, FUJIFILM Corp. (Japan); Tomohiko Asano, National Defense Medical College (Japan)

Transrectal ultrasonography (TRUS) is the most popular imaging modality in the diagnosis and therapy of prostate cancer. TRUS-guided prostate biopsy is mandatory for histological diagnosis in patients with elevated serum PSA (prostate-specific antigen), but its diagnostic accuracy is not satisfactory due to low resolution of TRUS. As a result, considerable number of patients is forced to have unnecessary repeated biopsy.

Photoacoustic (PA) imaging technique is ready to provide microvascular network imaging using hemoglobin as intrinsic optically absorbing molecules. We developed an original TRUS-type PA probe consisting of micro-convex array transducer with optical illumination system provided co-registered PA and ultrasound images. TRUS-type PA imaging has the advantage of much higher resolution and contrast than those of Doppler TRUS.

The purpose of this study is to demonstrate clinical feasibility of the transrectal PA image. We performed a clinical trial to compare the image of cancerous area by transrectal PA and that in TRUS Doppler during prostate biopsy. Obtained prostate biopsy cores are stained with anti-CD34 antibodies to have a map of microvascular distribution and confirm its...
consistency with PA images and pre-biopsy MRI findings. Our study demonstrated that transrectal identification of tumor angiogenesis under co-registered photoacoustic and ultrasound images was easier than that under TRUS alone. We successfully found a consistent relationship between PA images and MRI. This research is partially supported by the Research on Development of New Medical Devices from Japan Agency for Medical Research and development, AMED.

9708-85, Session PTuE

**Evaluation of Fabry-Perot polymer film sensors made using hard dielectric mirror deposition**

Jens Buchmann, Technische Univ. Berlin (Germany); Edward Z. Zhang, Univ. College London (United Kingdom); Chris Scharfenorth, Bastian Spannekrebs, Technische Univ. Berlin (Germany); Claus Villringer, Technische Hochschule Wildau (Germany); Jan Lauffer, Technische Univ. Berlin (Germany) and Charité Universitätsmedizin Berlin (Germany)

Fabry-Perot (FP) polymer film sensors offer high acoustic sensitivity, small element sizes, broadband frequency response and optical transmission to enable high resolution, backward mode photoacoustic imaging. Typical approaches to sensor fabrication involve the deposition of stacks of alternating dielectric materials to form interferometer mirrors, which are separated by a polymer spacer. If hygroscopic soft dielectric materials are used, a protective polymer layer is typically required. In this study, methods for the deposition of water-resistant, hard dielectric materials onto polymers were explored to improve the robustness and performance of the sensors. This involved the optimisation of the fabrication process, the optical and acoustic characterisation of the sensors, and a comparison of the frequency response with the output of an acoustic forward model. The mirrors, which were separated by a 20 µm parylene spacer, consisted of eight double layers of Ta2O5 and SiO2 deposited onto polymer substrates using temperature-optimised electron vapour deposition. The free spectral range of the interferometer was 32 nm, its finesse FR = 90, and its visibility V = 0.72. The noise-equivalent pressure was 0.3 kPa (20 MHz bandwidth). The measured frequency response was found to be more resonant around 30 MHz compared to sensors with soft dielectric mirrors. This was in agreement with the output of a forward model of the sensor. The model also suggests a significant increase in bandwidth (55 MHz, -3 dB), which can be attributed to improved acoustic impedance matching. The sensors were used in a PA scanner to acquire 3-D images in tissue phantoms and in vivo.

9708-86, Session 13

**Functional multi-scale photoacoustic remote sensing microscopy**

 Parsin Haji Reza, Kevan Bell, Wei Shi, Roger J. Zemp, Univ. of Alberta (Canada)

We introduce a novel multi-scale photoacoustic remote sensing (PARS) imaging system. Our system can provide optical resolution details for superficial structures as well as acoustic resolution for deep-tissue imaging down to 5 cm, in a non-contact setting. PARS system does not require any contact with the sample or ultrasound coupling medium. The optical resolution PARS (OR-PARS) system uses optically focused pulsed excitation with optical detection of photoacoustic signatures using a long-coherence interrogation beam co-focused and co-scanned with the excitation spot. In the OR-PARS initial pressures are sampled right at their subsurface origin where acoustic pressures are largest. The Acoustic resolution PARS (AR-PARS) picks up the surface oscillation of the tissue caused by generated photoacoustic signal using a modified version of Michelson interferometry. By taking advantage of 4-meters polarization maintaining single-mode fiber and a green fiber laser we have generated a multi-wavelength source using stimulated Raman scattering. Remote functional imaging using this multi-wavelength excitation source and PARS detection mechanism has been demonstrated. The oxygen saturation estimations are shown for both phantom and in vivo studies. Images of blood vessel structures for an In vivo chicken embryo model is demonstrated. The Phantom studies indicates ~3 µm lateral and ~300µm lateral resolution for OR-PARS and AR-PARS respectively. To the best of our knowledge this is the first dual modality non-contact optical and acoustic resolution system used for in vivo imaging.

9708-87, Session 13

**Acoustic and photoacoustic microscopy imaging of single leukocytes**

Eric M. Strohm, Michael Moore, Michael C. Kolios, Ryerson Univ. (Canada)

A combined acoustic and photoacoustic microscope was used to image single leukocytes with micrometer resolution. Contrast was provided though the cell biomechanical properties (ultrasound) and optical absorption (photoacoustics), which were then used to identify different cell types. In this hybrid acoustic/photoacoustic microscope, an ultrasound transducer with 1 GHz central frequency was positioned above the sample, and a 532nm fiber-coupled pulsed laser was focused from below using a 20x objective. The GHz frequencies (for ultrasound) and small laser focal spot (for photoacoustics) provided a resolution near 1 µm in both modalities. The fiber-coupling produced output from 532 to 620 nm through stimulated Raman scattering and the wavelength was selected using bandpass filters. A blood smear was prepared and stained with Wright-Giemsa, a metachromatic dye commonly used to visually identify leukocytes. Stained neutrophils, lymphocytes and monocyes were imaged using a raster scan first with ultrasound at 1 GHz, then with photoacoustics at 532nm and then 600nm.
9708-88, Session 13

**Fully integrated reflection-mode photoacoustic/two-photon microscopy in vivo**

Liang Song, Wei Song, Yang Zhang, Wei Zheng, Shenzhen Institute of Advanced Technology (China)

Using a water-immersion optical objective in conjunction with a miniature 40-MHz ultrasonic transducer, we developed reflection-mode photoacoustic microscopy with a transverse resolution as high as 320 nm. Here, we further integrated two-photon microscopy capability into the system to enable multimodality in vivo biomedical imaging at submicron resolution. As a result, the system is capable of tri-modality label-free imaging of microvasculature, collagen, and cell morphology, based on the contrast of optical absorption, second-harmonic generation, and autofluorescence, respectively. In addition, we demonstrated simultaneous microscopic imaging of neuron and microvasculature in the brain cortex of a living mouse, which may offer new opportunities for studying the mechanisms of neurovascular coupling.

9708-89, Session 13

**Mechanisms and models for photoacoustic remote sensing microscopy**

Roger J. Zemp, W. Shi, Parsin Haji Reza, Univ. of Alberta (Canada)

We recently introduced photoacoustic remote sensing (PARS) microscopy as an all-optical non-contact optical-resolution modality with absorption-based photoacoustic contrast. A pulsed excitation beam is optically focused into a sample then the resulting photoacoustic signal is sensed using a confocal long-coherence probe beam right at the source of the large pressures generated. Several mechanisms are proposed to explain the source of these large signals, including surface-displacements, local refractive-index step-modulation, scatterer displacements, and photothermal mechanisms. We carefully model each of these mechanisms and predict the fraction of modulated light from each. Experimental measurements detect ~0.1% of the incident interrogation light is modulated and this is confirmed with theoretical modulation calculations. We provide experimental evidence that pressure-induced refractive-index step modulation and scatter position modulation may be highly significant when using traditional optical microscopy alone.

9708-90, Session 13

**Super-resolution photoacoustic imaging of single gold nanoparticles**

Seunghyun Lee, Pohang Univ. of Science and Technology (Korea, Republic of); Owoong Kwon, Sungkyunkwan Univ. (Korea, Republic of); Mansik Jeon, Kyungpook National Univ. (Korea, Republic of); Jae jung Song, Minguk Jo, Junwoo Son, Sunghee Kim, Pohang Univ. of Science and Technology (Korea, Republic of); Yunseok Kim, Sungkyunkwan Univ. (Korea, Republic of); Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Photoacoustic imaging (PAI) is an emerging hybrid imaging modality that can provide a strong optical absorption contrast using the photoacoustic (PA) effect, and breaks through the fundamental imaging depth limit of existing optical microscopy such as optical coherence tomography (OCT), confocal or two-photon microscopy. In PAI, a short-pulsed laser is illuminated to the tissue, and the PA waves are generated by thermoelastic expansion. Despite the high lateral resolution of optical-resolution photoacoustic microscopy (OR-PAM) thanks to the tight optical focus, the lateral resolution of OR-PAM is limited to the optical diffraction limit, which is approximately a half of the excitation wavelength. Here, we demonstrated a new super-resolution photoacoustic nanoscopy system by breaking the optical diffraction limit. The conventional microscopes with nanoscale resolutions such as a scanning electron microscope (SEM) and transmission electron microscope (TEM) are typically used to image the structures of nanomaterials, but these systems should work in a high vacuum environment and cannot provide the optical properties of the materials. Our newly developed PA nanoscopic system provides the optical properties with a nanoscale resolution in a normal atmosphere. We have photoacoustically imaged single gold nanoparticles with an average size of 80 nm in diameter and shown their PA expansion properties individually. The lateral resolution of this system was better than 30 nm. Therefore, this tool will provide an unprecedented optical absorption property with an accurate nanoscale resolution and greatly impact on materials science and nanotechnology field.

9708-91, Session 13

**Non-linear photoacoustic and fluorescence microscopy using a modulated laser diode**

Gregor Langer, Thomas Berer, Research Ctr. for Non Destructive Testing GmbH (Austria)

We present simultaneous photoacoustic and fluorescence microscopy using a modulated laser diode. Superposition of harmonic and continuous wave (cw) excitation leads to a non-linear behavior of the signals which we exploit to enhance the resolution in photoacoustic and fluorescence microscopy. Photoacoustic waves and modulated fluorescence are generated in chromophores by using a laser diode with a wavelength of 405nm at modulation frequencies in the Megahertz range. Additionally, a cw offset radiation can be superposed. The excitation light is focused to the sample using a high NA objective. Fluorescence is collected by the same objective in a confocal configuration and measured by an avalanche photo diode. Photoacoustic waves are recorded on the opposite site of the sample using a focusing transducer. Imaging is facilitated by means of a scanning stage. Signals are recorded using a lock-in technique, allowing recording the fundamental and harmonic frequency responses of the photoacoustic and fluorescence signals. The fundamental and harmonic responses of the photoacoustic and fluorescence signals depend on the cw intensity and on the modulated intensity. This behavior can be used to improve the spatial resolution in fluorescence and photoacoustic microscopy.

9708-92, Session 14

**Multibeam Fabry Perot photoacoustic scanner for fast 2D and 3D imaging**

Nam Trung Huynh, Olumide Ogunlade, Edward Z. Zhang, Benjamin T. Cox, Paul C. Beard, Univ. College London (United Kingdom)
The planar Fabry Perot (FP) photoacoustic scanner has been shown to provide exquisite high resolution 3D images of soft tissue structures in vivo to depths up to approximately 10mm. However a significant limitation of current embodiments of the concept is low image acquisition speed. This is because the FP sensor is optically addressed by sequentially scanning a single focused interrogation laser beam over its surface in order to map the incident photoacoustic waves. Only a single A-line per excitation pulse is therefore acquired resulting in long acquisition times. For example, a typical 2D scan comprising 10,000 A-lines over a 10mm x 10mm scan area takes approximately 4 minutes using an excitation laser with a PRF of 50Hz. Although acceptable for applications where the target is relatively static (e.g. imaging anaesthetised mice), acquisition times of this duration reduces throughput and precludes direct visualisation of dynamic physiological events. Perhaps most importantly, it also inhibits the clinical utility of the technology since the inevitable motion-related artefacts that would arise with such low frame rates would compromise image quality.

To increase acquisition speed, a novel multi-beam scanner architecture has been developed. This enables a line of equally spaced 8 interrogation beams to be scanned simultaneously across the FP sensor and the photoacoustic signals detected in parallel. A specific challenge relates to the fact that the FP sensor optical thickness is non uniform; the optimal bias wavelength is therefore different for each beam. In order to minimise the sensitivity variation across the beams this variation would produce when using a single interrogation laser, a new approach to optimally biasing the sensor has been developed. This is based on forming a map of the sensor optical thickness and identifying the optimum spatial alignment of the 8 interrogation beams via a statistical analysis of the sensitivity distribution over the scan area. In addition, an excitation laser operating at 200Hz was used. The combination of parallelising the detection and the high PRF of the excitation laser has enabled dramatic reductions in image acquisition time to be achieved. A 3D image obtained with our previous 1st generation scanner that would take 4 minutes to acquire, can now be acquired in 8 seconds. Moreover 2D images at video rates (20fps) are now possible. In order to demonstrate the fast 3D imaging capability of the system, in vivo volumetric images of the human palm have been obtained. To demonstrate the 2D video rate imaging capability, images of dynamic phantoms and in vivo hemodynamic changes in the major arteries and veins of the forearm were acquired. It is anticipated that the significant increase in acquisition speed provided by this new scanner design will pave the way to new biomedical applications of the FP sensor technology, particularly in clinical medicine.

**9708-94, Session 14**

**Enhanced field of view in-vivo photoacoustic tomography using orthogonal Fabry-Pérot planar sensor arrays**

Robert J. Ellwood, Edward Z. Zhang, Paul C. Beard, Benjamin T. Cox, Univ. College London (United Kingdom)

Planar Fabry-Pérot (FP) ultrasound sensor arrays have been used to produce in-vivo photoacoustic images of exquisite quality due to their broad detection bandwidth and dense spatial sampling. However, a single planar FP sensor provides a modest 3D field of view due to limited sensitivity to signals originating from depth. This limits the use of a single planar array for certain applications such as whole body small animal imaging. Planar sensors also suffer from a limited detection aperture, due to their planarity, which results in significant limited-view artefacts in the reconstructed image.

In this work we present a novel sensor geometry that allows a greater field of view by placing a second sensor orthogonal to the first. This not only captures data from the deeper lying regions of interest, but also mitigates the limited view effect. A previous proof-of-principle study has shown that this results in significantly improved images for targets in the sensing region. Here we describe a second-generation optimised system with improved sensor design and faster acquisition speed due to simultaneous sampling. An optimal illumination geometry, designed to match the field of view of sensors, has also been implemented. As there is no exact one-step reconstruction algorithm for this arrangement, an iterative reconstruction approach was used.

The ability of the system to produce high resolution images with reduced artefacts was demonstrated using phantoms, as well as in-vivo. The abdomen of a mouse model was imaged using the orthogonal sensor geometry and demonstrates the quality of images of internal organs and vasculature that can be achieved using this new system due to its improved field of view.

**9708-93, Session 14**

**Photoacoustic imaging with ultra-sensitive plano-concave optical microresonator detectors**

James A. Guggenheim, Edward Z. Zhang, Paul C. Beard, Univ. College London (United Kingdom)

Most photoacoustic scanners use piezoelectric detectors but these have two key limitations. Firstly, they are optically opaque, inhibiting backward mode operation. Secondly, it is difficult to achieve adequate detection sensitivity with the small element sizes needed to provide near-omnidirectional response as required for tomographic imaging. Planar Fabry-Perot (FP) ultrasound sensing etalons can overcome both of these limitations and have proved extremely effective for superficial (<1cm) imaging applications. To achieve small element sizes (<100um), the etalon is 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. To overcome this, novel planoconcave micro-resonator sensors have been fabricated using precision ink-jet printed polymer domes with curvatures matching that of the laser wavefront. By providing near-perfect beam confinement, we show that it is possible to approach the maximum theoretical limit for finesse (f) imposed by the etalon mirror reflectivities (e.g. f=400 for R=99.2% in contrast to a typical planar sensor value of f<50).

This yields an order of magnitude increase in sensitivity over a planar FP sensor with the same acoustic bandwidth. Furthermore by eliminating beam walk-off, viable sensors can be made with significantly greater thickness than planar FP sensors. This provides an additional sensitivity gain for deep tissue imaging applications such as breast imaging where detection bandwidths in the low MHz can be tolerated. For example, for a 250 µm thick planoconcave sensor with a -3dB bandwidth of 5MHz, the measured NEP was 4 Pa. This NEP is comparable to that provided by mm scale piezoelectric detectors used for breast imaging applications but with more uniform frequency response characteristics and an order-of-magnitude smaller element size. Following previous proof-of-concept work, several important advances towards practical application have been made. A family of sensors with bandwidths ranging from 3MHz to 20MHz have been fabricated and characterised. A novel interrogation scheme based on rapid wavelength sweeping has been implemented in order to avoid previously encountered instability problems due to self-heating. Finally, a prototype microresonator based photoacoustic scanner has been developed and applied to the problem of deep-tissue (>1cm) photoacoustic imaging in vivo. Imaging results for second generation microresonator sensors (with R = 99.5% and thickness up to -800um) are compared to the best achievable with the planar FP sensors and piezoelectric receivers.

**9708-95, Session 14**

**Resolution and contrast of an optical full-field holographic system for fast non-contact photoacoustic tomography**

Michael Münter, Jens Horstmann, Christian Buj, Benedikt Schmarbeck, Univ. zu Lübeck (Germany); Ralf Brinkmann, Univ. zu Lübeck (Germany) and Medizinisches
Photoacoustic (PA) imaging has significant potential in interventional medicine for applications in which a miniature probe is inserted into the body via a needle or catheter for diagnostic or guidance purposes. Conventional PA probes typically employ a combination of an optical fibre for delivery of the excitation laser light and piezoelectric ultrasound detectors. However, this approach presents several technical challenges, particularly for applications that involve probe insertion via a fine needle or catheter and thus are highly miniaturised (~500 μm). With piezoelectric based PA probes, it is difficult to achieve the necessary level of miniaturisation due to the need to offset the opaque detector from the delivery fibre. Achieving adequate wideband detection sensitivity with the small element sizes required for a miniature probe can also be challenging. Moreover, technical complexity and cost make it difficult to manufacture at low unit cost for disposable use.

In this study, we report the fabrication and characterisation of a highly miniaturised (~150 μm outer diameter), all-optical, forward-looking PA probe. The probe consists of a single optical fiber for delivering the excitation light and a wideband Fabry-Perot (F-P) ultrasound sensor formed at the distal end by multi-layer optical coating. The transparent nature of the sensor avoids the need to offset it from the fiber tip allowing realization of a PA probe with an unprecedented level of miniaturization limited only by the diameter of the fiber. With the use of a miniature graded index (GRIN) lens, the ultrasonic field proximal to the F-P cavity we can achieve high acoustic sensitivity by reducing the phase dispersion and walk-off of the highly divergent interrogation beam as it undergoes multiple reflections in the F-P cavity. The GRIN lens also confers the benefit of high lateral spatial resolution by confining the photoacoustic excitation light to spot sizes as small as 30 μm, with foci as far as 1mm from the distal end. The probe has an acoustic bandwidth of 50 MHz and sub-kPa noise equivalent pressure. Using tissue phantoms and ex-vivo tissues, we demonstrate the potential of the probe for applications such as guiding needles used to deliver regional anaesthesia where a highly miniaturised probe is required.

9708-97, Session 14
Grueneisen relaxation photoacoustic microscopy in vivo
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Optical-resolution photoacoustic microscopy (OR-PAM) can achieve submicron lateral resolution by tightly focusing the excitation light, while the axial resolution is still limited by the frequency bandwidth of the ultrasonic transducer. The Grueneisen relaxation effect, in which the Grueneisen parameter changes within the thermal relaxation time following a laser impulse heating, can provide excellent axial resolution due to its optical sectioning property. Based on this effect, Grueneisen relaxation photoacoustic microscopy (GR-PAM) was developed and demonstrated ex vivo. Here, we present for the first time in vivo imaging of mouse brains with implanted axial resolution based on GR-PAM. An intensity-modulated continuous-wave (CW) S32 nm laser thermally heated the in-focus absorber. Another S32 nm pulsed laser, which is aligned confocally with the CW laser, generated the photoacoustic (PA) signal from the absorber. The difference between the amplitudes of the photoacoustic signals with and without heating was used for image reconstruction. The achieved axial resolution is ~12.5 μm, which is much better than the acoustically determined axial resolution value for a 20 MHz-bandwidth ultrasound transducer. The system was demonstrated by imaging a blood-filled tube ex vivo and blood vessels of mouse brains in vivo. The blood-filled tube diameter obtained from the PA image by GR-PAM is 105 μm, which is much closer to its actual diameter (100 μm) than the value from conventional OR-PAM (160 μm). This axial resolution improvement was further validated in imaging mouse brains in vivo, and yielded significantly narrower axial profiles of the vessels. This in vivo demonstration of imaging by GR-PAM might inspire more applications in PA biomedical imaging and sensing.

9708-98, Session 14
Miniature fiber optic probe for minimally invasive photoacoustic sensing
Sunish J. Mathews, Edward Z. Zhang, Adrien E. Desjardins, Paul C. Beard, Univ. College London (United Kingdom)
A Monte Carlo investigation on quantifying the retinal pigment epithelium melanin concentration by photoacoustic ophthalmoscopy

Xiao Shu, Hao F. Zhang, Wenzhong Liu, Northwestern Univ. (United States)

The melanin in the retinal pigment epithelium (RPE) protects retina and other ocular tissues by photo-screening and acting as antioxidant and free radical scavenger. It helps maintain normal visual functions since human eye is subjected to lifelong high oxygen stress and photon exposure. Loss of the RPE melanin weakens the protection mechanism and jeopardizes ocular health. Local decrease in the RPE melanin concentration is believed to be both a cause and a sign of early-stage age-related macular degeneration (AMD), the leading blinding disease in developed world. Current technology cannot quantitatively measure the RPE melanin concentration which might be a promising marker in early AMD screening. Photoacoustic ophthalmoscopy (PAOM), as an emerging optical absorption-based imaging technology, can potentially be applied to measure the RPE melanin concentration if the dependence of the detectable photoacoustic (PA) signal amplitudes on the RPE melanin concentrations is verified. In this study, we tested the feasibility of using PA signal ratio from RPE melanin and the nearby retinal blood vessels as an indicator of the RPE melanin variation. A novel whole eye optical model was designed and Monte Carlo modeling of light (MCML) was employed. We examined the influences on quantification from PAOM axial resolution, the depth and diameter of the retinal blood vessel, and the RPE thickness. The results show that the scheme is robust to individual histological and illumination variations. This study suggests that PAOM is capable of quantitatively measuring the RPE melanin concentration in vivo.

High speed photoacoustic imaging with fast OPO Laser at 1.7 µm

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Acute cardiovascular events are mostly due to a blood clot or thrombus induced by the sudden rupture of vulnerable atherosclerotic plaques within coronary artery walls. Based on the high optical absorption contrast of the lipid rich plaques within the vessel wall, intravascular photoacoustic (IVPA) imaging at 1.7 µm spectral band has shown promising capabilities for detecting of lipid composition, but the translation of the technology for in vivo application is limited by the slow imaging speed. In this work, we will present a high speed integrated IVPA/US imaging system with a 500 Hz optical parametric oscillator laser at 1725 nm (5 nm linewidth). A miniature catheter with 1.0 mm outer diameter was designed with a polished 200 ?m multimode fiber and an ultrasound transducer with 45 MHz center frequency. Two optical illumination methods by gradient-index (GRIN) lens and ball lens are introduced and compared for higher spatial resolution. At 1725 nm, atherosclerotic rabbit abdominal aorta was imaged at two frame per second, which is more than one order of magnitude faster than previous reported IVPA imaging. Furthermore, by wide tuning range of the laser wavelength from 1680 nm to 1770 nm, spectroscopic photoacoustic analysis of lipid-mimicking phantom and an human atherosclerotic artery was performed ex vivo.
Cancer cell killing and immunotolerance reversal by PDT and PTT: role of ER stress (Invited Paper)

Mladen Korbelik, BC Cancer Research Ctr. (Canada)

Detection of tumors by photodynamic therapy (PDT) and other therapies producing targeted localized traumatic stress including phototherapy (PTT) is based on the infliction of endoplasmic reticulum (ER)-localized stress in treated cells. Such type of insult provokes the launching of unfolded stress response (UPR) in the ER in the attempt of re-establishing homeostasis. Unresolved presence of damaged proteins in ER instigates the activation of signal transduction pathways prompting the transition from adaptive to lethal phase of ER stress response. The latter promotes cell death by orienting the process of autophagy to its lethal form and by boosting apoptosis. Of further critical importance is that the intensified reticular UPR is associated with the engagement of trafficking of damage-associated molecular patterns (DAMPs) that results in their cell surface expression and/or extracellular release securing the establishment of immunogenic cell death (ICD) that enables the breakdown of tumor immunotolerance. It will be described how these recent mechanistic revelations inspire the development of novel approaches for amplifying the impact of above-described events for strengthening the clinical efficacy of therapies such as PDT and PTT in destruction of both localized and disseminated cancerous lesions.

Photodynamic therapy and anti-tumor immune response (Invited Paper)

Michael R. Hamblin, Wellman Ctr. for Photomedicine (United States)

Photodynamic therapy (PDT) uses the combination of non-toxic dyes and harmless visible light to produce highly reactive oxygen species that destroy tumors. The ideal cancer treatment should target both the primary tumor and the metastases with minimal toxicity. This is best accomplished by educating the body’s immune system to recognize the tumor as foreign so that after the primary tumor is destroyed, distant metastases will also be eradicated. PDT may accomplish this feat and stimulate the long-term, specific anti-tumor immunity. PDT causes an acute inflammatory response, the rapid induction of large amounts of necrotic and apoptotic tumor cells, induction of damage-associated molecular patterns (DAMPs) including heat-shock proteins, stimulates tumor antigen presentation to naïve T-cells, and generation of cytotoxic T-cells that can destroy distant tumor metastases.

By using various syngeneic mouse tumors in immunocompetent mice, we have studied specific PDT regimens related to tumor type as well as mouse genotype and phenotype. We have investigated the role of tumor-associated antigens in PDT-induced immune response by choosing mouse tumors that express more defined antigenically-and biologically occurring cancer tests antigen, and oncogenic virus-derived antigen.

We studied the synergistic combination of low-dose cyclophosphamide and PDT that unmasks the PDT-induced immune response by depleting the immunosuppressive T-regulatory cells. PDT combined with immunostimulants (toll-like receptor ligands) can synergistically maximize the generation of anti-tumor immunity by activating dendritic cells and switching immunosuppressive macrophages to a tumor rejection phenotype. Tumors expressing defined tumor-associated antigens with known MHC class I peptides allows anti-tumor immunity to be quantitatively compared.

Therapeutic effect of photodynamic therapy combined with targeted delivery of silencing vascular endothelial growth factor (Invited Paper)

Yih-Chih Hsu, Chung Yuan Christian Univ. (Taiwan)

Photodynamic therapy 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 tumours oxygen-independent hypoxic conditions. Vascular endothelial growth factor (VEGF) is one of the primary factors that affect tumor angiogenesis. Another emerging treatment to cure cancer is the use of interference RNA to silence a specific mRNA sequence. Such treatment requires a delivery system such as liposomes. The nanoparticle size measured was about 30 nm. Cellular uptake study was performed to verify that the nanoparticles have a sigma receptor mediated pathway. Non-targeted LCP NPs did not show significant difference with or without haloperidol but has a lower intensity as than targeted LCP NPs. These results confirm that LCP NPs have a receptor mediated pathway.

Cell viability was found to decrease at 25 nM of transfected VEGF siRNA. Combined therapy of PDT and VEGF siRNA showed significant response as compared with PDT and gene therapy alone. In vivo toxicity assay with mice treated with targeted LCP NPs containing control siRNA or VEGF siRNA and non-targeted LCP NPs containing VEGF siRNA did not show any significant difference with the PBS injected group which suggests that there is no toxicity with the dose. It suggests that PDT combined with targeted gene therapy has a potential mean to achieve better therapeutic outcome.

Anti-tumor immune response in glioblastoma induced by 5-ALA-based PDT? (Invited Paper)

Ronald Sroka, Laser-Forschungslabor (Germany) and Klinikum der Univ. München (Germany); Herbert Stepp, LIFE Ctr. (Germany) and Klinikum der Univ. München (Germany); Heike Pohla, Klinikum der Univ. München (Germany); Robert Kammerer, Friedrich-Loeffler-Institut (Germany); Alexander Buchner, Patricia Müller, LIFE Ctr. (Germany) and Klinikum der Univ. München (Germany); Sara Abdel Hamid, LIFE Ctr. (Germany) and The German Univ. in Cairo (Egypt) and Klinikum der Univ. München (Germany); Wolfgang Zimmermann, LIFE Ctr. (Germany) and Klinikum der Univ. München (Germany)

5-aminolevulinic acid (5-ALA) based stereotactic interstitial Photodynamic Therapy (iPDT) of glioblastoma in some cases resulted in longtime survivals (1, 2). As the accumulation of the photosensitizer protoporphyrin IX (PpIX) is very heterogeneous within the tumor and also the application of light inevitably shows large variations throughout the treated tumor volume, it must be assumed that parts of the tumor volume receive insufficient doses of either drug (PpIX) or light or both. Surviving tumor cells would almost inevitably cause recurrence. As this is not always observed following 5-ALA iPDT, the hypothesis of the induction of an immune response may be justified.

In vitro experiments with sublethal PDT, we performed oligonucleotide microarray analyses to investigate, whether up- or downregulated gene
9709-30, Session PMon

**Temperature distribution in target tumor tissue and photothermal tissue destruction during laser immunotherapy**

Austin Doughty, Aamir M. Hasanjee, Connor West, Kegan Silk, Feifan Zhou, Wei R. Chen, Univ. of Central Oklahoma (United States)

Laser Immunotherapy (LIT) is a novel cancer treatment modality for late-stage metastatic cancers that applies a synergistic effect between laser irradiation to expose tumor elements and an immunoadjuvant to instigate a systemic immune response to the cancer. As there is no molecule or protein common to all cancers, LIT relies on the generation of a local temperature gradient within the tumor to cause an escalating degree of cellular destruction and expose all elements of the cancer to the host immune system. The host immune system then selects from the available biomarkers and launches a curative immune response against the cancer. This study, in particular, investigates the photothermal cell destruction in an attempt to determine its correlation with the laser induced temperature gradient in tissue. As a result, LIT treatment could then be optimized for maximum subcellular exposure with minimal tissue damage and reduce painful side-effects in patients. Additionally, this study aims to accurately observe the photothermal effect induced by laser irradiation, both superficially and internally, and to correlate tissue temperatures measured using different methods. This would allow the thermal effect to be monitored in real-time during treatment non-invasively through the use of infrared imaging. By doing this, LIT operators could better ensure a potent response and more accurately predict patient outcome.

9709-31, Session PMon

**Optimized acquisition time of spectra in quantitative x-ray fluorescence (XRF) analysis of gold nanoparticles (GNPs)**

Liqiang Ren, Di Wu, Yuhua Li, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States); Bin Zheng, Hong Liu, The Univ. of Oklahoma (United States)

X-ray fluorescence (XRF) is a promising spectroscopic technique to characterize imaging contrast agents with high atomic numbers such as gold nanoparticles (GNPs) inside small objects. Its utilization for biomedical applications, however, is greatly limited to experimental research due to the longer measurement time. The objective of this study is to determine an optimized acquisition time, at which the spectral data is of sufficient statistical properties while the radiation dose is minimized. For this purpose, a theoretical criterion that distinguished the net fluorescence signal from the statistical fluctuations of scattering background with a 99.7% confidence level was derived, based on photon counting statistics and error propagation. Then, using a typical laboratory XRF setting consisting of a pencil-beam X-ray and a single spectrometer, spectral data were acquired by exciting the prepared GNP solutions with various concentrations of 0.1%, 0.2%, 0.5%, 1% and 2% by weight. In order to analyze the statistical properties while the radiation dose is minimized, our preliminary investigation has encapsulated the cisplatin into lipid-based nanoparticle with size around 21 nm and examined few mice of the second worldwide cause of death, treating cancer remains a challenge although the mechanism of causing cancer has not fully understood. Our preliminary investigation has encapsulated the cisplatin into lipid-based nanoparticle with size around 21 nm and examined few mice of the second worldwide cause of death, treating cancer remains a challenge although the mechanism of causing cancer has not fully understood. Our preliminary investigation has encapsulated the cisplatin into lipid-based nanoparticle with size around 21 nm and examined few mice of the second worldwide cause of death, treating cancer remains a challenge although the mechanism of causing cancer has not fully understood. 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9709-34, Session PMon

Alterations of morphology of immune organs and peripheral blood indicators under the influence of gold nanoparticles in rats

Alla B. Bucharskaya, Svetlana S. Pakhomy, Olga V. Zlobina, Galina N. Maslyakova, Olga V. Matveeva, Irina O. Bugaeva, Nikita A. Navolok, Saratov State Medical Univ. (Russian Federation); Boris N. Khlebtsov, Vladimir A. Bogatyrev, Nikolai G. Khlebtsov, Institute of Biochemistry and Physiology of Plants and Microorganisms (Russian Federation); Valery V. Tuchin, N.G. Chernyshevskiy Saratov State Univ. (Russian Federation)

The best way to improve the efficiency and selectivity of laser treatment of cancer is to use a photothermal sensitization of tumors by gold nanoparticles of different size and structure. The aim of the work was to study the effect of the prolonged peroral administration of gold nanoparticles with different sizes (1-3 nm, 15 nm and 50 nm) on morphological changes in immunogenesis organs and indicators of peripheral blood of laboratory animals in experiments. The experiment was conducted on 24 white mongrel male rats weighing 180-220 g, gold nanoparticles sizes 1-3, 15 and 50 nm were administered orally for 15 days at a dosage of 190 µg/kg of animal body weight. The gold nanoparticles were conjugated with polyethylene glycol to increase their biocompatibility and bioavailability. The size-dependent decrease of the number of neutrophils and lymphocytes was noted in the study of peripheral blood, especially pronounced after administration of gold nanoparticles with size of 50 nm. The stimulation of lymphocyte and myelocytic series of hematopoiesis was recorded at morphological study of the bone marrow. The signs of strengthening of the processes of differentiation and maturation of cellular elements were showed in lymph nodes, which was reflected in the increasing number of immunoblast and large lymphocytes.

The morphological changes of quantitative relations cellular components in immunogenesis organs identify the activation of the migration, proliferation and differentiation of immune cells, indicating the presence of immunomodulating effect of gold nanoparticles.

9709-35, Session PMon

DC vaccine generated by photodynamic therapy for squamous cell carcinoma

Xiuli Wang, Haiyan Zhang, Jie Ji, Shanghai Skin Disease Hospital (China)

Dendritic cell (DC) based vaccine has emerged as a promising immunotherapy for cancers. Photodynamic therapy (PDT), an established cancer treatment strategy, can cause immunogenic apoptosis to induce an effective antitumor immune response. In this study, we developed a DC-based cancer vaccine using immunogenic apoptotic tumor cells induced by 5-aminolevulinic acid (ALA) mediated PDT. We observed the maturation of DCs potentiated by ALA-PDT treated tumor cells, including morphology maturation (enlargement of dendrites and increase of lysosomes), phenotypic maturation (upregulation of surface expression of MHC-II, DC80, and CD86), and functional maturation (enhanced capability to secrete IFN-γ and IL-12, and to induce T cell proliferation). Most interestingly, PDT-induced apoptotic tumor cells are more capable in potentiating maturation of DCs than PDT-treated or freeze/thaw treated nectrotic tumor cells. ALA-PDT-DC vaccine mediated by apoptotic cells provided protection against tumor in mice, far stronger than that of DC vaccine obtained from freeze/thaw treated tumor cells. Immunohistochemistry showed that PDT-DC vaccine could induces increases in CD4+ and CD8+ T cells in the tumor cells injected region. Flow cytometry assays showed that the ratio of CD4+ and CD8+ T cells in spleen increased significantly in PDT-DC vaccine treated mice.

Our results indicate that immunogenic apoptotic tumor cells can be more effective in enhancing DC-based cancer vaccine, which could improve the clinical application of PDT-DC vaccines.

9709-36, Session PMon

Regulatory T cell effects in antitumor laser immunotherapy: a mathematical model and analysis

Sean M. Laverty, Bryan A. Dawkins, Univ. of Central Oklahoma (United States)

Understanding the role of regulatory T cells (Tregs) in antitumor laser immunotherapy is crucial for improving treatment efficacy. We present a mathematical model of the adaptive immune response initiated by laser treatment of cancerous tumors in an animal model. We explicitly model populations of dendritic cells, cytotoxic T cells, primary tumor cells, and metastatic tumor cells. Though not modeled explicitly, the effects of Tregs can be analyzed by their influence on model parameters. Tregs have been shown to directly kill antigen presenting cells, to interfere with the antigen presentation process, to kill activated cytotoxic T cells in the tumor microenvironment, and to decrease proliferation rates for cytotoxic T cells upon their departure from lymph nodes. By varying key model parameters relating to these processes, we analyze the effects of Tregs on tumor burden dynamics. Simulation results from these numerical experiments are compared with available animal model treatment data. Additionally, tumor burden dynamics of our model simulations are partitioned with a systematic method of assigning clinical treatment outcomes based on criteria such as peak primary tumor burden, tumor recurrence, and level of overall tumor clearance by the end of the simulated treatment period. We will use our clinical treatment outcome partitioning criteria to identify patterns in the underlying model parameters. Ultimately, we will show how clinical treatment outcomes relate to varying levels Treg suppression.

9709-37, Session PMon

Distinguishing oxygenated and deoxygenated fetal blood using fluorescence lifetime imaging on stained placental sections

Teng Luo, Xiao Peng, Danying Lin, Junle Qu, Shenzhen Univ. (China)

The use of conventional fluorescence microscopy for characterizing tissue pathological states is limited by overlapping spectra and the dependence on excitation power and fluorophore concentration. Fluorescence lifetime imaging (FLIM) has previously been shown to provide contrast between normal and diseased tissues. This work reports the use of fluorescence lifetime to aid the pathological distinction between the umbilical arterial deoxygenated and nutrient-depleted fetal blood and the umbilical venous oxygenated and nutrient rich blood from H&E stained placental tissue sections. Oxygenated blood in umbilical vein was found to have larger lifetimes in the range from 0 ps to 200 ps while deoxygenated blood in umbilical artery has smaller lifetimes distributed from 0 ps to 150 ps. The use of conventional fluorescence microscopy for characterizing tissue pathological states is limited by overlapping spectra and the dependence on excitation power and fluorophore concentration. This work reports the use of fluorescence lifetime imaging (FLIM) has previously been shown to provide contrast between normal and diseased tissues. This work reports the use of fluorescence lifetime to aid the pathological distinction between the umbilical arterial deoxygenated and nutrient-depleted fetal blood and the umbilical venous oxygenated and nutrient rich blood from H&E stained placental tissue sections. Oxygenated blood in umbilical vein was found to have larger lifetimes in the range from 0 ps to 200 ps while deoxygenated blood in umbilical artery has smaller lifetimes distributed from 0 ps to 150 ps. The results suggest that the proposed method can be used to supplement the traditional histopathological examination of oxygen-saturated hemoglobin relative to unsaturated hemoglobin in the blood. This is the first study to employ fluorescence lifetime imaging to evaluate the oxygen saturation from pathologists' view point.
Toxicity induced by upconversion nanoparticles in mouse macrophages

Xiao Peng, Shuai Ye, Guangsheng Wang, Yuliang Tian, Maozhen Xiong, Wei Yan, Dong Wang, Jun Song, Junle Qu, Shenzhen Univ. (China)

In recent studies, the rapid development and growing demand for biological applications of upconversion nanoparticles (UCNPs) requires a better understanding of their cytotoxicity and biocompatibility. In this study, we synthesized a series of NaYF4 UCNPs with a facile thermal decomposed method. These nanoparticles are identified to be uniform with the sizes around 20 nm. To study their potential cytotoxicity on mammalian cells, we used Raw264.7 cells, a mouse macrophage cell line involved in in vivo nanoparticle disposition process, to perform a series of biological tests, such as cell viability assay, proliferation assay and apoptosis test. Results have shown that UCNPs induced less cytotoxicity than quantum dots at certain concentrations, indicating an improved utility in further biological applications.
Laser speckle rheology (Invited Paper)

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

Most pathological conditions are accompanied by alterations in tissue stiffness. For instance, myocardial infarction is caused by rupture of mechanically-compromised atherosclerotic plaques. In a variety of clotting disorders that cause bleeding or thrombosis, coagulation defects are associated with changes in clot stiffness. For tumor diagnosis, mechanical cues have long been used to detect regions of elevated stiffness by palpation. At the cellular level, altered mechanical properties of the tumor microenvironment have been shown to regulate malignant transformation and cancer cell proliferation. Therefore the significant evidence on the role of mechanical factors on disease initiation and progression calls for development of technologies for biomechanical evaluation of tissue in situ.

My talk will describe a new optical approach, Laser Speckle Rheology (LSR) that quantifies the viscoelastic properties of tissue in situ. In LSR, the sample is illuminated with coherent light and time-varying laser speckle patterns are acquired using a CMOS camera. Laser speckle that occurs by the interference of coherent light scattered from the sample, is exquisitely sensitive to the Brownian motion of light scattering particles, in turn influenced by the viscoelastic susceptibility of the medium. By calculating the mean squared displacements from the temporal evolution of laser speckle fluctuations we can derive tissue viscoelastic modulus in a non-contact manner. In this talk, I will investigate three clinical and biological applications employing the LSR approach for cardiology, blood diagnostics and cancer biology applications.

Ex vivo multiscale quantitation of skin biomechanics in wild-type and genetically-modified mice using multiphoton microscopy

Stéphane Bancelin, Institut National de la Recherche Scientifique (Canada) and Ecole Polytechnique (France); Barbara Lynch, Ecole Polytechnique (France); Christelle Bonod-Bidaud, Institut de Génomique Fonctionnelle de Lyon (France); Guillaume Ducourthial, Sotiris Psilodimitrakopoulos, Lab. d’Optique et Biosciences (France) and Ecole Polytechnique (France); Petr Dokladal, Ctr. de Morphologie Mathématique (France); Jean-Marc Allain, Ecole Polytechnique (France); Marie-Claire Schanne-Klein, Lab. d’Optique et Biosciences (France) and Ecole Polytechnique (France); Florence Ruggiero, Institut de Génomique Fonctionnelle de Lyon (France)

Soft connective tissues such as skin are made of more than 90% of extracellular matrix proteins, fibrillar collagens being the major structural component. The diameter and organization of collagen fibers depend on tissues and provide mechanical support to residing cells. Defective or aberrant collagen synthesis typically disrupt tissue mechanical properties or tissue cohesion by altering cell interactions with the extracellular matrix. Such changes in tissue biomechanics have a dramatic impact in development, wound repair and ageing. As a typical example, the classic form of Ehlers-Danlos syndrome is a connective tissue disorder caused by mutations in collagen V genes, mainly characterized by skin hyperextensibility.

In this work, we developed a unique interdisciplinary approach combining advanced techniques in mechanics, optics, image processing and biology to investigate the relationship between the skin microstructure and its macroscopic mechanical property. Mechanical measurements were performed while imaging optical sections of ex vivo mouse skin, using multiphoton microscopy, and specific image processing, based on mathematical morphology algorithms, was implemented to quantify tissue microstructure and correlate it with stress/stretch relationship. This multiscale quantitative characterization of skin mechanics enabled us to challenge the theoretical explanation of the microstructural origin of the skin macroscopic mechanical response. Finally, we took advantage of two well-characterized mouse strains to unravel the role of collagen V in skin mechanics.

This approach may be generalized to other diseases or tissues, or to guide engineering of tissue substitutes with appropriate biomechanical responses and therefore opens avenues for the investigation of the complex biomechanics of collagen-rich tissues.

Compression optical coherence elastography for improved diagnosis of disease (Invited Paper)

Brendan F. Kennedy, Philip Wijesinghe, Lixin Chin, Andrea Curatolo, Shaghayegh Es’haghian, Wes M. Allen, Luke Frewer, Arash Arabshahi, Karol Karnowski, David D. Sampson, The Univ. of Western Australia (Australia)

Optical coherence elastography (OCE) is emerging as a potentially useful tool in the identification of a number of diseases. In our group, we are developing OCE techniques based on compressive loading. Typically, these techniques employ a quasi-static mechanical load introduced by uniaxially compressing a sample with a rigid plate. The resulting deformation of the sample is measured using phase-sensitive detection and the axial strain is estimated from the slope of displacement over a finite depth in the sample, providing qualitative mechanical contrast. In this talk, an overview of our work will be given and some of the outstanding challenges described. Our group’s work in OCE can broadly be divided into four streams, each of which will be described in detail in the talk: system development; techniques; quantification; and applications.

• System development: The phase-sensitive OCE method we have developed will be described, as well as a high resolution optical coherence microscopy-based elastography system suitable for imaging cellular-scale mechanical properties.

• Techniques: In addition to presenting techniques to estimate strain, our approaches to imaging tissue viscoelasticity and nonlinearity will be described. A technique to segment elastograms based on strain heterogeneity will be presented.

• Quantification: Methods under development to quantify tissue stiffness in compression OCE will be described. This work is enabled by optical palpation and solutions to the forward and inverse elasticity problems.

• Applications: Three applications areas will be described: intraoperative assessment of tumour margins, mapping stiffness in tumour biology and assessing the stiffness of cardiovascular tissue in an animal model.
The endogenous fluorescence of fibroblast in collagen gels as indicator of stiffness of the extracellular matrix

Juan Pablo Padilla-Martinez, Antonio Ortega-Martinez, Walfre Franco, Wellman Ctr. for Photomedicine (United States)

The stiffness or rigidity of the extracellular matrix (ECM) regulates cell response. Established mechanical tests to measure stiffness, such as indentation and tensile tests, are invasive and destructive to the sample. Endogenous or native molecules to cells and ECM components, like tryptophan and cross-links of collagen, display fluorescence upon irradiation with ultraviolet light. Most likely, the concentration of these endogenous fluorophores changes as the stiffness of the ECM changes. In this work we investigate the endogenous fluorescence of collagen gels containing fibroblasts as a non-invasive non-destructive method to measure stiffness of the ECM. Human fibroblast cells were cultured in three-dimensional gels of type I collagen (50,000 cells/ml). This construct is a simple model of tissue contraction. During contraction, changes in the excitation-emission matrix — a fluorescence map in the 240-520/290-530 nm range — of constructs were measured with a spectrofluorometer, and changes in stiffness with a common indentation test over 16 days. Results show that a progressive increase in fluorescence of the 290/340 nm excitation-emission pair correlates with a progressive increase in stiffness (r=0.9). The fluorescence of this excitation-emission pair is ascribed to tryptophan and variations in the fluorescence of this pair correlate with cellular proliferation. In this tissue model, the endogenous functional fluorescence of proliferating fibroblast cells is a biomechanical marker of stiffness of the ECM.

Mapping ECM nanotopology via PS-OCT measurements of gold nanorod diffusion

Richard L. Blackmon, The Univ. of North Carolina at Chapel Hill (United States); Brian S. Chapman, Joseph B. Tracy, North Carolina State Univ. (United States); Patricia Casbas-Hernandez, Rupinder Sandhu, Melissa Troester, Amy L. Oldenburg, The Univ. of North Carolina at Chapel Hill (United States)

The mammary gland is comprised of biopolymers like collagen, forming an extracellular matrix (ECM) that is created and maintained by mammary fibroblasts. Changes in mechanical properties of ECM via remodeling of its nanotopography play a critical role in cancer progression. Here, we describe a method to map the nanoscale porosity of ECM using diffusion-sensitive optical coherence tomography (DS-OCT) by spatially resolving the weakly-constrained diffusion of gold nanorods (GNRs). Spectral-domain, polarization-sensitive OCT was used to collect DS-OCT images. For each DS-OCT image, an ensemble of 100 M-mode scans (4000x1024 pixels) was collected by incrementing the scanning beam 20 μm laterally over 2 mm. Dynamic light scattering theory was used to calculate GNR diffusion rates, which were spatially resolved to 20μx4.65μm in x and z, respectively. DS-OCT imaging was first performed on homogeneous collagen samples with densities of 2, 5, and 8 mg/mL premixed with GNRs (83x22nm), revealing a decrease in GNR diffusion as pore size decreased. Multi-layered samples comprised of 2 and 8 mg/mL collagen were also imaged, demonstrating the ability of DS-OCT to sense heterogeneous porosity. Finally, DS-OCT was employed on 3D mammary fibroblast cultures with 0, 50, and 100 cells/μL seed densities in collagen:Matrigel, revealing increasing spatial heterogeneity of ECM for increasing cell seed densities. Diffusion rates in both collagen and ECM were highly correlated with pore size measurements obtained using scanning electron microscopy (SEM, R=0.968). DS-OCT demonstrates a novel tool to spatially resolve the mechanical properties of the ECM in a minimally-invasive way.

Online monitoring of mechanical properties of three-dimensional tissue engineered constructs for quality assessment

Yvonne Reinwald, Keele Univ. (United Kingdom); Pierre O. Bagnaninchi, The Univ. of Edinburgh (United Kingdom); Ying Yang, Yanny Baba Ismail, Alicia J. El Haj, Keele Univ. (United Kingdom)

To regenerate tissues, various approaches have been established including the delivery of cell-seeded three-dimensional scaffolds. Their development and maturation is promoted through the utilisation of bioreactors aiming to mimic the growth environment cells and tissues experience in vivo. Monitoring the maturation of tissue constructs during culture and their performance prior to implantation into the patient is important for defining their quality and manufacturing criteria. Key properties include scaffold integrity and mechanical properties. Therefore, novel non-invasive three-dimensional (3D) imaging modalities are required providing rapid results and translational solutions. Elastography allows the mapping of mechanical properties by measuring the deformation in correlation to external mechanical loading. However, it is limited in terms of resolution and sensitivity. To overcome these drawbacks elastography has been coupled with optical coherence tomography into a new image modality known as optical coherence elastography (OCE). In this study, we applied OCE to monitor mechanical properties of 3D tissue constructs while being mechanically stimulated in the hydrostatic force bioreactor. Agarose gels have been infiltrated into porous 3D printed or salt-leshached scaffolds to mimic heterogeneous mechanical properties of cell-seeded constructs. Phase-resolved elastography algorithms were applied to the image datasets. The displacement and strain maps resulting from the applied hydrostatic pressure in the constructs have been generated and using the magnitude of applied hydrostatic pressure their Young’s modulus has been calculated. Our data suggest that OCE enables the real-time non-invasive monitoring of the mechanical properties of tissue constructs and the effect of the mechanical force on cellular activities during dynamic culture.

Laser speckle micro-rheology for biomechanical evaluation of breast tumors

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

The stiffness of the extra cellular matrix (ECM) is recognized as a key regulator of cancer cell proliferation, migration and invasion. Therefore technologies that quantify ECM stiffness with micro-scale scale resolution will likely provide important insights into neoplastic progression. Laser Speckle Micro-Rheology (LSM) is a novel optical tool for measuring tissue viscoelastic properties with micro-scale resolution. In LSM, speckle images are collected through an objective lens by a high-speed camera. Spatio-temporal correlation analysis of speckle frames yields the intensity autocorrelation function, g2(τ), for each pixel, and subsequently a 2D map of viscoelastic modulus, G’(τ) is reconstructed. Here, we investigate the utility of LSM for micro-mechanical evaluation of the ECM in human breast lesions. Specimens collected 18 women undergoing lumpectomy or mastectomy were evaluated with LSM. Because collagen is the key protein associated with ECM stiffness, G’(τ) maps obtained from LSM were compared with collagen content measured by second harmonic generation (SHG) microscopy. Regions of low G’(τ), identified by LSM, corresponded to low-intensity SHG signal and adipose tissue. Likewise, regions with high G’(τ) in LSM images matched high intensity SHG signal caused by desmoplastic collagen accumulation. Quantitative regression analysis demonstrated a strong, statistically significant correlation between G’(τ) and SHG signal intensity (R=0.66 p< 0.01). These findings highlight the capability of LSM
for quantifying the ECM micro-mechanics, potentially providing important insights into the biomechanical regulators of breast cancer progression.

9710-8, Session 3

**Acoustic radiation force optical coherence elastography (Invited Paper)**

Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States)

We report on the development of an acoustic radiation force optical coherence elastography (ARF-OCE) technology to characterize tissues biomechanical properties. Knowledge of tissue mechanical properties provides valuable medical information in disease diagnosis and prognosis. There is a close correlation between tissue elasticity and pathology. For example, in atherosclerosis, measurement of tissue biomechanical properties has the potential to differentiate between various plaque components. Furthermore, tissue mechanical properties provide critical information to assess the vulnerability of plaques. The stress in the cap increases with decreasing thickness and increasing macrophage infiltration. High strain locations in the vessel wall indicate the presence of vulnerable plaques. We have applied the ARF-OCE to image post-mortem human coronary artery with atherosclerosis. The result demonstrates the potential of ARF-OCE as a non-invasive method for imaging and characterizing vulnerable plaques.

In addition, we have developed an acoustic radiation force orthogonal excitation optical coherence elastography (ARFOE-OCE) method for the visualization of shear wave and the calculation of the shear modulus based on the Doppler OCT variance method. The ARFOE-OCE method allows direct visualization of the shear wave propagation inside tissue, and measurement of the shear wave propagation speed allows direct map of tissue mechanical properties. ARFOE-OCE also provides a mean to quantify tissue mechanical properties at depth beyond the OCT imaging range. The ARF-OCE technology will have a broad range of clinical applications, including imaging and characterizing cardiovascular atherosclerotic lesions, imaging and diagnosing of early stage cancer, and imaging and evaluating ophthalmic diseases such as keratoconus and age-related macular degeneration.

9710-9, Session 3

**Dynamic phase-sensitive optical coherence elastography at a true kilohertz frame-rate**

Manmohan Singh, Zhaolong Han, Thomas Hsu, Jiasong Li, Alexander Schill, Chih-Hao Liu, Chen Wu, Raksha Raghunathan, Achuth Nair, Kirill V. Larin, Univ. of Houston (United States)

Measuring the mechanical properties of tissues can provide valuable information for detecting and monitoring the progression of disease. Various techniques have been proposed to quantify the biomechanical properties of tissues, but each has its limitations. In this work we demonstrate the first use of dynamic phase-sensitive optical coherence elastography (OCE) at a true kilohertz frame-rate. The OCE system utilized a Fourier Domain Mode Locked (FDML) laser with an A-scan rate of ~1.5 MHz. Three techniques were evaluated for their ability to obtain the mechanical properties of tissue-mimicking phantoms: focused air-pulse induced elastic wave imaging, resonant acoustic vibrography, and compressional micro-elastography. The use of an ultra-fast FDML laser source reduced the acquisition time of an elastogram significantly for each of the aforementioned techniques by acquiring successive B-scans (B-M mode). Finite element modeling and uniaxial mechanical testing were conducted to validate the OCE measurements, and the results were in good agreement. Furthermore, the acoustic vibrography and micro-elastography results showed that these two techniques were able to discern inclusions embedded in the phantoms.

9710-10, Session 3

**Ultra-high speed all optical shear wave imaging optical coherence elastography**

Shaozhen Song, Bao-Yu Hsieh, Wei Wei, Tueng Shen, Matthew O’Donnell, Ruikang K. Wang, Univ. of Washington (United States)

Optical Coherence Elastography (OCE) is a non-invasive testing modality that maps the mechanical property of soft tissues with high sensitivity and spatial resolution using phase-sensitive optical coherence tomography (PS-OCT). Shear wave OCE (SW-OCE) is a leading technique that relies on the speed of propagating shear waves to provide a quantitative elastography. Previous shear wave imaging OCT techniques are based on repeated M-B scans, which have several drawbacks such as long acquisition time and repeated wave stimulations. Recent developments of Fourier domain mode-locked high-speed swept-source OCT system has enabled enough speed to perform kHz B-scan rate OCT imaging. Here we propose ultra-high speed, single shot shear wave imaging to capture single-shot transient shear wave propagation to perform SW-OCE. The frame rate of shear wave imaging is 16 kHz, at A-line rate of 1.62 MHz, which allows the detection of high-frequency shear wave of up to 8 kHz. The shear wave is generated photo-thermal-acoustically, by ultra-violet pulsed laser, which requires no contact to OCE subjects, while launching high frequency shear waves that carries rich localized elasticity information. The image acquisition and processing can be performed at video-rate, which enables real-time 3D elastography. SW-OCE measurements are demonstrated on tissue-mimicking phantoms and porcine ocular tissue. This approach opens up the feasibility to perform real-time 3D SW-OCE in clinical applications, to obtain high-resolution localized quantitative measurement of tissue biomechanical property.

9710-11, Session 3

**High speed optical coherence elastography for human skin deformation studies**

Xuesong Hu, Raman Maiti, Robert A. Byers, The Univ. of Sheffield (United Kingdom); Lutz Gerhardt, Philips Research (Netherlands); Matt J. Carré, Roger Lewis, The Univ. of Sheffield (United Kingdom); Steven E. Franklin, Philips Research (Netherlands) and The Univ. of Sheffield (United Kingdom); Stephen J. Matcher, The Univ. of Sheffield (United Kingdom)

A dual-channel optical coherence tomography (OCT) system with an ultra-high speed of 500 kHz A-scan rate was developed for measuring the biomechanical properties of human finger-pad and forearm skin. Such an OCT system operates at a center wavelength of 870 nm with a spectral bandwidth of 200 nm resulting in a very good axial resolution of 2.7 µm. The measured sensitivity and sensitivity roll-off of the system were -90 dB and -10 dB/mm, respectively. In order to achieve 500 kHz A-scan rate, we synchronized two Basler sprint CCD cameras, i.e., each running at 250 kHz, at two separated optical channels. Elastographic B-scan images of the human finger-pad and forearm skin were constructed by using 1000 A-scans. Deformation of the human finger-pad, while pressed against a transparent optical glass plate under the action of 0.5-24 N force, was examined both at the surface and sub-surface. However, when measuring the deformation of the forearm skin the forearm was loaded in tension using a tape with forces between 1.5-4.5 N. Biomechanical properties, i.e., finger-pad/glass interface contact area, thickness of the epidermis layer, etc., as a function of applied pressure were measured with this ultra-high speed OCT system. The same experiments were performed on a 1500 nm commercial OCT system (Vivosight, Michelson Diagnostics Ltd.) with a low A-scan rate of 20 kHz. Results obtained from the Vivosight system were discussed for comparison. Finally, we discussed the potential use of the high speed system for “speckle-tracking”.

9711-1, Session 3

**3D high-speed optical coherence elastography with picosecond-based laser pulsing**

Matthew O’Donnell, Matthew Rizzo, Matthew Rizzo, Ruikang K. Wang, Univ. of Washington (United States)

Using a 1 kHz, 3 ns laser pulse source, we acquire 2D phase images at 10 kHz, then perform 3D image acquisition by scanning the sample in the X-Y plane while acquiring phase images at each X-Y location. The phase images are then unwrapped to obtain the strain field. The system was tested on phantoms for its ability to detect inclusions and its sensitivity to sample stiffness. Preliminary results indicate that the system was capable of detecting inclusions with dimensions as small as ~1 mm. The system was also tested on human skin samples. The results showed that the system was able to detect variations in skin stiffness, which could potentially be used for disease diagnosis. Further work is needed to improve the spatial resolution of the system and to test its performance on a wider range of tissue samples.
Lorentz force megahertz optical coherence elastography
Chen Wu, Manmohan Singh, Zhaolong Han, Raksha Raghunathan, Chih-Hao Liu, Jiasong Li, Alexander Schill, Kirill V. Larin, Univ. of Houston (United States)

Optical Coherence Elastography (OCE) has emerged as a promising technique for tissue biomechanical property quantification. This study first demonstrates the feasibility of using Lorentz force to induce the elastic wave inside tissue to study elasticity in combination of Megahertz Optical Coherence Elastography system (MHz OCE). Lorentz force can be generated as the electrical current flow through the biological tissues in a magnetic field, and the force will result in tissue deformation. The localized tissue deformation cause the propagation of elastic wave which can be detected with a phase-sensitive OCE system at 1.5 million A-lines per second. In this study, first we use this method to study the elasticity of different concentration of agar sample, and then perform the experiments on the porcine liver. The Lorentz force OCE results are validated by the mechanical compressional tests in the end. The possibility of Lorentz force excitation bring us a new method to perform dynamic elastography based on elastic wave propagation in biological tissues. Due to the high frame-rate of the Megahertz OCE system, the elastic wave propagation can be recorded with single excitation. The results demonstrate that Lorentz force MHz OCE can be applied to study the elasticity of biological tissue effectively and have the potential for clinical study in the future.

Continuous-wave stimulated Brillouin spectroscopy in scattering media at 780 nm
Itay Remer, Alberto Billenca, Ben-Gurion Univ. of the Negev (Israel)

Quantitative probing of the mechanical properties of scattering media by Brillouin spectroscopy is an emerging field of research. At present, Brillouin spectrometers typically detect spontaneous Brillouin backscattered signals from the sample using setups that comprise virtually imaged phased arrays (VIPAs) cascaded in cross-axis configuration or heated molecular absorption cells prior to the VIPA. These experimental arrangements are necessary in order to significantly suppress the strong elastic scattering background from the medium.

In this talk, we present a different approach for Brillouin spectroscopy of scattering matter based on stimulated Brillouin scattering (SBS) amplification. Unlike spontaneous Brillouin scattering, SBS amplification does not show elastic scattering background due to the resonant nature of the amplification process, thereby providing excellent spectral contrast. We demonstrate that the use of two continuous-wave distributed feedback lasers at 780 nm in a counter-propagating SBS amplifier geometry is useful for acquiring high signal-to-noise ratio SBS spectra of Intralipid solutions at concentrations that yield up to 3 scattering events for photons propagating through the sample. Potential applications of SBS spectroscopy in inelastic mechanical quantification of thin tissue sections and biopolymers will be discussed.

Ultra-high spectral extinction Brillouin spectroscopy for turbid tissue measurements
Jitao Zhang, Antonio Fiore, Univ. of Maryland, College Park (United States); Peng Shao, Seok-Hyun Yun, Wellman Ctr. for Photomedicine (United States); GiulianoScarcelli, Univ. of Maryland, College Park (United States)

Brillouin spectroscopy allows non-invasive measurement of the mechanical properties of a sample by measuring the spectra of acoustically induced light scattering therein, and thus has been widely investigated for biomedical application. Recently, the development of fast Brillouin spectrometry based on virtually-imaged phased array (VIPA) has made in-situ measurement of biomedical sample possible. However, one limitation of current Brillouin technique is the low spectral extinction, which limits the measurement to nearly transparent sample. In order to measure turbid sample, multistage VIPA can be cascaded to gain spectral extinction. For example, spectral extinction of -80 dB was achieved using three-stage VIPA; however, this approach significantly sacrificed measurement throughput. In this work, we develop a novel spectrometer that achieves high extinction without significant signal loss. To achieve this goal, we combine a two-stage VIPA spectrometer with a triple-pass Fabry-Perot interferometer. The triple-pass Fabry-Perot interferometer acts as a band-pass filter with -3 GHz bandwidth and -35-dB spectral extinction. Therefore, the overall extinction of this spectrometer greatly surpasses 80 dB with only -20% excess loss. We demonstrated the performance of this spectrometer measuring background-free Brillouin spectra from Intralipid solutions and within chicken tissue.

High-speed elasticity-specific nonlinear Brillouin imaging/sensing via time-resolved optical (BISTRO) measurements
ZhaoKai Meng, Charles Ballman, Georgi I. Petrov, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Viscoelastic properties of living cells are often directly related to the cell types and their physiological conditions. Unfortunately, all the currently existing methods for analysis of viscoelastic properties of cells, such as micropipette aspiration, atomic force microscopy and optical tweezers are intrinsically slow, limiting their applicability to study large population of cells, which are often needed for either fundamental or clinical studies. Brillouin spectroscopy, an emerging technique in biomedical spectroscopy and imaging, is a non-invasive elasticity-specific probing technique based on inelastic light scattering originated from phonon-photon interactions. Nevertheless, the throughput of spontaneous Brillouin spectroscopy is also limited by the weak signal strength. In this report, by incorporating the concept of impulsive stimulated Brillouin scattering (IBBS), we report a Brillouin Imaging and Sensing system via Time-Resolved Optical (BISTRO) measurements. The IBBS concept is based on the optically generated phonons and the simultaneous optical heterodyne detection of these phonons using Bragg diffraction. In IBBS, a pair of external laser pulses coherently generates phonons on which the probe pulse is inelastically scattered. Therefore, the signal strength is coherently enhanced and can be substantially stronger than the spontaneous Brillouin signal. We will prove the principle of the BISTRO system by presenting example microscopic images and flow/cell cytometry results.

A Fabry-Perot etalon-based notch filter for background cleaning in Brillouin microscopy
Peng Shao, Sebastien Besner, Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States) and Harvard Medical School (United States); Giuliano Scarcelli, Univ. of Maryland, College Park (United States); Seok-Hyun Yun, Wellman Ctr. for Photomedicine (United States)
Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States) and Harvard Medical School (United States)

In Brillouin scattering imaging, rejection of background noise due to elastic scattering and reflections from optical components is crucial. This is because Brillouin signal is weak, and the signal frequency shift compared with source laser line is very small. Therefore the line of interest is very easy to be contaminated. Whereas physical blocking of undesired component in a dispersed spectrum is used, to filter out background optically provides better stability. Conventional optical filter techniques, such as dielectric-stack filters, holographic volume filters, Lyot filters etc. normally have a stopband-width (bandstop filter) or edge-width (edge filter) ranging from a few nanometers to tens of nanometers despite high rejection rate. They cannot be implemented in Brillouin imaging due to the small wavelength shift (<1 pm).

We report a Fabry-Perot etalon-based notch filter for background cleaning in Brillouin imaging. The notch filter takes advantage of multiple reflections of the light beam with a Fabry-Perot etalon to achieve high rejection with narrow bandwidth. The theoretical rejection rate is multiple time of the rejection of one reflection of the etalon. We demonstrated a laser line suppression of > 40 dB while with < 40% power loss with experiments. Width of the stopband at -30 dB rejection level is ~ 1 GHz. This method is not wavelength specific. One etalon may be implanted to a wide spectrum of laser wavelengths. Furthermore, it does not require heating as gaseous notch filters. Our method can also be implemented in a Fabry-Perot scattering, fluorescent imaging and other imaging techniques in which line of interest is close to the laser source.

9710-17, Session 4

Cell biomechanical properties in 2D and 3D with Brillouin microscopy

Giuliano Scarcelli, Univ. of Maryland, College Park (United States); Seok Hyun Yun, Harvard Medical School (United States)

We present Brillouin optical microscopy for noncontact, label-free, and 3D interrogation of live cell mechanical properties. This technique is based on the interaction of light with spontaneous acoustic phonons. By measuring the optical frequency shift of the scattered light, the longitudinal modulus of the medium is determined. Mechanically, Brillouin interaction can be thought of as a test that measures the ratio of uniaxial stress and strain in confined compression conditions at high frequency. We show that Brillouin microscopy is sensitive to liquid-solid regulation within cells under different osmotic conditions and directly validate our measurements to gold-standard micro-indentation. Moreover, we show Brillouin microscopy can detect intracellular mechanical changes due to actin cytoskeletal changes such as polymerization, branching and network disruption. Finally, enabled by the non-contact nature of the technique, we investigate the mechanical behavior of cells cultured on 2D flat substrates vs when they are embedded in 3D meshwork of proteins.

9710-51, Session 4

Quantification of plaque stiffness by Brillouin microscopy

Giuseppe Antonacci, Ryan Pedrigi, Rob Krams, 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.

Hitherto Brillouin microscopy has been shown the ability to provide unique stiffness maps of biological samples, such as the human lens, in a non-destructive manner. In this work, we present results obtained using our Brillouin microscope to map the stiffness variations in the walls of blood vessels in particular when atherosclerotic plaques are formed. The stiffness of the membrane that covers the plaques is critical in developing acute myocardial infarction yet it is not currently possible to credibly assess its stiffness due to lack of suitable methods.
Combined optical coherence tomography and optical coherence elastography for glomerulonephritis classification

Chih-Hao Liu, Yong Du, Mannohman Singh, Chen Wu, Zhaolong Han, Jiasong Li, Qais Mohammadzai, Raksha Raghunathan, Thomas Hsu, Shezaan Noorani, Anthony Chang, Chandra Mohan, Univ. of Houston (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States) and Samara State Aerospace Univ. (Russian Federation)

Glomerulonephritis caused by anti-glomerular basement membrane disease (anti-GBM) is a rapidly progressing disease with a relatively high mortality, which is due to delayed diagnosis. Traditional diagnostic techniques such as blood examination, urine analysis, and tissue biopsy can be time consuming. Ultrasound imaging and computed tomography (CT) imaging can also provide valuable information for glomerulonephritis diagnosis with shorter examination times, but their limited spatial resolutions lead to a reduced disease detection sensitivity. Optical coherence tomography (OCT) is a well-established noninvasive imaging technique that provides superior resolution (micron scale) as compared to ultrasound and CT. Pathological changes in tissue properties can be detected based on optical metrics obtained from the OCT signal, such as optical attenuation and speckle variance. Furthermore, OCT does not rely on ionizing radiation as with CT imaging. In addition to morphological changes, the elasticity of the kidney can be altered due to nephritis. In this work we demonstrate the first use of combining OCT and optical coherence elastography (OCE) to quantify the difference in tissue properties between healthy and nephritic murine kidneys. Although disease detection by OCT imaging alone could identify the diseased tissue, its classification accuracy was insufficient for clinical use. After combining the optical metrics with elasticity, the classification accuracy improved from 76% to 95%. These results show that OCT combined with OCE can be a robust tool capable of detecting nephritis.

Depth-dependent displacement sensitivity analysis and the influence of Doppler angle for quantitative assessment of mechanical properties using phase-sensitive spectral domain optical coherence tomography

Gillian M. Lynch, Hrebesh M. Subhash, Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Optical coherence elastography (OCE) is a promising new method to assess the mechanical properties of tissue and materials by applying stress or mechanical stimulation and detecting the tissue displacement using optical coherence tomography (OCT). OCE has very unique features in that it is a non-contact, non-invasive, high resolution imaging method, and it can provide real time depth-resolved imaging capability in highly scattering specimens. Several types of OCE techniques have been demonstrated, based on both amplitude and phase information. Recently, a dynamic elastography technique based on phase-sensitive OCT has been demonstrated for the assessment of biomechanical properties of soft tissues with high sensitivity. In comparison to amplitude-based OCE, phase-based OCE can provide very high displacement sensitivity on the order of sub-nanometre scale. However, the displacement sensitivity of typical OCT systems are directly related to the overall signal-to-noise ratio (SNR) and their phase stability. Moreover, the estimation of the Doppler angle is also crucial for the accurate determination of the sample displacement, which is related to the sample surface shape. In this study, we evaluate the significance of these parameters for the quantitative assessment of the mechanical properties of tissue-mimicking phantoms with a phase-sensitive spectral domain OCT system.

Robust strain mapping in optical coherence elastography by combining local phase-resolved and cumulative displacement measurements

Vladimir Y. Zaitsev, Alexander L. Matveyev, Lev A. Matveev, Grigory V. Gellikonov, Institute of Applied Physics of the RAS (Russian Federation); Ekaterina Gubarkova, Natalia D. Gladkova, Nizhny Novgorod State Medical Academy (Russian Federation); Alex Vitkin, Univ. of Toronto (Canada)

We report a novel hybrid method of robust strain mapping in compressional optical coherence elastography (OCE) using combined phase measurements on sub-wavelength-scale and cumulative pixel-scale displacement tracking. In contrast to majority of compressional OCE methods, the proposed approach does not use initial reconstruction of displacements for subsequent differentiation in order to obtain local strains. On the contrary, local strains are used for tracking cumulative displacements that can be efficiently compensated to enable more accurate estimates of local strains. As a result, such an approach does not suffer from the phase-wrapping problem for super-wavelength displacements despite the fact that the tracked displacements may be essentially greater than the optical-wavelength scale and even the pixel scale. Such a significantly extended range of measurable displacements and strains (in comparison with conventional direct phase measurements) confers significant robustness of the proposed strain-mapping method with respect to both additive noise and decorrelation noise produced by displacements and strains.

The main advantages of the proposed approach are illustrated by the results of numerical simulations based on a realistic model of deformation-induced speckle-pattern evolution earlier proposed by the authors. Experimental demonstrations of obtaining strain maps for phantoms and real biological tissues are also presented. The robustness of the proposed method looks promising for practical realization of compressional elastography in the conditions of manually-produced deformation of the tissue, when application of stable periodic sources of deformation is impossible and thus improvement of the signal-to-noise ratio by many-period averaging cannot be utilized.

Mechanical characterization of mouse diaphragm with optical coherence elastography reveals fibrosis-related change of muscle stiffness

Shang Wang, James A. Loehr, Irina V. Larina, George G. Rodney Jr., Baylor College of Medicine (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States) and Samara State Aerospace Univ. (Russian Federation)

The diaphragm, composed of skeletal muscle, plays an important role in respiration through its dynamic contraction. Genetic and molecular studies of the biomechanics of mouse diaphragm can provide great insights into improved understanding and potential treatment of disorders that lead to diaphragm dysfunction (i.e. muscular dystrophy). However, due to the relatively small tissue size, mechanical assessment of mouse diaphragm under its proper physiological conditions has been challenging. Here, we present the application of optical coherence elastography (OCE) for quantitative elastic characterization of ex vivo mouse diaphragm. Phase-sensitive optical coherence tomography was combined with a focused air-puff system to image and measure the elastic wave propagation from tissue surface. Experiments were performed on wildtype and dystrophic mouse diaphragm tissue containing different levels of fibrosis. The OCE
Alterations in microstructure of cornea under thermo-mechanical effect of 1.56 microns laser radiation

Olga I. Baum, Emil N. Sobol, Institute on Laser and Information Technologies (Russian Federation); Andrey V. Bolshunov, Vladimir I. Siplivy, Research Institute of Eye Diseases (Russian Federation)

The new approach of the nonablative correction of the eye refraction under laser-induced stress relaxation in cornea is presented. The ring-shaped laser beam with various ring diameters allows obtaining controllable alterations of the eye refraction with axial symmetry. Atomic Force Microscopy, measurements of cornea surface and Light Scattering measurements have been used to study structure alterations in sclera due to non-uniform laser heating with wavelength 1.56 microns. At ring-shaped distribution of intensity of laser radiation, the tension and temperature of surface of cornea has also ring-shaped distribution, that results in heating and deformation of cornea on its periphery. This leads to the absence of any pathological changes in central part of cornea. The measurements of the surface curvature of the cornea during and after ring-shaped laser treatment have demonstrated controllable alterations in the eye refraction. Optimal laser settings, allowing to obtain refraction correction without tissue damage and denaturation have been established. Theoretical model is developed to estimate laser settings for desirable changes in the eye refraction.

Magnetomotive optical coherence elastography for thermal therapy dosimetry

Pin-Chieh Huang, Marina Marjanovic, Darold R. Spillman Jr., Boris M. Odintsov, Stephen A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Biomechanical properties of tissues have been utilized for disease detection, diagnosis, and progression, however they have not been extensively utilized for therapy dosimetry. Magnetic hyperthermia aims to kill cells and ablate tumors using magnetic nanoparticles (MNPs) either injected in or targeted to tumors. Upon application of an appropriate AC magnetic field, MNPs can heat target tissue while sparing non-targeted healthy tissue. However, a sensitive monitoring technique for the dose of magnetic hyperthermia is needed to prevent overtreatment and collateral injury. During hyperthermia treatments, the viscoelastic properties of tissues are altered due to protein denaturation, coagulation, and tissue dehydration, making these properties candidates for dosimetry. Magnetomotive optical coherence elastography (MM-OCE) utilizes MNPs as internal force transducers to probe the biomechanical properties of tissues. Therefore, we aim to evaluate the hyperthermia dose based on the elastic changes revealed by MM-OCE.

In this study, MNPs embedded in tissues were utilized for both hyperthermia and MM-OCE measurements. Tissue temperature and elastic modulus were obtained, where the elastic modulus was extracted from the resonance frequency detected by MM-OCE. Results showed a correlation between stiffness and temperature change following treatment. To investigate the thermal-dose-dependent changes, intervals of hyperthermia treatment were repeatedly performed on the same tissue sequentially, interspersed with MM-OCE. With increasing times of treatment, tissue stiffness increased, while temperature rise remained relatively constant. These results suggest that MM-OCE may potentially identify reversible and irreversible tissue changes during thermal therapy, supporting the use of MM-OCE for dosimetric control of hyperthermia in future applications.

Revealing anisotropic properties of cornea at different intraocular pressures using optical coherence elastography

Jiasong Li, Manmohan Singh, Zhaolong Han, Chen Wu, Chih-Hao Liu, Raksha Raghunathan, Achuth Nair, Univ. of Houston (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States) and National Research Tomsk State Univ. (Russian Federation)
Variations in the orientation of collagen fibrils in the cornea can lead to subsequent alterations in the biomechanical anisotropic properties of the cornea. Furthermore, the mechanical properties of the cornea can heavily be influenced by the intraocular pressure (IOP). In this work, the elastic anisotropy and hysteresis of in situ porcine corneas in the whole eye-globe configuration was evaluated while cycling IOP using a noncontact method of phase stabilized swept source optical coherence elastography (PhS-SOCE).

The home-built PhS-SOCE system imaged focused air-pulse induced elastic waves in stepped radial directions. Due to the high displacement sensitivity of the PhS-SOCE system, the elastic wave amplitude was minimal (µm scale). The IOP was cycled between 15 and 30 mmHg using a closed-loop feedback system while the OCE measurements were performed, and the Young’s modulus was translated from the wave velocity. Thus, the mechanical anisotropy and hysteresis of the cornea while cycling IOP was quantified. Our results demonstrated that the elastic anisotropy became apparent at IOP ≥ 20 mmHg, and that there were distinct radial angles of greater and lesser stiffness. Furthermore, results showed that there was indeed a measurable elastic anisotropic hysteresis while cycling IOP.

Our method of noncontact OCE was utilized to detect and assess the anisotropy and hysteresis of corneal elasticity as a function of IOP. Due to the small amplitude of the elastic wave and noninvasive measurement, this method may be useful for in vivo corneal studies that can provide information regarding corneal health and integrity.

9710-3, Session 7

Cells might not see where they are but they certainly feel the mechanics of their microenvironment! (Keynote Presentation)

Dennis E. Discher, Univ. of Pennsylvania (United States)

Soft tissues such as fat bear little physical stress, whereas stiffer tissues like muscle and bone sustain high stress. We have begun to uncover systematic relationships between such tissue properties and differentiation processes, having first shown that a soft matrix helps specify soft tissue lineages while a stiff matrix helps specify stiff tissue lineages [1]. Proteomics analyses of embryonic and mature tissues [2] have now revealed that while collagen directly determine tissue elasticity E the nucleoskeletal protein lamin-A follows polymer physics-type scaling versus E. Lamin-A has been reported for decades to vary widely between tissues, and mutations in lamin-A cause diseases of multiple stiff tissues as well as accelerated aging syndromes with defects in stiff tissue repair. Differentiation of various stem cell types is generally modulated by lamin-A levels downstream of matrix E and soluble factors such as retinoids [2,3], and we have uncovered multiple pathways that are co-regulated by lamin-A. Complementary insights are obtained from analyses in stem cells of the contractile cytoskeleton which not only physically stresses the nucleus but often contributes to key polarized processes of stem cell lineages [4]. Matrices and forces [5] thus combine with growth factor control of lineages, lamin-A, and cell fates.


9710-29, Session 8

Inverse problems in biomechanical imaging (Invited Paper)

Assad A. Oberai, Rensselaer Polytechnic Institute (United States)

It is now well recognized that a host of imaging modalities (a list that includes Ultrasound, MRI, Optical Coherence Tomography, and optical microscopy) can be used to “watch” tissue as it deforms in response to an internal or external excitation. The result is a detailed map of the deformation field in the interior of the tissue. This deformation field can be used in conjunction with a material mechanical response to determine the spatial distribution of material properties of the tissue by solving an inverse problem. Images of material properties thus obtained can be used to quantify the health of the tissue. Recently, they have been used to detect, diagnose and monitor cancerous lesions, detect vulnerable plaque in arteries, diagnose liver cirrhosis, and possibly detect the onset of Alzheimer’s disease. In this talk I will describe the mathematical and computational aspects of solving this class of inverse problems, and their applications in biology and medicine.

In particular, I will discuss the well-posedness of these problems and quantify the amount of displacement data necessary to obtain a unique property distribution. I will describe an efficient algorithm for solving the resulting inverse problem. I will also describe some recent developments based on Bayesian inference in estimating the variance in the estimates of material properties. I will conclude with the applications of these techniques in diagnosing breast cancer and in characterizing the mechanical properties of cells with sub-cellular resolution.
A comparative study of shear wave speed estimation techniques in optical coherence elastography applications

Fernando Zvieticovich, Jaining Yao, Ying-Ju Chu, Univ. of Rochester (United States); Panomsak Meemon, Suranaree Univ. of Technology (Thailand); Jannick P. Rolland, Kevin J. Parker, Univ. of Rochester (United States)

Optical Coherence Elastography (OCE) is a widely investigated noninvasive technique for estimating the mechanical properties of tissue. In particular, vibrational OCE methods aim to estimate the shear wave velocity generated by an external stimulus in order to calculate the elastic modulus of tissue. In this study, we compare the performance of five different acquisition and processing techniques for estimating the shear wave speed in tissue-mimicking phantoms. Accuracy, contrast to noise ratio, and resolution are measured in simulations and experiments for all cases. The first two techniques make use of one piezo-electric actuator for propagating a continuous sine wave, and a tone-burst (TB) of 400 Hz over the gelatin phantom by measuring its localized motion using phase-sensitive OCE. The other remaining techniques make use of one additional actuator located on the opposite side of the region of interest in order to create an interference pattern. When both actuators have the same frequency, a standing wave pattern is generated. Otherwise, when there is a frequency difference df between both actuators, a crawling wave pattern (CWP) is generated and propagates with less speed than a shear wave, which makes it suitable for being detected by the 2D cross-sectional OCE imaging. If df is not small compared to the operational frequency, the crawling wave travels faster and a sampled version of it (SCWP) is acquired by the system. Preliminary results suggest that TB (error < 7%) and SCWP (error < 3%) techniques are more accurate when compared to mechanical measurement test results.

Experimental classification of surface waves in optical coherence elastography

Fernando Zvieticovich, Jaining Yao, Jannick P. Rolland, Kevin J. Parker, Univ. of Rochester (United States)

Various types of waves are produced when a harmonic force is applied to a semi-infinite half space elastic medium. In particular, surface waves are perturbations with transverse and longitudinal components of displacement that propagate in the boundary region at the surface of the elastic solid. Shear wave speed estimation is the standard for characterizing elastic properties of tissue in elastography, however, the penetration depth of Optical Coherence Tomography (OCT) is typically measured in millimeters constraining the measurement region of interest to be near the surface. Plane harmonic Rayleigh waves propagate in solid-vacuum interfaces while Scholte waves exist in solid-fluid interfaces. Theoretically, for an elastic solid with a Poisson's ratio close to 0.5, the ratio of Rayleigh to shear wave speed is 95%, and 84% for the Scholte to shear wave. Our study demonstrates the evidence of Rayleigh waves propagating in the solid-air boundary of a tissue-mimicking elastic phantom. A 1 cycle sinusoidal tone-burst of 400 Hz was excited over the phantom by using a piezo-electric actuator. The wave propagation was detected with a phase-sensitive OCT system, and its speed was measured by tracking the most prominent peak of the tone along in time and space. Similarly, this same experiment was repeated with a water interface. In order to get the shear wave speed in the material, mechanical compression tests were conducted in samples of the same phantom. A 93.6% Rayleigh-shear and 80.1% Scholte-Shear speed ratio were measured during experiments. Results were complemented with numerical simulations demonstrating both types of surface waves.

A three dimensional solution for laser-induced thermoelastic deformation of the viscoelastic medium

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Absorption of laser energy on the surface of soft biological tissues leads to fast heating, rapid thermoelastic expansion, and generation of elastic waves in the tissue. After the elastic transients have decayed, the tissue reaches equilibrium, although quasi-steady-state thermoelastic stresses still exist. Both dynamic and quasi-steady-state stresses can induce ablation when the laser-induced stresses exceed the strength of the material. Recently, laser-induced deformation was proposed as a way to generate strain in the tissue to measure tissue mechanical properties. In combination with measuring tissue response using optical coherence tomography, such an approach could be an effective method for noncontact assessment of tissue mechanical properties.

We have derived an axially symmetric three-dimensional analytical solution for the thermoelastic displacements and stresses in the viscoelastic layer irradiated by the laser beam. The solution was obtained for Gaussian and top-hat radial temperature profiles on the upper surface of the viscoelastic layer. Two time regimes were considered: quasi-steady-state and dynamic tissue response. Analytical solutions to these problems were derived using the Hankel transform in the integral form. The proposed theoretical model was used to calculate the dynamic and static distributions of the laser-induced stresses and displacements in the layer. The influence of layer thickness on the stress and strain distribution was evaluated. The proposed analytical solution could be used in photomechanical models of laser ablation, as well as to predict mechanical and photooacoustic tissue response to laser excitation.

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Quantitative optical coherence elastography as an inverse elasticity problem

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Quantitative elasticity imaging, which retrieves elastic modulus maps from tissue, is preferred to qualitative strain imaging for acquiring system- and operator-independent images and longitudinal and multi-site diagnoses. Quantitative elasticity imaging has already been demonstrated in optical elastography by relating surface-acoustic and shear wave speed to Young's modulus via a simple algebraic relationship. Such approaches assume largely homogeneous samples and neglect the effect of boundary conditions.

We present a general approach to quantitative elasticity imaging based upon the solution of the inverse elasticity problem using an iterative technique and apply it to compression optical coherence elastography. The
Computational optical palpation: micro-scale force mapping using finite-element methods

Philip Wijesinghe, David D. Sampson, Brendan F. Kennedy, The Univ. of Western Australia (Australia)

Accurate quantification of forces, applied to, or generated by, tissue, is key to understanding many biomechanical processes, fabricating engineered tissues, and diagnosing diseases. Many techniques have been employed to measure forces; in particular, tactile imaging – developed to spatially map palpation-mimicking forces – has shown potential in improving the diagnosis of cancer on the macro-scale. However, tactile imaging often involves the use of discrete force sensors, such as capacitive or piezoelectric sensors, whose spatial resolution is often limited to 1-2 mm. Our group has previously presented a type of tactile imaging, termed optical palpation, in which the change in thickness of a compliant layer in contact with tissue is measured using optical coherence tomography, and surface forces are extracted, with a micro-scale spatial resolution, using a one-dimensional spring model. We have also recently combined optical palpation with compression optical coherence elastography (OCE) to quantify stiffness. A main limitation of this work, however, is that a one-dimensional spring model is insufficient in describing the deformation of mechanically heterogeneous tissue with uneven boundaries, generating significant inaccuracies in measured forces. Here, we present a computational, finite-element method, which we term computational optical palpation. In this technique, by knowing the non-linear mechanical properties of the layer, and from only the axial component of displacement measured by phase-sensitive OCE, we can estimate, not only the axial forces, but the three-dimensional traction forces at the layer-tissue interface. We use a non-linear, three-dimensional model of deformation, which greatly increases the ability to accurately measure force and stiffness in complex tissues.

Measurement of strain and strain rate in embryonic chick heart using spectral domain optical coherence tomography

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It is important to measure embryonic heart myocardial wall strain and strain properties of the right and left ventricle walls. These properties were characterized by a smooth growth of the stiffness and myocardial tissue resistivity at a relatively low strain against reduction in their strength and elastcity from 31-40 to 61-70 years. It was found that tissue of the left ventricle at 61-70 years had a lower stretchability and strength when compared with the tissues of the right ventricle and septum. These data expands understanding of the morphological organization of the heart ventricles, which is very important for the development of personalized medicine. Taking into account individual, age and sex differences of the heart ventricle tissue biomechanical characteristics allows to rationally choose the type of materials of patches during reconstructive operations on the heart.
rate for understanding the mechanisms of embryonic heart development. Optical coherence tomography (OCT) can provide depth resolved images with high spatial and temporal resolution, which makes it have the potential to reveal the complex myocardial activity in the early stage embryonic heart. We develop a novel method to measure strain in embryonic chick heart based on spectral domain OCT images and subsequent image processing. We perform 4D(x,y,z,t) scanning on the outflow tract (OFT) of chick embryonic hearts in HH18 stage (~3 days of incubation). Only one image sequence acquired at the special position is selected based on the Doppler blood flow information where the probe beam penetrates through the OFT perpendicularly. For each image of the selected sequence, the cross-section of the myocardial wall can be approximated as an annulus. The OFT is segmented with a semi-automatic boundary detection algorithm, thus the area and mean circumference of the annular myocardial wall can be achieved. The myocardial wall thickness was calculated using the area divided by the mean circumference, and then the strain was obtained. The results demonstrate that OCT can be a useful tool to describe the biomechanical characteristics of the embryonic heart.

9710-48, Session PSun
Skin surface and sub-surface strain and deformation imaging using OCT and DIC
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Bio-mechanical properties of the human skin deformed by external forces at difference skin/material interfaces attract much attention in medical research. For instance, such properties are important design factors when one designs a healthcare device, i.e., the device might be applied directly at skin/device interfaces. In this paper, we investigated the bio-mechanical properties, i.e., interface contact areas, thickness of the epidermis layer, etc., of the human finger-pad and forearm skin as a function of applied pressure by utilizing two non-invasive techniques, i.e., optical coherence tomography (OCT) and digital image correlation (DIC). The commercial OCT system called Vivosight (Michelson Diagnostics Ltd.) operates at a center wavelength of 1300 nm with a spectral bandwidth of 110 nm resulting in an axial resolution of 10 μm. Such an OCT system offered a B-scan image of a 15 μm lateral resolution and a 2 mm imaging depth while an A-scan rate of 20 kHz was realized in the OCT system. On the other hand, the DIC imaging system used in this study has a 5 MP AIV Pike camera (2452?2452 pixels) with a 5 frame s?1 image capture rate. Skin deformation results of the human finger-pad and forearm skin were obtained while pressed against a transparent optical glass plate with the action of 0.5-24 N force and loaded in tension using a tape with shear forces between 1.5-4.5 N, respectively. The obtained OCT images showed the deformation results beneath the skin surface, however, DIC images gave overall information of strain at the surface.

9710-50, Session PSun
Phase-sensitive optical coherence elastography with acoustic radiation force impulse excitation
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Many diseases such as a cancer affect the biomechanical properties of normal tissues. Therefore, the accurate measurement of this biomechanical change is crucial to diagnosis it in the early stage as well as to characterize tissue characteristics itself. Various approaches based on acoustic radiation force impulse imaging have been studied for ultrasound elastography. Here, we propose phase-sensitive optical coherence tomography-based elastography, which utilizes acoustic radiation force impulse in the axial direction. The spectral domain optical coherence tomography system consists of an 826.4 nm broadband superluminescent diode laser and a 1.2 mm outer diameter micro-motorized catheter. To detect the local elastic properties, we perform a “step-by-step” mode at 1 revolution per second, which can potentially be increased to > 10 revolutions/s. The beam is scanned in a limited number (up to 50) of angular steps, at each of which the beam position is held stable. When the laser beam is stable, the phase difference across a variable number of A-lines can be computed to assess the elastic displacement. Choosing a proper window delay, local elastic tissue displacement and strain can be quantified based on the phase shift. We conducted ex-vivo experiments with a cylindrical phantom where the elastic property changes at different angular positions. A syringe pump was used to generate variable pressure loading, which is synchronized to the motor driving signal. The experimental results show that the elastic displacements are detected to be different at different angular positions. The results of elastic properties detection in human artery will also be demonstrated.

9710-49, Session PSun
Micromotor OCT enables catheter-based assessment of vascular elasticity
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Here, we present the first catheter-based optical coherence elasticity measurement using a newly developed super fast intravascular optical coherence tomography (OCT) system. The system is based on a 1.5 MHz Fourier Domain Mode Locked laser and a 1.2 mm outer diameter motorized catheter. To detect the local elastic properties, the micro-motor is programmed to actuate the laser beam in a “step-by-step” mode at 1 revolution per second; which can potentially be increased to > 10 revolutions/s. The beam is scanned in a limited number (up to 50) of angular steps, at each of which the beam position is held stable. When the laser beam is stable, the phase difference across a variable number of A-lines can be computed to assess the elastic displacement. Choosing a proper window delay, local elastic tissue displacement and strain can be quantified based on the phase shift. We conducted ex-vivo experiments with a cylindrical phantom where the elastic property changes at different angular positions. A syringe pump was used to generate variable pressure loading, which is synchronized to the motor driving signal. The experimental results show that the elastic displacements are detected to be different at different angular positions. The results of elastic properties detection in human artery will also be demonstrated.

9710-36, Session 10
A study on the relation between tissue deformation and optical properties measured by multi-diameter fiber reflectance spectroscopy on an optical phantom
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Many forms of elastic scattering spectroscopy have been used in non-invasive disease diagnosis. Measurements with spectroscopy systems such as Single Fiber Reflectance spectroscopy (SFR) require direct contact between the probe and tissue. Previous studies have reported effects of the probe pressure on the measured spectrum [Reif et al., JBO 13(1), 2008] while it is difficult to compare and quantify the various results due to different spectroscopic techniques and pressure application geometries used. We believe it is the tissue deformation generated by the pressure application, rather than the pressure itself that influences the optical properties.

As the first step to study the relation between tissue deformation and the optical properties a silicone elastomer based flat-surface optical phantom will be made. Scattering will be included by adding titanium dioxide particles and silicon oil will be added to mimic tissue-like mechanical properties. Multi-diameter single fiber reflectance spectroscopy [Ute et al., OL 37, 2012] will be used to extract ? and ? s. The laboratory setup is designed to generate a unidirectional force that is perpendicular to the surface of the phantom. It is based on a motorized translation stage which will induce the deformation. A pressure sensor is included to measure the pressures induced on the phantom meanwhile.

In our study, we will measure the optical property changes of the phantom as a function of deformation and pressure and correlate these changes with tissue mechanical properties. This will give us insight of the connection between optical and mechanical property and help us to obtain a better understanding the relation between tissue deformation and optical properties.

Our group previously introduced Polarized Spatial Frequency Domain Imaging (PSFDI), a wide-field, reflectance imaging technique which we used to empirically map fiber direction in bovine pulmonary heart valve leaflets (PHVL) without optical clearing or physical sectioning of the sample. Presented is an extended analysis of our PSFDI results using an inverse Mueller matrix model of polarized light scattering that allows additional maps of fiber orientation distribution, along with instrumentation permitting increased imaging speed for dynamic PHVL fiber measurements.

We imaged PHVL with PSFDI, and then compared our inverse model results to the same leaflets optically cleared and imaged with small angle light scattering (SALS). We used our improved imaging speed to observe PHVL subjected to various loading conditions using a biaxial stretching device. The linearized data analysis algorithm produced maps of fiber orientation, fiber orientation orientation, attenuation, and birefringence of 2 megapixel PHVL image sets in under 10 seconds. The static PHVL images showed distinct regional variance of fiber orientation distribution, matching our SALS results. Our dynamic imaging experiment showed trackable changes in the fiber microstructure of PHVL under loading. Our new PSFDI analysis model and instrumentation allows characterization of fiber structure within heart valve tissues (as validated with SALS measurements), along with imaging of dynamic fiber remodeling. The experimental data will be used as inputs to our constitutive models of PHVL tissue to fully characterize these tissues’ elastic behavior, and has immediate application in determining the mechanisms of structural and functional failure in PHVLs used as bio-prosthetic implants.
Mechanisms of laser-induced stress relaxation for temporal and permanent reshaping of cartilage

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Cartilage is a rigid tissue with high internal stress and property of shape memorization.

Cartilage possesses also a property of temporal plasticity which allows changing its shape without dramatic alterations in permanent cartilage structure and mechanical properties.

Stress relaxation in cartilage is a basis of the prospective laser technologies of cartilage reshaping and regeneration. Laser reshaping of cartilage is applied for correction of nasal septum, epiglottis and ear deformities, manufacturing of cartilage implants for trachea surgery.

Laser-induced stress relaxation via modification of fine structure of cartilage may promote tissue regeneration and repair.

The aim of the paper is to consider various mechanisms of stress relaxation which provide permanent and temporal reshaping with stable or unstable effect of laser medical treatment.

Temporal effect can be achieved via bound-to free transition of water state, creating soluble crystals and non-stable pores which will be overgrown in the living tissue.

Permanent effect can be achieved via local modification of proteoglycan substructure and formation of stable pores. We have shown that positive ions in the tissue liquid play important role in the stabilization of laser-modified cartilage structure.

We develop two theoretical models describing stress relaxation (a) via pore formation, and (b) as a diffusion limited process.

The results of the experimental studies of the laser-induced stress relaxation and structural modification of cartilage using AFM, TEM, thermo-mechanical testing, and OCT elastometry will be presented. Finally, the recommendation for laser settings providing stable results in laser reshaping will be discussed.

Three-dimensional mapping of shear modulus using acoustic radiation force orthogonal excitation optical coherence elastography (ARFOE-OCE)

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In this study the shear wave induced by an orthogonal acoustic radiation force (ARF) is detected by an optical coherence tomography (OCT) Doppler variance method. The ARF remotely generates the vibration perpendicular to the OCT detection direction inside a tissue-equivalent phantom. The shear wave propagation in a two-dimensional (2D) plane perpendicular to the ARF excitation direction is visualized. Subsequently, we construct a three-dimensional (3D) map of the shear modulus after visualizing the shear wave propagation in a series of 2D planes. Benefiting from the approximate uniformity of the ARF along the ultrasound axial direction in a range of several millimeters, this method can map the shear modulus without movement of the ultrasound transducer and the use of the array transducer and, thus, reduces the system complexity. An orthogonal ARF excitation allows this method to measure the shear modulus at a deeper specimen, compared with previous parallel ARF excitation. Instead of the Doppler phase method, the use of OCT Doppler variance method sensitive to the perpendicular vibration can simplify the data processing without the phase wrapping correction. A homogeneous agar phantom and a two-layer agar phantom are detected by the ARFOE-OCE system, and the measured shear moduli are compared with the values from a mechanical test system. The results show that the ARFOE-OCE system has the ability to determine the 3D map of the shear modulus in a deeper phantom with a simpler system setup and data processing.

Speckle-free elasticity imaging with moving acoustic radiation force and phase-sensitive optical coherence tomography

Bao-Yu Hsieh, Shaozhen Song, Thu-Mai Nguyen, Soon
Phase-sensitive optical coherence tomography (PhS-OCT) can be utilized for quantitative shear wave elastography by speckle tracking. However, current approaches cannot directly reconstruct elastic properties in speckle-less or speckle-free regions, for example within the crystalline lens in ophthalmology. Investigating the elasticity of the crystalline lens could help the understanding and management of presbyopia pathologies related to biomechanical properties. We propose to reconstruct the elastic properties in speckle-less regions by sequentially launching shear waves with moving acoustic radiation force (mARF), and then detecting the displacement at a specific speckle-generating position with PhS-OCT. A linear ultrasound array (the center frequency is 5 MHz) interfaced with a programmable imaging system was designed to launch shear waves by mARF. The acoustic sources were electronically controlled and laterally translated to create shear waves, and PhS-OCT operating in M-B mode with a 125-kHz A-line rate was used for displacement detection by 1-D speckle tracking. Local displacements were calculated and stitched sequentially based on the distance between acoustic source and detection beam. Then, shear wave speed, and the associated elasticity map, were reconstructed based on a time-of-flight algorithm. In this study, partial speckle-free phantoms were made to demonstrate the capability of this imaging method to reconstruct the elastic properties of speckle-free regions. Results showed that harder inclusions within the speckle-free region could be detected, suggesting that this imaging method may be able to detect the elastic properties of the crystalline lens.

9710-44, Session 11

Characterizing tissue stiffness at the tip of a rigid needle using an all-optical force sensor

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Each year, in the Netherlands alone, more than 50,000 percutaneous procedures are performed for treatment or for removal of tissue from possibly diseased organs, of which 30% return non-diagnostic due to erroneous needle targeting, often as a result of non-homogeneity of the penetrated tissue. In this study, we aim to facilitate needle targeting by assessing the tissue in front of the needle based on its mechanical properties. A probe that can identify tissues via real-time measurements of their mechanical properties is placed at the tip of the needle. The probe, actuated by a remote system at the distal part of the needle, employs the bending of a micro-machined cantilever fabricated on top of an optical fiber. The displacement of the cantilever, imposed by pressing a micro-bead (r = 75 μm) glued at the tip of the cantilever against the tissue, is interrogated by Fabry-Pérot interferometry and converted to force acted on the tissue in real-time. The force transducer is able to perform in harsh environments due to its monolithic design and all-optical working principle. Using our setup, load-indentation curves were obtained during needle insertion in several gelatin-based specimens. We demonstrate the ability of our device to detect and quantify layers of varying stiffness and to successfully locate tissue boundaries in animal tissue embedded in gelatin. Furthermore, a diagnostic measurement can be made by quantifying intra-organ tissue stiffness at the needle target location.
9711-1, Session 1

**Quantitative long term measurements of burns in a rat model using spatial frequency domain imaging and laser speckle imaging (Invited Paper)**

Adrien Ponticorvo, Rebecca A. Rowland, Melissa L. Baldado, Gordon T. Kennedy, Rolf B. Saager, Bernard Choi, Anthony J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

The ability to accurately assess burn wound severity in a timely manner is a critical component of wound management as it dictates the course of treatment. While full thickness and superficial burns can be easily diagnosed through visual inspection, burns that fall in between these categories are difficult to classify. Additionally, the ability to better quantify different stages of wound healing from a burn of any severity would be important for evaluating the efficacy of different treatment options. Here we present a longitudinal (28 day) study that employs spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI) as non-invasive technologies to characterize in-vivo burn wounds and healing in a murine model. Burn wounds were created using an established technique of a brass comb heated to a given temperature and applied for a set amount of time. They were imaged immediately after the initial injury and then at 2, 4, 7, 14, 21, and 28 days following the injury. Biopsies were taken on the day of the injury in order to verify the extent of the burn damage as well as at different time points after the injury in order to visualize different stages of inflammation and healing. The results of this study suggest that the reduced scattering coefficient measured using SFDI and blood flow as measured using LSI have the potential to provide useful metrics for quantifying the severity of burn injuries as well as track the different stages associated with wound healing progression.

9711-2, Session 1

**Lasing within live cells for barcode-type cell tagging and tracking**

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Laser light has become an indispensable tool in today’s biomedical research with applications ranging from spectroscopy and in vivo imaging to biosensing and surgery. However, direct generation of laser light by or within biological samples has so far required macroscopic external resonator structures or has relied on process of random lasing.

Here, we demonstrate that microscopic whispering gallery mode (WGM) resonators doped with a fluorescent dye can generate laser light within individual, freely migrating, live cells. By using confocal fluorescence microscopy we prove that the micron-sized resonators are fully internalized by a great number of cell types via the natural process of endocytosis. On pumping the internalized WGM resonators with nanojoule light pulses, green laser emission is generated inside the cells.

Characteristic features in the lasing spectrum of our cell laser provide each cell with a unique barcode-type tag. Importantly, the spectral information can be collected by applying only a single pump pulse, thus making this technique compatible with high throughput detection schemes. To demonstrate the applicability of the intracellular laser tags, we performed cell tracking experiments over extended time periods and analysed the corresponding changes in the laser characteristics. Finally, we describe an easy and reliable method to increase the uptake efficiency of WGM resonators by non-phagocytic cells.

9711-3, Session 1

**Time evolution of trapped single cell microorganism**

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The combination of optical tweezers and Raman spectroscopy is called Raman tweezers. A single focused beam is utilized as an excitation source and also as a source for generation of an optical trap. Raman tweezers have been recently used in variety of bio-applications as a very useful tool for their non-contact and non-destructive features.

Using Raman tweezers allows us to follow the response of cells on external stimuli in the real time. Based on 2D correlation analysis we are able to observe reactions of each biomolecules inside of cells. Our work is focused on the time-course detection of the metabolic process within cells exposed to different types of stress. As an investigated candidate bacterial strains which are capable of accumulation of very high amounts of polyhydroxyalkanoates (PHAs) in cytoplasm were selected. For this purpose, we have utilized bacterial strain of Cupriavidus necator.

The information contained in a series of dynamic spectra, could be shown by correlation analysis and can help to describe the base of dynamic process inside cells. Dynamic processes can be caused by various changes like a nutrition process or for example a thermal effect.

9711-4, Session 1

**Live cell phase imaging under whole blood shear flow using oblique back-illumination**

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A focus of transplant immunology research is observing endothelial cell (EC) interactions with immune cells in the presence of immune modulators in flowing blood. Current optical imaging modalities used to observe these interactions (Zernike phase contrast (PC) or differential interference contrast (DIC) microscopy) have limited performance when imaging ECs under physiological whole blood flow conditions due to unfavorable optical properties of erythrocytes. Oblique back-illumination microscopy (OBM) provides high-contrast phase-gradient images of near-surface structures with such strongly scattering backgrounds. Here we present an OBM setup for imaging ECs under physiological flow and shear conditions using a
commercial flow chamber.

The imaging setup comprises a commercial inverted microscope outfitted with a pair of optical fibers delivering strobed illumination from near-infrared light emitting diodes (NIR LEDs). 3D-printed fiber guides mounted to a 10x phase contrast objective position the optical fibers in a range of positions and incidence angles. OBM and PC images were obtained of a monolayer of adherent cells under physiological whole blood flow conditions. The influence of illumination fiber incidence angle, fiber separation, flow chamber depth, and illumination wavelength on OBM image quality was assessed.

Our findings show that Zernike phase contrast microscopy cannot image through more than tens of micrometers of whole blood due to high optical scattering and absorption. In contrast, OBM produces high-quality images of adherent cells and red blood cells (RBCs) under 20 dynes/cm2 shear stress and 0.3 – 18 mL/min blood flow in commercial flow chambers of heights 100 – 800 µm, respectively. These results suggest that OBM has the potential to be used instead of Zernike for observing EC interactions with immune cells.

9711-5, Session 1

Investigation of cell-matrix interactions in ovarian cancer via multiphoton excited fabrication of 3D image-based biomimetic stromal models

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A profound remodeling of the extracellular matrix (ECM) occurs in human ovarian cancer but it unknown how this affects tumor growth, where this understanding could lead to better diagnostics and therapeutic approaches. We investigate the role of these ECM alterations by using multiphoton excited (MPE) polymerization to fabricate biomimetic models to investigate operative cell-matrix interactions in invasion/metastasis.

First, we create nano/microstructured gradients mimicking the basa lamina to study adhesion/migration dynamics of ovarian cancer cells of differing metastatic potential. We find a strong haptotactic response that depends on both contact guidance and ECM binding cues. While we found enhanced migration for more invasive cells, the specifics of alignment and directed migration also depend on cell polarity. We further use MPE fabrication to create collagen scaffolds with complex, 3D submicron morphology. The stromal scaffold designs are derived directly from “blueprints” based on SHG images of normal, high risk, and malignant ovarian tissues. The models are seeded with different cancer cell lines and this allows decoupling of the roles of cell characteristics (metastatic potential) and ECM structure and composition (normal vs cancer) on adhesion/migration dynamics.

We found the malignant stroma structure promotes enhanced migration and proliferation and also cytoskeletal alignment. Creating synthetic models based on fibers patterns further allows decoupling the topographic roles of the fibers themselves vs their alignment within the tissue. These models cannot be synthesized by other conventional fabrication methods and we suggest the MPE image-based fabrication method will enable a variety of studies in cancer biology.

9711-6, Session 1

Quantitative imaging of light-triggered DOXorubicin release using spatial frequency domain imaging

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The efficacy of chemotherapy is related to the concentration of drug that reaches tumor sites. Doxorubicin (DOX) is a common anti-cancer drug that is also approved for use in liposomal form for the treatment of ovarian cancer. We recently developed a porphyrin-phospholipid (PoP)-liposome system that enables on demand release of DOX from liposomes using near infrared irradiation to improve DOX bioavailability. Owing to its intrinsic fluorescence, it is possible, and desirable, to quantify DOX concentration and distribution, preferably noninvasively. Here we quantified DOX distribution following light-triggered drug release in phantoms and an animal cancerous using spatial frequency domain imaging. This study demonstrates the feasibility of non-invasive quantitative mapping of DOX distributions in target areas.

Fluorescence lifetime imaging of NAD(P)H measures metabolic enzyme activity in cells

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The fluorescence lifetime of the metabolic co-enzyme NAD(P)H provides a label-free measure of cellular metabolism. NAD(P)H has a two-exponential lifetime decay due to its free and protein-bound forms, which have a short and long lifetime, respectively. However, the biochemical significance of changes in the bound lifetime of NAD(P)H remains unclear. In order to address this issue, the lifetime of NAD(P)H bound to specific enzymes were measured using two-photon microscopy and time-correlated single photon counting. Our hypothesis is that the bound NAD(P)H fluorescence lifetime is influenced by preferred protein binding was supported by measuring the NAD(P)H lifetime in human breast cancer cells (BT474).

BT474 cells were treated with FX11, a competitive inhibitor of NADH binding to lactate dehydrogenase A, which caused a significant decrease in the long lifetime of NAD(P)H in the cell cytoplasm versus untreated controls after 24 hours (p<0.05). Dichloroacetate, a small molecule activator of pyruvate dehydrogenase, caused a significant increase in the long lifetime of NAD(P)H in BT474 cells (p<0.05) after 24 hours. These inhibitors did not cause a significant change in the non-cancerous mammary epithelial cell line MCF-10A, up to 48 hours. These results confirm that changes in the protein-bound lifetime of NAD(P)H may be attributed to changes in the activity of enzymes that bind NAD(P)H, and that the bound NAD(P)H lifetime could provide a label-free method to probe changes in the activity of specific metabolic pathways.

Detection of particle flow patterns in tumor by directional spatial frequency analysis

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Drug delivery to tumors is well known to be chaotic and limited, partly from dysfunctional vasculature, but also because of microscopic regional variations in composition. Modeling the transport of nanoparticle therapeutics, therefore must include not only a description of vascular permeability, but also of the movement of the drug as suspended in tumor
interstitial fluid (TIF) once it leaves the blood vessel. Understanding of this area is limited because we currently lack the tools and analytical methods to characterize it. We have previously shown that directional anisotropy of drug delivery can be detected using Directional Fourier Spatial Frequency (DFSFS) Analysis. Here we extend this approach to generate flow line maps of nanoparticle transport in TIF relative to tumor ultrastructure, and show that features of tumor spatial heterogeneity can be identified that are directly related to local flow isometries. The identification of these regions of limited flow may be used as a metric for determining response to therapy, or for the optimization of adjuvant therapies such as radiation pre-treatment, or enzymatic degradation.

9711-9, Session 1
Forensic applications of in vivo Raman spectroscopy: determination of post-mortem interval in animal models
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The determination of the post-mortem-interval (PMI) is critical to any murder investigation. Existing techniques such as evaluation of drop in body temperature, formation of hypostasis and lividity, muscle relaxation, cadaveric spasm and blood coagulation are subjective, time consuming and can be performed only in a laboratory, resulting in delayed and less effective investigation. The precision of determining PMI using these traditional techniques is 25 hours for the first 100 hours. Thus, there is a need for rapid, objective techniques that can determine PMI at the site of crime. Several groups have explored spectroscopic techniques for the determination of PMI. Ping et.al. have reported PMI determination using DNA degradation in tissues probed by Raman microspectroscopy in the range of 48-72 hours using kidney and liver tissue - that would require dissection of the body. In the current study, we evaluate the possibility of determining PMI using in vivo Raman spectroscopy. The study, we acquired spectra from thigh of same mice (n=5) immediately after death by cervical dislocation, 5 hours after death and 24 hours after death. We analyzed the data using Principal-Component-Analysis (PCA) and Principal-Component-Linear-Discriminant-Analysis (PC-LDA). PCA showed clusters of 0, 5 and 24 hours PMI spectra, although slight overlap was observed between these groups. Using PC-LDA, 0, 5 and 24 hours PMI spectra could be classified with 78, 67 and 67% efficiency respectively after cross validation. Results suggest feasibility of determining PMI using in vivo Raman spectroscopy within the range of 5 hours.

9711-10, Session 2
An integrated optofluidic platform for assessing biologics
Perry Schein, Dakota O’Dell, David Erickson, Cornell Univ. (United States)

Protein therapeutics are a rapidly growing portion of the pharmaceuticals market and have many significant advantages over traditional small molecule drugs. As this market expands, however, critical regulatory and quality control issues remain, most notably the problem of protein aggregation. Individual target proteins often aggregate into larger masses which trigger an immune response in the body, which can reduce the efficacy of the drug for its intended purpose, or cause serious anaphylactic side-effects. Although detecting and minimizing aggregate formation is critical to ensure an effective product, aggregation dynamics are often highly complicated and there is little hope of reliable prediction and prevention from first principles. This problem is compounded for aggregates in the sub-visible range of 100 nm to 10 micrometers where traditional techniques for detecting aggregates have significant limitations. Here, we present an integrated optofluidic platform for detecting nanoscale protein aggregates and characterizing interactions between these aggregates and a reference surface. By delivering light to a solution of proteins with an optical waveguide, scattered light from individual protein aggregates can be detected and analyzed to determine the force profile between each particle and the waveguide surface. Unlike existing methods which only determine size or charge, our label-free screening technique can directly measure the surface interaction forces between single aggregates and the glass substrate. This direct measurement capability may allow for better empirical predictions of the stability of protein aggregates during drug manufacturing and storage.

9711-11, Session 2
Elasticity measurements to evaluate optical damages induced in red blood cells optically trapped using near-infrared lasers
Marcos A. S. de Oliveira, Diogenes S. Moura, Adriana Fontes, Renato E. de Araujo, Univ. Federal de Pernambuco (Brazil)

Optical tweezers have been used to trap and manipulate individual cells and also to measure their mechanical properties. In this work, we evaluated the damages on red blood cells (RBCs) optically trapped by near-infrared lasers beams operating at 785 nm or 1064 nm. Near-infrared (NIR) lasers beams have been used to a variety of biophotonic applications, such as Raman micro-spectroscopy. A homemade computer controlled optical tweezers system was setup in a microscope equipped with a CCD camera and a motorized XY stage. After trapping, the cell was maintained under NIR illumination for 1 or 2 minutes and its elasticity was than evaluated. In our usual experiments with RBCs, the association of the optical tweezers system and image processing techniques allows rapid (20 s) and reproducible evaluation of cell deformability, without damages. RBCs were obtained from healthy volunteers and diluted in their own donor serum. On the evaluation of optical damages a significant number (tens of hundreds) of cells were analyzed by the optical trapping. Our results have revealed an increase up to around 50% on RBCs rigidity, after 1 minute of 10 mW optical trapping, and about 100% for 2 minutes, for the 785 nm wavelength. The measurements provided evidence that RBCs have their biomechanical properties strongly affected by the NIR radiation, depending on the time of trapping. We also observed significant changes as a function of the wavelength. Our results establish new limits for RBC evaluation by laser techniques.

9711-12, Session 3
Evaluation of local MSC therapeutic impact in osteogenesis imperfecta using deep tissue single cell ablation with a multicolor femtosecond fiber laser source
Kayvan F. Tehrani, The Univ. of Georgia (United States); Charles P. Lin, Wellman Ctr. for Photomedicine (United States); Luke J. Mortensen, The Univ. of Georgia (United States)

Osteogenesis imperfecta (OI) is a congenital disease where a mutation in the gene encoding collagen type I reduces bone deposition yielding weak brittle bones. One potential therapeutic strategy is the transplantation of mesenchymal stem cells (MSCs). MSCs are able to differentiate into cells that generate bone, cartilage, and fat. When culture expanded, they are promising as therapeutics for inflammatory and bone diseases. For inflammatory disease, secreted factors released in the first 24-48 hours of transplantation are thought to be responsible for the therapeutic impact. However, for congenital bone diseases like OI, healthy donor cell integration and contribution to bone might be important. Although MSC therapy
for OI is promising, it is unclear if the transplanted cells function by local
contribution to bone or by a systemic response.
To evaluate the local impact of transplanted MSCs on bone deposition, we
established an intravital microscope using a multicolor femtosecond laser
for 2 photon imaging and ablation. The 775 nm and 960 nm excitation
pulses allow simultaneous imaging of second harmonic generation,
labeled MSCs, and recipient cells. Using the 775 nm line, we determined
the ablation threshold for cell knockout at range of depths in the bone
marrow and evaluated damage to the surrounding tissue by quantifying
autofluorescence and longitudinally tracking cell morphology. MSCs were
transplanted in OI mice, and half of the MSCs in the skull were ablated, with
local bone thickness measured to determine local cell contribution. Results
suggest future MSC targeting for bone formation.

9711-13, Session 3

Monitoring stem cell differentiation in
phase contrast imaging
Katherine P. Dempsey, KP Lam, Keele Univ. (United Kingdom)
Understanding the mechanisms behind the proliferation of Mesenchymal
Stem cells (MSCs) can offer a greater insight of the behaviour of these cells
throughout their life cycles. Traditional methods of determining the rate of
MSC differentiation rely on population based studies over an extended time
period. However, such methods can be inadequate as they are unable to
track cells as they divide; in autologous cell therapies for osteoarthritis, for
example, the development of biological assays that predict in vivo functional
activity and biological action are particularly challenging. Here further
research is required to define non-histochemical biomarkers which provide
correlations between cell survival and predictive functional outcome. This
paper proposes a method combining Canny edge detection with advanced
texture-based analysis to facilitate cells tracking in in vitro, time-lapsed
microscopy. The effectiveness of this technique was examined in the context
of unlabelled, phase contrast (PC) imaging, with the goal of tracking and
labeling stem cells as they differentiate. The results obtained are analysed
and compared with existing methods including the typical fluorescent-
based approaches of cell labelling. In addition to achieving greater accuracy,
early results show that the proposed algorithm offers the distinct advantage
that it is amendable to real-time implementation.

9711-14, Session 3

Cell sheets image validation of phase-
diversity homodyne OCT and effect of the
light irradiation on cells
Naoko Senda, Kentaro Osawa, Hitachi, Ltd. (Japan)
Optical coherence tomography (OCT) is one of powerful 3D tissue imaging
tools with no fluorescence staining. We have reported that Phase-Diversity
Homodyne OCT developed in Hitachi could be useful for non-invasive
regeneration tissue evaluation test. The OCT enables cell imaging because
of high resolution (axial resolution: 2.6 μm, lateral resolution: ~1 μm in the
air), whereas conventional OCT was not used for cell imaging because of
low resolution (10-20 μm). Furthermore, the OCT has advantage over other
3D imaging devices in cost because the light source and the objective were
originally used as an optical pickup of compact disc.
In this report, we aimed to assess effectiveness and safety of Phase-
 Diversity Homodyne OCT cell imaging. Effectiveness of OCT was evaluated
by imaging a living cell sheet of human oral mucosal epithelial cells. OCT
images were compared with reflection confocal microscopy (RCM) images,
because confocal optical system is the highest resolution (<1 μm) 3D in vivo
imaging technique. Similar nuclei images were confirmed with OCT and
RCM, which suggested the OCT has enough resolution to image nuclei inside
a cell sheet. Degree of differentiation could be estimated using OCT images,
which becomes possible because the size of cells depends on distribution of
differentiation. Effect of the OCT light irradiation on cells was studied using
NIH/3T3 cells. Light irradiation, the exposure amount of which is equivalent
to OCT, had no impact on cell shape, cell viability, and proliferation rate. It
suggested that the light irradiation has no cell damage under the condition.

9711-15, Session 3

Non-disruptive measurement system of
cell viability in bioreactors containing
artificial vascular networks
Florian Rudek, Bryan L. Nelsen, Westsächsische
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Westsächsische Hochschule Zwickau (Germany)
and Fraunhofer IWS Dresden (Germany); Alexander
Kabardiadi, Westsächsische Hochschule Zwickau
(Germany); Peter Hartmann, Westsächsische Hochschule
Zwickau (Germany) and Fraunhofer IWS Dresden
(Germany)
Current bioreactor technology used to grow viable tissue cultures for drug
testing has a fundamental limit to the thickness of tissue it can sustain.
Nutrient and oxygen delivery, as well as removal of metabolic waste through
osmosis can only sustain a tissue sample thickness of about 0.2 μm. To aid
in nutrient delivery, and therefore produce more physically realistic tissue
cultures, newer bioreactor technology is utilizing artificial vascular networks.
This work is designed to test the efficacy of such artificial vascular networks
by non-disruptively measuring cell viability through certain natural markers
produced within the cell during normal metabolic processes.
Nicotinamide adenine dinucleotide (NADH/NADPH) is a coenzyme naturally
consumed and produced during a cell's metabolic processes and has
thoroughly been studied as a means to determine the metabolic state
of a cell. Measuring the fluorescence of NADH within the cell provides
a non-disruptive marker for measuring cell viability. Since the measurement
process is optical in nature, NADH fluorescence also provides a pathway
for sampling at different measurement depths within a given tissue
sample. The measurement system itself utilizes a specially designed fiber
with a channel for excitation laser delivery and independent channels for
fluorescence collection. The signal is then spectrally filtered and delivered
to a silicon photodetector array which provides high detection efficiency, a
time resolution of 600 ps, and a spatial resolution of 4x4 pixels for faster
measurement throughput. The system developed in this study should be
scalable to large-scale operations for drug-tissue interaction testing.

9711-16, Session 3

Confocal microscopy and
electrophysiological study of single
patient corneal endothelium cell cultures
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Pugliese, Univ. degli Studi di Firenze (Italy); Roberto Pini,
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The characterization of the ion channels in corneal endothelial cells and the
elucidation of their involvement in corneal pathologies would lead to the
identification of new molecular target for pharmacological treatments and
the clarification of corneal physiology. The corneal endothelium is an
amitotic cell monolayer with a major role in preserving corneal transparency
and in regulating the water and solute flux across the posterior surface of
the cornea. Although endothelial cells are non-excitable, they express a range of ion channels, such as voltage-dependent Na+ channels and K+ channels, L-type Ca2 channels and many others. Interestingly, purinergic receptors have been linked to a variety of conditions within the eye but their presence in the endothelium and their role in its pathophysiology is still uncertain. In this study, we were able to extract endothelial cells from single human corneas, thus obtaining primary cultures that represent the peculiarity of each donor. Corneas were from tissues not suitable for transplant in patients. We characterized the endothelial cells by confocal microscopy, both within the intact cornea and in the primary endothelial cells cultures. We also studied the functional role of the purinergic system (adenosine, ATP and their receptors) by patch clamp recordings and confocal time-lapse microscopy. Our results indicate that the application of purinergic compounds modulates the amplitude of outward, voltage-dependent, membrane currents of the isolated endothelial cells. These findings may lead to the proposal of new therapies for endothelium-related corneal diseases.

9711-17, Session 3
Filamentation and spatiotemporal distribution of extracellular polymeric substances: role on X.fastidiosa single cell adhesion and biofilm formation
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Biofilms can be defined as a community of microorganisms attached to a surface, living embedded in a self-produced matrix of hydrated extracellular polymeric substances (EPS) which comprises most of the biofilm mass. We have recently used an extensive pool of microscopy techniques (confocal fluorescence, electron and scanning probe microscopies) at the micro and nanoscales in order to create a detailed temporal observation of Xylella fastidiosa biofilm formation, using both wild type strain and Green Fluorescent Protein (GFP)-modified cells of this citrus phytopathogen. We have identified three different EPS compositions, as well as their spatial and temporal distribution from single cell to mature biofilm formation stages. In the initial adhesion stage, soluble-EPS (S-EPS) accumulates at cell polar regions and forms a surface layer which facilitates irreversible cell attachment and cell cluster formation. These small clusters are subsequently connected by filamentous cells; further S-EPS surface coverage facilitates cell attachment and form filaments, leading to a floating framework of mature biofilms. The important role of EPS in X.fastidiosa biology was further investigated by immunolabelling experiments to detect the distribution of XadA1 adhesin, which is expressed in early stages of biofilm formation and released in outer membrane vesicles. This protein is located mainly in S-EPS covered areas, as well as on the filaments, indicating a molecular pathway to the enhanced cell attachment previously observed. These results suggest that S-EPS may thus represent an important target for disease control, slow plant colonization by the bacteria, keeping the plant more productive in the field.

9711-19, Session 3
Movement of bacteria in urban microfluidics: a method for biosimulation of complex traffic
Viola Tokarova, McGill Univ. (Canada); Ben Libberton, Univ. of Liverpool (United Kingdom); Ondrej Kaspar, McGill Univ. (Canada); Sylvain Martel, Ecole Polytechnique de Montréal (Canada); Dan V. Nicoula, McGill Univ. (Canada)
No Abstract Available

9711-18, Session PMon
Measurement of muscle food spoilage using fluorescence imaging
Binlin Wu, Southern Connecticut State Univ. (United States)

Safety and quality of muscle foods such as meat and poultry have been a major concern for public dietary health considerations. However, the current techniques are time-consuming and labor-intensive. A device that can detect and measure the degree of food spoilage rapidly and accurately is highly desired. In this study, a conventional fluorescence microscope equipped with a digital color camera is employed to evaluate the technique for meat spoilage detection using fluorescence imaging. Fresh meat samples stored at different temperatures are measured by the fluorescence microscope at successive storage times which correspond to a decay in meat freshness. Selective wavelengths are used for excitation. Fluorescence images due to intrinsic tissue emission are acquired in three color channels (RGB) of the digital camera. Multivariate data analysis algorithm such as nonnegative matrix factorization (NMF) is used to unmix the fluorescence signal due to different native fluorophores such as collagen, elastin, reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). Relative concentrations of these native fluorophores are retrieved to correlate with the degree of spoilage over time. Support vector machine (SVM) is used to classify different types of meat samples such as fresh meat, semi-fresh meat and spoiled meat. The method may potentially be extended to design a portable device which utilizes a conventional color camera such as a cell phone camera for data acquisition and analysis, and achieve spoilage detection in situ.

9711-53, Session PMon
Enhancement of trastuzumab penetration using atorvastatin and cyclophosphamide to Her2+ NCI N87 xenograft mouse model
Jin Su Kim, Kyung Deuk Cho, Kook-Hyun Yu, Korea Institute of Radiological & Medical Sciences (Korea, Republic of)

To investigate the effect of atorvastatin and cyclophosphamide for enhancement of Alexa Fluor 488 conjugated trastuzumab on the accumulation and the microdistribution of Alexa 488 trastuzumab. Groups of nude mice (n = 4-5 / group) were inoculated with Her2 positive NCI-N87 tumor cells. When the tumor size reached ~200 mm3, Alexa-488- Trastuzumab was intravenously injected single dose (150 Î¼g) for control and cyclophosphamide (70 mg/kg) were intraperitoneally injected, and 12 μg/day of atorvastatin in a 0.2 ml volume (roughly equivalent to 40 mg/day in human treatment) was administered via oral gavage. Tumors were harvested, flash frozen and sliced (8 μm) 3 days after Alexa 488 trastuzumab injection. Microscopic imaging was performed using a EVOS FL auto cell imaging system. The penetration of Alexa-488-trastuzumab up to ~80 μm depth from the vessel and tumor surface was calculated by the area-under-curve (AUC) analysis. Circularity and fractal dimension were measured to identify the difference of morphological changes after treatment between groups. Overall micro-distribution of vessels was also assessed in terms of vascular density. Accumulation of Alexa-488-trastuzumab per tissue area was 1.72 ± 0.01 AU/µm2 for the control group, 3.10 ± 0.05 AU/µm2 for cyclophosphamide treatment group, and 2.76 ± 0.02AU/µm2 for atorvastatin treatment group. The accumulation of Alexa-488-trastuzumab was 80.1% or 60.1% more when cyclophosphamide or atorvastatin was administered, respectively, than the
control group (P < 0.0001). The penetration of Alexa-488-trastuzumab to 80-μm was 15% or 100% higher when cyclophosphamide or atorvastatin was administered, respectively, than the control group (P < 0.0001).

Circularity near the vessel was significantly lower than the circularity far from the vessel for both the cyclophosphamide and atorvastatin groups (P < 0.05). There was no difference of circularity for the control group. The fractal dimension near the vessel was significantly higher than that of the far field region from the vessel for both the cyclophosphamide and atorvastatin treatment groups (P < 0.05). There was no difference of vascular density among the groups.

Co-administration of cyclophosphamide or atorvastatin with Alexa-488-trastuzumab resulted in significantly improved trastuzumab antibody penetration in the near field region from the vessel.

9711-54, Session PMon

PCA-HOG symmetrical feature based diseased cell detection

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A histogram of oriented gradient feature (HOG) is applied to the field of diseased cell detection, which can detect diseased cells in high resolution tissue images rapidly, accurately and efficiently. Firstly, motivated by symmetrical cellular forms, a new HOG symmetrical feature based on the traditional HOG feature is proposed to meet the condition of cell detection. Secondly, considering the high feature dimension of traditional HOG feature leads to plenty of memory resources and long runtime in practical applications, a classical dimension reduction method called principal component analysis (PCA) is used to reduce the dimension of high-dimensional HOG descriptor. Because of that, computational speed is increased greatly, and the accuracy of detection can be controlled in a proper range at the same time. Thirdly, support vector machine (SVM) classifier is trained with PCA-HOG symmetrical features proposed above. At last, practical tissue images is detected and analyzed by SVM classifier.

In order to verify the effectiveness of this new algorithm, it is practically applied to conduct diseased cell detection which takes 200 pieces of H&E-Thematoxylin & eosin high resolution staining histopathological images collected from 20 breast cancer patients as a sample. The experiment shows that the average processing rate can be 25 frames per second and the detection accuracy can be 92.1%.

9711-55, Session PMon

Turn-and-run of crawling cells mediated by membrane ruffling with two different time scales

Taeseok D. Yang, Youngwoon Choi, Kyong-Jin Lee, Korea Univ. (Korea, Republic of)

The movement of a crawling cell is driven by extension of the actin cytoskeleton showing a ruffling motion at a leading edge. A typical characteristic of the ruffling is well illustrated by a coordinated cycle of plasma membrane protrusion, adhesion, and the release of the preceding adhesion sites. In this study, by using microglial cells extracted from a central nervous system (CNS) of a rat, we found that periodic membrane ruffling with two different time scales was involved in crawling and turning of the freely crawling cells. First, the periodic membrane ruffling, which is spatially biased toward a turning direction, by actin dynamics with a short time scale (about 6 seconds) was observed to determine the turning direction of a cell. Second, the dorsal fin ruffling, of which the wave is generated at the cell body, by myosin contraction with a long time scale (about 2 minutes) was observed and led to progression of the cell body. Interestingly, two types of periodic membrane ruffling were similarly observed for cells of which turning motions were inhibited on a micro-patterned 1-D substrate. This implies that the periodic ruffling drives an active cell migration and thus mediates the turn-and-run motions of crawling cells.

9711-56, Session PMon

Automated particle identification through regression analysis of size, shape and colour

Juan Carlos Rodriguez Luna, Jonathan Cooper, Steven Neale, Univ. of Glasgow (United Kingdom)

Rapid point of care diagnostic tests and tests to provide therapeutic information are now available for a range of specific conditions from the measurement of blood glucose levels for diabetes to card agglutination tests for parasitic infections. Due to a lack of specificity these test are often then backed up by more conventional lab based diagnostic methods for example a card agglutination test may be carried out for a suspected parasitic infection in the field and if positive a blood sample can then be sent to a lab for confirmation. The eventual diagnosis is often achieved by microscopic examination of the sample, this paper we propose a computerized vision system for aiding in the diagnostic process.

We will show the detection and classification of different types of cells in a diluted blood sample using regression analysis of their size, shape and colour. The first step is to define the objects to be tracked by a Gaussian Mixture Model for background subtraction and binary opening and closing for noise suppression. After subtracting the objects of interest from the background the next challenge is to predict if a given object belongs to a certain category or not. This is a classification problem, and the output of the algorithm is a Boolean value (true/false). As such the programme should be able to “predict” with reasonable level of confidence if a given particle belongs to the kind we are looking for or not. We show the use of a binary logistic regression analysis with three continuous predictors: size, shape and color histogram.

9711-57, Session PMon

Label-free separation of arteries and veins using two-photon excitation autofluorescence microscopy

Wei Zheng, Shenzhen Institute of Advanced Technology (China)

Optical imaging and separating of arteries and veins is definitely important for assessment, diagnosis and treatment of blood vessel related diseases. Many morphological and biochemical characteristics of blood vessels have been employed to identify vessel types, such as the anatomic difference, the flow velocity fluctuation of blood, and the variety of reflection colors. Unfortunately, the success of these strategies greatly depends on the individual experience of experimenter. There is a lack of obvious biomarker which could be used to solely identify the vessel types without human experience. Fluorescence microscopy is a prevailing technique. Elaborate design of the staining method has the potential to distinguish different blood vessels. However, to our knowledge, no studies have reported that the arteries and veins could be identified utterly based on the autofluorescence of blood vessel itself. Our previous studies have reported that the two-photon excited autofluorescence of hemoglobin could be applied to image vascular network. In this study, we keep on that research. We find the autofluorescence signal of elastin fiber in arterial wall provides a remarkable contrast mechanism to identify arteries and veins. Therefore, we could simultaneously image and separate arteries and veins using two-photon excitation microscopy without exogenous fluorescent agent. This method is objective and effective. Using this method, the two-photon excited autofluorescence microscopy of arterial network and venous network in mouse dorsal skin and mouse cortex will be presented.
could provide diagnostic information that is not possible with current viscoelastic characterization of red blood cell properties at the microscale. Properties and haemoglobin content at the microscale are observed. The removal of haemoglobin from the red blood cells. Changes in both viscoelasticity and mechanical properties have been induced in a number of diseases such as sickle cell anaemia and malaria. For both of these applications, maintaining the mechanical integrity of the red blood cells is critical to their long-term survival in circulation. Although techniques such as atomic force microscopy (AFM) and aspiration have been applied, there is currently not an accepted mechanical characterization technique for red blood cells. In this work, we apply Brillouin spectroscopy to characterize the mechanical properties of red blood cells and red blood cell ghosts. Brillouin spectroscopy involves inelastic coupling of light with phonons (sound waves) and enables non-contact viscoelastic characterization of samples at the microscale. Recent advances in instrumentation have allowed us to develop a Brillouin micro-spectroscopy system capable of measuring spectral shifts on the order of 100s of MHz. The Brillouin measurements can be combined with Raman spectroscopy to simultaneously determine biochemical characterization such as the presence of haemoglobin. Red blood cells are ghosted using standard techniques that remove haemoglobin from the red blood cells. Changes in both viscoelastic and mechanical properties and haemoglobin content at the microscale are observed. The viscoelastic characterization of red blood cell properties at the microscale could provide diagnostic information that is not possible with current techniques.

Characterization of red blood cells using Brillouin spectroscopy

Sandra C. Bustamante-Lopez, Swansea Univ. (United Kingdom) and Texas A&M Univ. (United States); Zhaokai Meng, Vladislav V. Yakovlev, Texas A&M Univ. (United States); Kenith E. Meissner II, Swansea Univ. (United Kingdom)

Erythrocytes, or red blood cells, transport oxygen to and carbon dioxide from the body’s tissues and organs. Red blood cell mechanical properties are altered in a number of diseases such as sickle cell anaemia and malaria. Additionally, modified red blood cell ghosts are being considered as a long-term, biocompatible carrier for drug delivery and for blood analyte sensing. For both of these applications, maintaining the mechanical integrity of the red blood cells is critical to their long-term survival in circulation. Although techniques such as atomic force microscopy (AFM) and aspiration have been applied, there is currently not an accepted mechanical characterization technique for red blood cells. In this work, we apply Brillouin spectroscopy to characterize the mechanical properties of red blood cells and red blood cell ghosts. Brillouin spectroscopy involves inelastic coupling of light with phonons (sound waves) and enables non-contact viscoelastic characterization of samples at the microscale. Recent advances in instrumentation have allowed us to develop a Brillouin micro-spectroscopy system capable of measuring spectral shifts on the order of 100s of MHz. The Brillouin measurements can be combined with Raman spectroscopy to simultaneously determine biochemical characterization such as the presence of haemoglobin. Red blood cells are ghosted using standard techniques that remove haemoglobin from the red blood cells. Changes in both viscoelastic and mechanical properties and haemoglobin content at the microscale are observed. The viscoelastic characterization of red blood cell properties at the microscale could provide diagnostic information that is not possible with current techniques.

Plasmonic enhanced whispering gallery mode biosensor using nanostructure

Seunghun Lee, Pusan National Univ. (Korea, Republic of)

High-quality (Q) factor whispering gallery mode (WGM) biosensors derive their unprecedented sensitivity from monitoring WGM wavelength shifts induced by biomolecules binding at sites of highly confined field intensities. Field strengths can be further amplified by introduction of metallic nanantennas which localize a fraction of the WGM field at analyte binding sites. Here we demonstrate this approach for single molecule detection by coupling WGMs to gold nanopost-antenna arrays on a planar substrate. We estimate the energy fraction of the microcavity field that localizes at the antenna site and we demonstrate sensitive single molecule detection over a wide frequency range by co-localizing field and analyte at the nanopost.
9711-63, Session PMon

Dielectrophoresis measurement of red blood cells (RBCs) exposed to oxidative stress using optical tweezer and microfluidic chip

HeeJae Jeon, Jong Jin Kim, Dae Sung Yoon, Beop-Min Kim, Korea Univ. (Korea, Republic of)

Red Blood Cells (RBCs) function as oxygen carriers for maintaining important vital activities with the human circulatory system, thus RBCs dysfunction has been suggested as a crucial risk factor for deadly disease. Stored RBCs show progressive structural and functional deteriorations including a decline in deformability and dielectrophoretic (DEP) force during blood banking/storage, which causes a malfunction in blood circulations and homeostasis. It may lead to clinical complications and adversely affect patient mortality. In this paper, we report that the Captopril has the protective and pharmaceutical effect on RBCs exposed to oxidative damage with hydrogen peroxide. The concentration of reactive oxygen species (ROS) has the important physiological roles in regulating normal cell functions. The Captopril acting as a membrane permeable thiol reducing agent on RBCs enhances preservation and recovery of damaged RBCs by potentiating the precise regulation of cellular ROS activity. We used optical tweezers with a micro-electrode device to accurately measure the quantified DEP forces of RBCs under various conditions. The DEP force is reported as an important physical factor that is known to be closely related to the RBC deformability often associated with freshness of the RBCs. Experiment results reveal that the Captopril is a very good protector from oxidative damages on the RBCs and accelerates the recovery of damaged RBCs treated by it. Accordingly, our application can be a promising diagnostic tool that effectively monitors the freshness of the stored RBCs and the efficiency of added drug for recovering the damaged RBCs.

9711-64, Session PMon

Diagnosis and management of hand arthritis using a mobile medical application

Fartash Vasefi, eTreat Medical Diagnostics Inc. (Canada); Farhad Akhbardeh, Univ. of North Dakota (United States); Nicholas B. MacKinnon, eTreat Medical Diagnostics Inc. (Canada); Kouhyar Tavakolian, David Bradley, Reza Fazel-Rezai, Univ. of North Dakota (United States)

A smartphone deployable mobile medical application has been presented that employs a camera, patient input, internet connectivity and cloud-based image processing to document and analyze physiological characteristics of hand osteoarthritis (OA). The application performs digital image processing that identifies hand fiduciary features as well as abnormal distal and proximal interphalangeal joints. The algorithm can also quantify finger angular deformities. The diagnostically relevant features measured by the mobile application can be applied to current diagnostic protocols such as the American College of Rheumatology (ACR) criteria for OA.

The mobile application performs the following core functions. It corrects and transforms the image of the hand to real-world spatial coordinates and then analyzes hand border data to provide coordinates of anatomical fiduciary points. These include coordinates of finger-tips, finger vertices, finger centerline, and hand base coordinates. The algorithm determines the centerline, finger joint coordinates, and from the anatomical fiduciary points measures other features. These include width of the fingers, location and size of joints, and finger joint angulation.

The algorithm has been tested with 100 hand images. Hand segmentation accuracy has been analyzed at different image resolutions. The OA diagnostic algorithm has been implemented with hand images at different disease stages.

9711-65, Session PMon

Vanishing point: a smartphone application that classifies pimples and estimates prognosis

Nicholas B. MacKinnon, eTreat Medical Diagnostics Inc. (Canada); Fartash Vasefi, eTreat Medical Diagnostics Inc. (United States)

A smartphone deployable mobile medical application is presented that uses the smartphone camera, patient input, internet connectivity and cloud-based image processing to analyze physiological characteristics of pimples, identify the type and stage of the blemish and predict when it will heal. The application targets isolated pimples as opposed to patches of acne. Repeat measurements of the same blemish over time are used to update and refine prediction of the “vanishing point”.

The algorithm performs the following core functions. It captures text information from user input to create a profile including age, gender and skin type as well as other information for storage in a user profile in a HIPAA compliant database. It captures an image of the blemish as well as image-related information entered by the user and transmits it to a cloud-based engine for image processing. The image-processing algorithm performs image segmentation to identify each blemish in the image and then analyzes each blemish to identify whether it is a papule, a pustule, a white comedone or a black comedone. Once the type of pimple is determined the pimple characteristics are compared to known values and the stage of development is determined. From this information the vanishing point is predicted.

We analyzed 40 images of pimples to determine classification accuracy for pimple type, by comparing them to classification by clinical experts. We then analyzed 10 pimples of each type to determine accuracy of staging by comparison to clinical experts. We then compared the accuracy of this prediction to actual eventual vanishing point as well as the accuracy of the updated prediction from images captured on subsequent days.

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9711-66, Session PMon

Embryonic stem cell counting on fluorescence images

Ali Furkan Kamanli, Sakarya Univ. (Turkey); Gökşen Çetinel,
In this paper, automatic cell counting method under microscopy is proposed. The counting process can be performed in two ways: The manual counting in which a specialist counts the cells with naked eye, and the automatic counting that utilizes the computer-based techniques. The counting process becomes exhausting, long and inaccurate when the counting performed by the specialist. Even though same cell image taking into account by the different specialist, different counting results can be obtained. The microscopy parameters are incorrect the edges of the cells will be shaly and some cells cannot be counted manually. Overlap is another difficulty that causes from the presence of many cells in a single scene. In these cases manual counting becomes slower, inaccurate and demanding task with naked eye. There are several techniques for dyeing the cells to turn them visible with naked eye. However, if the concentration is more than normal, two or more lighter points can overlap which causes the difficult and inaccurate cell counting. Because of the all above mentioned problems, the cell counting process must be performed automatically and the proposed automatic cell counting methods must be improved.

We propose an automatic stem cell counting method by utilizing same image processing techniques that appropriate the frame of the method. Stem cell sections were obtained under the fluorescence microscopy. In the pre-processing stage color image is split into channels. The effectiveness of the proposed method is evaluated by performing numerous computer simulations. It is shown that the proposed method gives promising results and can eliminate the subjectivity originated from the manual counting. The method is tested on a three different database at different noise level validated by the specialists. After pre-processing step to fill in the overlapped sections of the cell boundaries hough transformation is applied to the cell image. Then histogram of the image partitioned in to four parts and the best combination is determined for watershed algorithm to obtain the most exact counting results. The aim is using watershed algorithm is to make the maximum points of cells more clear. Finally, maximum points of the cell image is counted and counting results are obtained.

9711-20, Session 4

Excitation-scanning hyperspectral imaging system for microscopic and endoscopic applications

Sam Mayes, Thomas C. Rich, Silas J. Leavesley, Univ. of South Alabama (United States)

Current microscopic and endoscopic technologies for cancer screening utilize white-light illumination sources. Hyper-spectral imaging has been shown to improve specificity and sensitivity when compared to white-light imaging in both microscopy and in vivo imaging. However, hyperspectral imaging methods have historically suffered from slow acquisition times due to the narrow bandwidth of spectral filters. Often, minutes are required to gather a full image stack. We have developed a novel approach called excitation-scanning hyperspectral imaging that provides 2-3 orders of magnitude increased signal strength. This reduces acquisition times significantly, allowing for live video acquisition. Here, we describe a preliminary prototype excitation-scanning hyperspectral imaging system that can be coupled to endoscopes or microscopes for hyperspectral imaging of tissues and cells. Our system is comprised of three subsystems: illumination, transmission, and imaging. The illumination subsystem employs light-emitting diode arrays to illuminate at different wavelengths. The transmission subsystem utilizes a unique geometry of optics and a liquid light guide. Software controls allow us to interface with and control the subsystems and components. Digital and analog signals are used to coordinate wavelength intensity, cycling and camera triggering. The testing of the system shows it can cycle 16 wavelengths at as fast as 1 ms per cycle. Additionally, more than 18% of the light transmits through the system. Our setup should allow for hyperspectral imaging of tissue and cells in real time.

9711-22, Session 4

Multi-mode optical dermoscopy (SkinSpect) for skin with mole analysis

(Final Paper)

Fartash Vasefi, Nicholas B. MacKinnon, Spectral Molecular Imaging, Inc. (United States); Rolf B. Saager, Anthony J. Durkin, Kristen M. Kelly M.D., Beckman Laser Institute and Medical Clinic (United States); Daniel L. Farkas, Spectral Molecular Imaging, Inc. (United States)

Purpose: To determine the performance of a polarization-sensitive hyperspectral imaging system (SkinSpect) designed to quantify melanin and hemoglobin concentration maps of skin with mole Materials and methods: A hyperspectral imaging system (SkinSpect) illuminates the human skin samples in-vivo with spectrally-programmable linearly-polarized light at 33 wavelengths between 468nm and 857 nm. Diffusely reflected photons are separated into collinear and cross-polarized image paths and images captured for each illumination wavelength. We have developed a method which combines two depth sensitive techniques: polarization, and hyperspectral imaging, to accurately determine the spatial distribution of melanin and hemoglobin oxygenation in a skin lesion. All the subjects have skin type I or II in this study. Melanin and hemoglobin concentration of 20 subjects with mole were measured by SkinSpect. We have validated the SkinSpect results by comparing melanin and hemoglobin concentrations with the Spatially Modulated Quantitative Spectroscopy (SMoQS) measurements.

Results: After the melanin correction step the correlation value decrease significantly from R = 0.84 to R = 0.07. The low correlation between melanin and total Hb shown in all 20 subjects (both mole and normal regions) validates the SkinSpect algorithm with melanin-hemoglobin absorption crosstalk. Melanin concentrations using SMoQS system has also shown no correlation with hemoglobin estimations.

Conclusion: We have built and used a polarization sensitive hyperspectral imager for tissue imaging. The acquired reflection spectra results using skin with moles suggest that the system is useful for extracting and quantifying relative hemoglobin concentrations in tissues independent of melanin concentrations by effectively reduce melanin-hemoglobin absorption crosstalk.

9711-23, Session 4

Hyperspectral in vivo fluorescence imaging with multi wavelength LED excitation

Siri Luthman, Isabel Quirós-Gonzalez, Sarah E. Bohndiek, Univ. of Cambridge (United Kingdom) and Cancer Research UK (United Kingdom)

Hyperspectral imagers (HSI) combine morphological and spectral information, yielding potential for real-time and high-throughput multiplexed fluorescent contrast agent imaging. Multiplexed readout from pathological targets, such as cell surface receptors overexpressed in cancer cells, could improve both sensitivity and specificity of tumour identification. For effective clinical translation of hyperspectral imaging there is, however, a need for compact and cost effective implementations of the technology.

We have implemented a low-cost wide-field multiplexed fluorescence imaging system for small animal imaging, which combines LED excitation (590, 655, 740nm) with a compact solid state HSI (600 – 1000nm). A key challenge for applying a reflectance based system in vivo is the separation of contrast agent fluorescence from the reflectance of excitation light. To overcome this challenge, we implemented a series of offline reflectance removal methods, prior to least-squares spectral unmixing. To select the optimal correction method, we made a quantitative comparison based on performance in well-plate contrast agent data. Here, we report for the first time the capability of our HSI system for non-invasive in vivo fluorescence.
imaging in small animals. We show that HSI enables quantitative unmixing of at least four fluorescent contrast agents simultaneously in living mice, when using appropriate reflectance correction. We demonstrate the successful unmixing of four fluorescent contrast agents – Alexa Fluor 610, 647, 700 and 750—both independently and when injected in a mixture solution. These data are promising for the future clinical application of HSI for multiplexed fluorescence contrast agent imaging.

9711-24, Session 4

In vivo perfusion assessment of an anastomosis surgery on porcine intestinal model

Hanh N. D. Le, Johns Hopkins Univ. (United States); Justin Opferman, Ryan Decker, Children’s National Health System (United States); Gyeong W. Cheon, Johns Hopkins Univ. (United States); Peter C. W. Kim, Children’s National Health System (United States); Jin U. Kang, Johns Hopkins Univ. (United States); Axel Krieger, Children’s National Health System (United States)

Anastomosis, the connection of two structures, is a critical procedure for reconstructive surgery with over 1 million cases/year for visceral indication alone. However, complication rates such as strictures and leakage affect up to 19% of cases for colorectal anastomoses and up to 30% for visceral transplantation anastomoses. Local ischemia plays a critical role in anastomotic complications, making blood perfusion an important indicator for tissue health and predictor for healing following anastomosis. In this work, we apply a real time multispectral imaging technique to monitor impact on tissue perfusion due to varying interrupted suture spacing and suture tensions. Multispectral tissue images at 470, 540, 560, 580, 670 and 760 nm are analyzed in conjunction with an empirical model based on diffuse reflectance process to quantify the hemoglobin oxygen saturation within the suture site. The investigated tissues for anastomoses include porcine small (jejunum and ileum) and large (transverse colon) intestines. Two experiments using interrupted suturing with suture spacing of 1, 2, and 3 mm and tension levels from 0 N to 2.5 N are conducted. Tissue perfusion at 5, 10, 20 and 30 min after suturing are recorded and compared with the initial normal state. The result indicates the contrast between healthy and ischemic tissue areas and assists the determination of suturing spacing and tension. Therefore, the assessment of tissue perfusion will permit the development and intra-surgical monitoring of an optimal suture protocol during anastomosis with less complications and improved functional outcome.

9711-25, Session 4

Feasibility for detection of autofluorescent signatures in rat organs using a novel excitation-scanning hyperspectral imaging system

Peter F. Favreau, Joshua A. Deal, David S. Weber, Thomas C. Rich, Silas J. Leaveseley, Univ. of South Alabama (United States)

The natural fluorescence (autofluorescence) of tissues has been noted as a biomarker for cancer for several decades. Autofluorescence contrast between tumors and healthy tissues has been of significant interest in endoscopy, leading to development of autofluorescence endoscopes capable of visualizing 2-3 fluorescence emission wavelengths to achieve maximal contrast. However, tumor detection with autofluorescence endoscopes is hindered by low fluorescence signal and limited quantitative information, resulting in prolonged endoscopic procedures, prohibitive acquisition times, and reduced specificity of detection. Our lab has designed a novel excitation-scanning hyperspectral imaging system with high fluorescence signal detection, low acquisition time, and enhanced spectral discrimination. In this study, we surveyed a comprehensive set of excised tissues to assess the feasibility of detecting tissue-specific pathologies using excitation-scanning. Fresh, untreated tissue specimens were imaged from 360 to 550 nm on an inverted fluorescence microscope equipped with a set of thin-film tunable filters (Semrock, A Unit of IDEX). Images were subdivided into training and test sets. Automated endmember extraction (ENVI 5.1, Exelis) with PCA identified endmembers within training images of autofluorescence. A spectral library was created from 8 endmembers. The library was used for identification of endmembers in test images. Our results suggest (1) spectral differentiation of multiple tissue types is possible using excitation-scanning; (2) shared spectra between tissue types; and (3) the ability to identify unique physiological features in disparate tissues from shared autofluorescent components. Future work will focus on isolating specific molecular signatures present in tissue spectra, and elucidating the contribution of these signatures in pathologies.

9711-26, Session 4

Investigate the variation in optical redox ratio of epicardial adipose tissue in patients with CAD or DM through autofluorescence metabolic molecular image

Lun-Zhang Guo, National Taiwan Univ. (Taiwan); Tsung-Dau Wang, Cardiovascular Ctr., National Taiwan Univ. Hospital (Taiwan) and National Taiwan Univ. (Taiwan); Jong-Wei Lin, Cardiovascular Ctr., National Taiwan Univ. Hospital (Taiwan); Tzu-Ming Liu, National Taiwan Univ. (Taiwan)

In recent years, it has been suggested that epicardial adipose tissue (EAT) plays an important role in development of coronary artery disease (CAD) and diabetes mellitus (DM). In this article, we used two-photon fluorescence microscope to measure the fluorescence metabolic image of EAT, which obtained from the patient with/without CAD/DM. We used 740nm and 890nm infrared light to excite the auto-fluorescence of metabolic molecules NADH and FAD respectively. We collected the fluorescence signal at wavelength 450nm to 500nm and 500nm to 550nm to obtain the metabolic image. Through the image, we computed the redox ratio (NADH/FAD) by analyzing the intensity. The preliminary result showed that the redox ratio increase in the patients with CAD. It indicates EAT adipocytes of patient with CAD have decreased cellular metabolic activity. But there were no significant variation of redox ratio in the patients with DM.

9711-27, Session 4

Light labeling with temporal intensity modulations for hyperspectral imaging

Scott R. Domingue, David G. Winters, Randy A. Bartels, Colorado State Univ. (United States)

We introduce a fundamentally new method of hyperspectral imaging (HI) entitled light labeling (LiLa), which eliminates the need to spatially disperse the light from each pixel within an interrogated field-of-view to capture spectroscopic information. LiLa is a multiplexed form of area- or spectral-scanning HI, where the spectroscopic axis is contained in a time-domain measurement through a wavelength-dependent intensity modulation encoded onto the illuminating power spectrum. The unique modulation frequency at each wavelength acts as a temporal label and is recoverable after a light-matter interaction, such as fluorescent absorption. Using LiLa, we demonstrate a new paradigm for absorptive imaging that is background-free. The intensity modulation frequency label for each wavelength within the excitation power spectrum is transferred to
Image analysis algorithms are compared to assess the feasibility of smartphone imaging for skin lesion self-imaging for screening, prior to SkinSpect analysis.

9711-30, Session 6

Cytometry metadata in XML

Robert C. Leif, Stephanie H. Leif, Newport Instruments (United States)

Introduction: The International Society for Advancement of Cytometry (ISAC) has created a standard for the Minimum Information about a Flow Cytometry Experiment (MIFlowCyt 1.0). CytometryML will serve as a common metadata standard for flow and image cytometry (digital microscopy). Methods: The MIFlowCyt data-types were created, as is the rest of CytometryML, in the XML Schema Definition Language (XSDL). The data-types are primarily based on the Flow Cytometry and the Digital Imaging and Communication (DICOM) standards. A small section of the code was formatted with standard HTML formatting elements (p, h1, h2, etc.). Results: 1) The part of MIFlowCyt that describes the Experimental Overview, as well as, substantial parts of several other major elements have been implemented as CytometryML XML schemas (www.cytometryml.org). 2) The feasibility of using MIFlowCyt to provide the combination of an overview, table of contents, and/or an index of a scientific paper or a report has been demonstrated. A sample electronic publication, EPUB, was created that could contain both MIFlowCyt metadata as well as the binary data.

Conclusions: The use of CytometryML technology together with XHTML5 and CSS permits the metadata to be directly formatted and together with the binary data to be stored in an EPUB container. This will facilitate: formatting, data- mining, presentation, data verification, and inclusion in structured research, clinical, and regulatory documents, as well as demonstrate in publications adherence to the MIFlowCyt standard, promote interoperability and should also result in the textual and numeric data being published using web technology without any change in composition.

9711-31, Session 6

Comprehensive guide to modern methods for processing and analyzing single molecule fluorescence data

Mélodie C. A. S. Hadzic, Danny Kowerko, Richard Börner, Sebastian L. B. König, Univ. Zürich (Switzerland); Mario Heidernätsch, Technische Univ. Chemnitz (Germany); Roland K. O. Sigel, Univ. Zürich (Switzerland)

Over the past decades, camera-based fluorescence detection at a single molecule level allowed to unveil conformational dynamics of many molecular systems involved in the cell machinery. Processing and analyzing the manifold sequences of single molecule images has therefore engendered a rich and diversified library of methods. Although performances of each single technique may be rigorously estimated by their authors, the absence of general comparison studies renders the appropriate selection more complex and time-consuming. The present study guides the experimentalist in this complicated choice, particularly in finding the approaches adapted to his own experimental data [1].

Twelve methods assigned to three processing steps, namely molecule detection, background signal correction and thermodynamic-kinetic model selection, were tested on synthetic data with variation of relevant conditions, readily quantifiable from experimental records as the molecular

Absorbing molecules and retained in the emitted fluorescent intensity, where the modulation frequencies are far below the optical carrier or typical fluorescent emission rates. The absorbed spectrum is thus retrievable from the temporal dynamics of the emission intensity, which is captured in the shot-noise limit. The diversity of additional applications directly enabled or enhanced by utilizing LiLα include high speed HI; spectroscopy in the mid-infrared, THz, and mm-wave; Fourier transform spectroscopy; high speed multidimensional spectroscopy, and pump-probe spectroscopy. The first two are of particular interest as they are straight-forward extensions of the demonstrated LiLα system and have the possibility of being transformative technological improvements for their respective fields; for example, increasing the HI frame rate far beyond the ceiling of state-of-the-art high speed cameras.

9711-28, Session 5

Contrast enhancement using differential spinning disc structured illumination in high resolution microendoscopy for imaging nuclear morphology in tissue (Invited Paper)

Pelham Keahey, Rebecca Richards-Kortum, Rice Univ. (United States)

Fiber-optic microendoscopes are a promising new technology to image changes in nuclear morphometry for in vivo diagnosis of precancer in epithelial tissue. Fiber-bundles are ideal imaging probes for in vivo use due to their small size and flexibility. However, contrast in images obtained with fiber-optic microendoscopes is limited by scattering in tissue that generates out-of-focus light. Confocal imaging techniques can improve image contrast by using optical sectioning to reduce out-of-focus light collected by fiber-bundle based imaging systems. Unfortunately, many confocal imaging strategies cannot be performed in real-time or require complex optics to be attached to the distal end of the fiber-bundle, which can make in vivo imaging mechanically impossible. Here we present an alternative high resolution microendoscopy capable of performing optical sectioning using differential spinning disc structured illumination. Optical sectioning can be performed in real-time at video rates without the need for any optics at the distal end of the fiber-bundle. An optical phantom was developed to quantify the sectioning capabilities of the system and we demonstrate contrast enhancement of nuclear morphology in mouse tissue.

9711-29, Session 5

Melanoma detection using smartphone and multimode hyperspectral imaging

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, Los Angeles (United States)

A goal of this project is to examine how effectively a technology continuum from a low cost, remotely deployable screening device to a more sophisticated (and more expensive) multimode imaging system can be implemented and integrated with clinical practice.

A smartphone deployable mobile medical application is presented employing smartphone camera, supplemented with a dermoscopy optical attachment, patient input, internet connectivity and cloud-based image processing to document and analyze physiological characteristics of melanocytic nevi suspicious for melanoma. The smartphone application captures cross-polarized images and performs digital image processing that quantifies chromophore distribution in tissue. Images are then captured by a multimode hyperspectral imaging system, SkinSpect, based on spectral imaging achieved using MEMS technologies (OneLight). We have previously shown this system to more accurately measure hemoglobin and melanin distribution in tissue by reducing crosstalk between the melanin and hemoglobin measurements. Images captured by the smartphone application will not have the ability to separate this crosstalk as well as the spectral system can but will be able to use some (multi)color imaging and polarization information. Relative accuracy and biological plausibility of the two systems and their image analysis algorithms are compared to assess the feasibility of smartphone imaging for skin lesion self-imaging for screening, prior to SkinSpect analysis.

9711-27, Session 5

Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues IX
surface density, the signal-to-noise ratio or the point spread function width. For each condition, we assessed algorithm efficiencies regarding accuracy, outcome reproducibility and calculation time.

To facilitate data production and evaluation, we developed a new and freely available graphical user interface, allowing flexible and realistic simulations and offering either multiple common approaches or compatible export solutions for a complete analysis. We demonstrate how to optimally parameterize nine molecule detection and background signal correction methods for typical experimental data, which can eventually be extended to new algorithms.

With this first independent and comprehensive study supported by open source software, we intend to open the way towards standardization of single molecule fluorescence data analysis and wish for higher compatibility between further developed approaches.


9711-32, Session 6

Directional spatial frequency analysis of lipid distribution in atherosclerotic plaque

Clyde R. Korn, Eric Reese, Lingyan Shi, Robert R. Alfano, Stewart Russell, The City College of New York (United States)

Atherosclerosis is characterized by the growth of fibrous plaques due to the retention of cholesterol and lipids within the artery wall, which can lead to vessel occlusion and cardiac events. One way to evaluate arterial disease is to quantify the amount of lipid present in these plaques, since a higher disease burden is characterized by a higher concentration of lipid. Although therapeutic stimulation of reverse cholesterol transport to reduce cholesterol deposits in plaque has not produced significant results, this may be due to current image analysis methods which use averaging techniques to calculate the total amount of lipid in the plaque without regard to spatial distribution, thereby discarding information that may have significance in marking response to therapy. Here we use Directional Fourier Spatial Frequency (DFSF) analysis to generate a characteristic spatial frequency spectrum for atherosclerotic plaques from C57 Black 6 mice both treated and untreated with a cholesterol scavenging nanoparticle. We then use the Cauchy product of these spectra to classify the images with a support vector machine (SVM). Our results indicate that treated plaque can be distinguished from untreated plaque using this method, where no difference is seen using the spatial averaging method. This work has the potential to increase the effectiveness of current in-vivo methods of plaque detection that also use averaging methods, such as laser speckle imaging and Raman spectroscopy.

9711-33, Session 6

Brain vascular image enhancement based on gradient adjust with split Bregman

Xiao Liang, Di Dong, Hui Hui, Liwen Zhang, Mengjie Fang, Jie Tian, Institute of Automation (China)

Light Sheet Microscope (LSM) is a high-resolution fluorescence microscopic technique which enables to observe the mouse brain vascular network clearly with antibody staining. However, micro-vessels are stained with few fluorescence antibodies and their signals are much weaker than large vessels, which make micro-vessels unclear in LSM images. In this work, we developed a vascular image enhancement method to enhance micro-vessel details which should be useful for vessel statistics analysis. Since gradient describes the edge information of the vessel, the main idea of our method is to maximize the gradient values of the enhanced image to make the micro-vessels sharper. Meanwhile, an optimum problem whose solution was the final enhanced image was formulated by designing an energy function similar to total variation model.

Our method contained two steps: 1) calculate the gradient image of LSM image, and then amplify high gradient values of the original image to enhance the vessel edge and suppress low gradient values to remove noises. To achieve this, we formulated a new optimum problem by adding the gradient image to total variation regularization. 2) The split bregman iteration method was used to deal with the L1-norm regularization problem and generate the final enhanced image. The main advantage of the split bregman method is that it has both fast convergence and low memory cost. In order to verify the effectiveness of our method, we applied our method to a series of mouse brain vascular images acquired from a commercial LSM system in our lab. The experimental results showed that our method could greatly enhance micro-vessel edges which were unclear in the original images.

9711-34, Session 6

Statistical image segmentation for the detection of skin lesion borders in UV fluorescence excitation

Antonio Ortega-Martinez, Juan Pablo Padilla-Martinez, Walfre Franco, Wellman Ctr. for Photomedicine (United States)

The skin contains several fluorescent molecules or fluorophores that serve as markers of structure, function and composition. UV fluorescence excitation photography is a simple and effective way to image specific intrinsic fluorophores, such as the one ascribed to tryptophan which emits at 345 nm upon excitation at 295 nm with a wavelength, and is a marker of cellular proliferation. Earlier, we built a clinical UV photography system to image cellular proliferation. In some samples, the naturally low intensity of the fluorescence can make it difficult to separate the fluorescence of cells in higher proliferation states from background fluorescence and other imaging artifacts -- like electronic noise. In this work, we describe a statistical image segmentation method to separate the fluorescence of interest. Statistical image segmentation is based on image averaging, background subtraction and pixel statistics. This method allows to better quantify the intensity and surface distributions of fluorescence, which in turn simplify the detection of borders. Using this method we delineated the borders of highly-proliferative skin conditions and diseases, in particular, allergic contact dermatitis, psoriatic lesions and basal cell carcinoma. Segmented images clearly define lesion borders. UV fluorescence excitation photography along with statistical image segmentation may serve as a quick and simple diagnostic tool for clinicians.

9711-35, Session 6

Limitations of fitting angular scattering from single cells

Xing Fan, Ashley E. Cannaday, Andrew J. Berger, Univ. of Rochester (United States)

The literature contains several reports of Mie-like fits to angular-domain elastic scattering measurements from multiple cells or isolated mitochondria. In these studies, the sampling volume typically contains hundreds or thousands of mitochondria, allowing for the size distribution of mitochondria to be modeled as a smooth function, (e.g. Gaussian or log-normal) with a small number of free parameters. In the case of a single-cell volume containing significantly fewer mitochondria, the true size distribution will no longer be as smooth. Increasing the number of free parameters can lead to unstable fits, however, as the forward-directed angular scattering pattern from such a population illuminated with 785 nm light is a monotonically decaying radial function with few distinct
features. Using simulations, we have investigated the limitations of modeling single-cell mitochondrial scattering using smooth population distributions of Mie scatterers. In different instances, the fidelity of the estimated size information can be limited by the number of organelles, the angular detection range, or the non-ideality of the data (both speckle and shot noise). We will describe the conditions under which each of these effects dominates. We will also discuss whether mean and standard deviation are the best sizes to report from single-Mie modeling, or if there are other size parameters that have greater fidelity to the true, non-smooth size distributions.

9711-36, Session 7

True 3D imaging method-holographic 3D display for future biomedical imaging (Invited Paper)
Hongyue Gao, Jicheng Liu, Shanghai Univ. (China)

3D display with more information shown is much better than 2D display. Current 3D techniques, such as stereoscopic 3D display, volumetric 3D display and so on, has some problems in them to be solved, and therefore they are not widely applied. Holographic display is a perfect 3D technology, which can provide realistic 3D images without any special eyewear for observers. It can be divided into static and dynamic holography. Large size, high definition, and full parallax 3D image can be reconstructed from a static hologram. However, the holograms recorded in materials usually are based on real objects. To make 3D holograms based on 3D models generated by computer or 3D images captured from objects, hologram print is a necessary. Therefore, we study hologram print, including computer generated hologram, holographic storage materials and hologram print system. Furthermore, we realize real time holographic 3D display in large size materials, which solves the problem that dynamic holography cannot reach video rate in materials. It is thought to be an important step to future holographic 3D television by some experts. In this paper, we will present our work on hologram print and holographic 3D video display. We hope that holographic 3D display can be used in biomedical 3D imaging and display to provide more information than 2D ones in the future.

9711-37, Session 7

One-step fabrication of multi-layered microcapsules by a tri-axial flow focusing device for microencapsulation of soluble drugs and imaging agents
Shuai Yuan, Ting Si, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Microcapsules have become attractive for microencapsulation of multiple drugs and imaging agents for image-guided therapy due to their efficient encapsulation properties. Recent advances in microfluidics methods, for example, poly(dimethylsiloxane) (PDMS)-based microchannels and glass capillaries, have enabled to prepare very high uniformity multi-layered microcapsules with unprecedented control and flexibility. However, their manufacturing process of devices is complex and the surface modification of devices is hard to control. Here we report a novel tri-axial flow focusing (TFF) method based on capillary flows that could one-step encapsulate drugs and imaging agents without modifying the surface of the device. The tri-axial needle manufactured by a laser beam welding process can be readily assembled, aligned and cleaned. The solutions prepared in advance are injected into different channels, and immiscible multiphase streams at the exit of the tri-axial needle flow through a single orifice, forming multilayered co-axial interfaces in the core of a high-speed driving coflowing stream. Eventually microcapsules can be obtained after the breakup of the liquid jet under hydrodynamic forces because of the jet instability. In this work, the formation of stable cone-jet mode and subsequent jet breakup in the TFF device can be observed by a set of equipments. The effects of main process parameters on the characteristics of the multi-layered cone-jet configurations are explored and different flow modes are identified. This one-step microencapsulation approach is available for fluids with a wide range of properties, and enables the full control of the thickness of each layer through the control of the flow rates of each of fluids. Moreover, this TFF process has high encapsulation efficiency, high throughput and low cost. The produced multi-layered microcapsules are capable of encapsulate multiple water-soluble drugs and imaging agents in the same shell. Therefore, this novel approach has great potential in applications such as drug delivery and image-guided therapy.

9711-38, Session 7

Tracking corneal epithelium stem cells using optical coherence tomography
Joseph Boadi, Stephen J. Matcher, Sheila MacNeil, The Univ. of Sheffield (United Kingdom); Virender S. Sangwan, LV Prasad Eye Institute (India)

The prevailing hypothesis for the existence and healing of the avascular corneal epithelium is that this layer of cells are continually produced by stem cells in the limbus and transported onto the cornea to mature into corneal epithelium. In the event that the cornea is damaged and the limbal stem cell population is severely reduced, this condition known as Limbal Stem Cell Deficiency and can lead to blindness. There are numerous treatments but most have high long term failure rates. Most treatment methods include the transplantation of limbal stem cells into damaged limbus with hope of repopulating the region and regenerating at healthy corneal epithelium. Optical Coherence Tomography (OCT) is well known for its high resolution in vivo images. A bespoke OCT has been built to investigate the trajectories of these limbal stem cells after transplantation to see whether they do repopulate the damaged limbus or not.

In the experimentation magno-labeuling was used to track the limbal stem cells. For the magno-labelling a mixture of limbal stem cells and cornea epithelium are cultured with super paramagnetic iron (Fe3O4) nanoparticles (20-30nm in size) for 24hours, to allow for uptake. The cells are then transplanted onto the denuded cornea. The transplanted cell mixture with the encapsulated magnetic nanoparticles is actuated with an external magnetic field 0.08T leading to a phase modulation on the signal. A Phase sensitive Magneto-motive OCT is used to locate the transplanted cells. The location of the cells with embed SPIOs were located both in 2D and 3D.

9711-39, Session 7

Fiber based imaging in bioengineered construct
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Microcapsules have become attractive for microencapsulation of multiple drugs and imaging agents for image-guided therapy due to their efficient encapsulation properties. Recent advances in microfluidics methods, for example, poly(dimethylsiloxane) (PDMS)-based microchannels and glass capillaries, have enabled to prepare very high uniformity multi-layered microcapsules with unprecedented control and flexibility. However, their manufacturing process of devices is complex and the surface modification of devices is hard to control. Here we report a novel tri-axial flow focusing (TFF) method based on capillary flows that could one-step encapsulate drugs and imaging agents without modifying the surface of the device. The tri-axial needle manufactured by a laser beam welding process can be readily assembled, aligned and cleaned. The solutions prepared in advance are injected into different channels, and immiscible multiphase streams at the exit of the tri-axial needle flow through a single orifice, forming multi-layered co-axial interfaces in the core of a high-speed driving coflowing stream. Eventually microcapsules can be obtained after the breakup of the liquid jet under hydrodynamic forces because of the jet instability. In this work, the formation of stable cone-jet mode and subsequent jet breakup in the TFF device can be observed by a set of equipments. The effects of main process parameters on the characteristics of the multi-layered cone-jet configurations are explored and different flow modes are identified. This one-step microencapsulation approach is available for fluids with a wide range of properties, and enables the full control of the thickness of each layer through the control of the flow rates of each of fluids. Moreover, this TFF process has high encapsulation efficiency, high throughput and low cost. The produced multi-layered microcapsules are capable of encapsulate multiple water-soluble drugs and imaging agents in the same shell. Therefore, this novel approach has great potential in applications such as drug delivery and image-guided therapy.
Monitoring cells and tissues through opaque and turbid media presents a major challenge for imaging bioengineered tissues. The fiber optics-based imaging system developed by our group offers a new approach for fluorescent imaging. A micro imaging channel is embedded in Polycaprolactone (PCL) electrospun scaffold, which allows for single mode fiber insertion and delivering laser excitation close to the fluorescent target. The emission is detected by an EMCCD detector allowing reconstruction of an image based on multiple excitation points within the bioengineered construct with a working distance of several centimeters. The objective of this study is to assess the effects that several physical and system parameters have on image reconstruction outcomes, using fluorescent beads on an opaque electrospun scaffold. Results indicate that scaffold thickness has a small impact on the image, whereas scaffold composition (collagen content), fluorophore spectra, and the reconstruction window have a large effect. These results suggest that a far-red fluorescence emission is preferential when using collagenous scaffold with thickness of up to 500 µm. Using these optimized parameters, we imaged fluorescently labeled cells on electrospun scaffold with a resolution of 15-20 µm and measured cell differentiation and scaffold surface coverage. Notably, these parameters can be applied to other bioengineered tissues for non-invasive monitoring both in vitro and in vivo.

9711-40, Session 7

Multi-contrast volumetric cell imaging based on optical projection tomography

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Optical projection tomography is a new optical imaging method for visualizing small biological specimens in three dimension. The most important advantage of OPT is to fill the gap between MRI and confocal microscope for the specimen having the range of 1-10 mm. Thus, it has been mainly used for whole-mount small animals and developmental study since this imaging modality was developed. The ability of OPT delivering anatomical and functional information of relatively large tissue in 3D has made it a promising platform in biomedical research. Recently, the potential of OPT spans its coverage to cellular scale. Even though there are increasing demand to obtain better understanding of cellular dynamics, only few studies to visualize cellular structure, shape, size and functional morphology over tissue has been investigated in existing OPT system due to its limited field of view.

In this study, we develop a novel optical imaging system for 3D cellular imaging with OPT integrated with dynamic focusing technique. Our tomographic setup has great potential to be used for identifying cell characteristic in tissue because it can provide selective contrast on dynamic focal plane allowing for fluorescence as well as absorption. While the dominant contrast of optical imaging technique is to use the fluorescence for detecting certain target only, the newly developed OPT system will offer considerable advantages over currently available method when imaging cellular molecular dynamics by permitting contrast variation. By achieving multi-contrast, it is expected for this new imaging system to play an important role in delivering better cytological information to pathologist.

9711-41, Session 7

Light-guide tunable snapshot spectrometer for biomedical applications

Ye Wang, Tomasz Tkaczyk, Michal E. Pawlowski, Rice Univ. (United States)

Snapshot hyperspectral imaging acquires special and spectral data without filtering and scanning, which has advantage of high collection efficiency. Here we present a proof-of-principle prototype of a fiber-based snapshot tunable spectrometer to provide high spectral sampling for biomedical application such as cell signaling or diagnostics. Image is collected by a custom 96 x 81 fiber bundle (25 x 27 mm) at input end. At the output end the fibers are divided into spatial groups with spaces in between as tunable dispersion zones. The image coming out is later scaled down by an image taper (to scale down the image size and allow smaller optical components), dispersed with a prism and captured by a CCD camera. An interpolation algorithm is used to locate each wavelength and reconstruct the image for each spectral channel. The fiber bundle is fabricated by aligning multi-mode bare fiber ribbons as matrix, gluing together in Teflon molds, laser cutting and polishing. The tunable dispersion zones between fiber groups are achieved by compressing resilient rubber layers. The system tunability allows trade-off between spatial and spectral samplings. Based on different resolution requirements, the imaging area for the system could be tuned from 96 x 81 to 96 x 39 pixels with the spectral resolution from low to high. We present preliminary results obtained with the spectrometer attached to an inverted microscope. Results were obtained for test fluorescence bead slides and stained fluorescence cell samples at different spectral sampling.

9711-42, Session 7

Vitamin C for stabilising biological lasers

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We report on efforts to improve the lifetime of biological lasers through the use of ascorbic acid (also commonly known as vitamin C). Fluorescent proteins and dyes, used in biological lasers, suffer from photobleaching due to the build-up of reactive oxygen species (ROS) which causes damage leading to a decrease in emission over time. This is an issue both for laser lifetime and cell health. It has previously been shown that ascorbic acid can be effective in reducing ROS levels in a variety of applications. For our experiments human embryonic kidney cells (HEK293), containing the fluorescent dye Calcein AM, were placed between two dielectric plane mirrors to form a laser cavity. The cells were pumped using the output of a Ti:Sapphire femtosecond OPO system, frequency doubled twice in BBO crystals, giving an output of 474 nm. Initial results have shown an increase in laser lifetime when ascorbic acid is added to cells indicating a reduction in the build-up of ROS.

9711-43, Session 7

Whole slide imaging of unstained tissue using lensfree microscopy

Sophie Nhu An Morel, Lionel Hervé, Thomas Bordy, Olivier Cioni, CEA-LETI (France); Antoine Delon, Univ. Grenoble Alpes (France) and Lab. Interdisciplinaire de Physique (France); Catherine Fromentin, LENS-Ctr. Hospitalier Dr. Schaffner (France); Jean-Marc Dinten, Cédric Allier, CEA-LETI (France)

Pathologist examination of tissue slides provides insightful information about a patient’s disease. Traditional analysis of tissue slides is performed under a binocular microscope, which requires staining of the sample and delays the examination. We present a simple cost-effective lensfree imaging method to record 2-4µm resolution wide-field (10 mm? to 6 cm?) images of unstained tissue slides.

The sample processing time is reduced as there is no need for staining. A wide field of view (10 mm?) lensfree hologram is recorded in a single shot and the image is reconstructed in 2s providing a very fast acquisition chain. The acquisition is multispectral, i.e. multiple holograms are recorded simultaneously at three different wavelengths, and a dedicated holographic reconstruction algorithm is used to retrieve both amplitude and phase. Whole tissue slides imaging is obtained by recording 130 holograms with X-Y translation stages and by computing the mosaic of a 25 x 25 mm?
reconstructed image. The reconstructed phase provides a phase-contrast-like image of the unstained specimen, revealing structures of healthy and diseased tissue. Slides from various organs can be reconstructed, e.g. lung, colon, ganglion, etc. To our knowledge, our method is the first technique that enables fast wide-field lensfree imaging of such unlabeled dense samples. This technique is much cheaper and compact than a conventional phase contrast microscope and could be made portable.

In sum, we present a new methodology that could quickly provide useful information when a rapid diagnosis is needed, such as tumor margin identification on frozen section biopsies during surgery.

9711-44, Session 7

Mapping molecular orientational distributions for biological sample in 3D

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Measuring molecular orientation properties is very appealing for scientists in molecular and cell biology, as well as biomedical research. Orientational organization at the molecular scale is indeed an important brick to cells and tissues morphology, mechanics, functions and pathologies. Recent work has shown that polarized fluorescence imaging, based on excitation polarization tuning in the sample plane, is able to probe molecular orientational order in biological samples; however this applies only to information in 2D, projected in the sample plane. To surpass this limitation, we extended this approach to excitation polarization tuning in 3D. The principle is based on the decomposition of any arbitrary 3D linear excitation in a polarization along the longitudinal z-axis, and a polarization in the transverse xy-sample plane. We designed an interferometer with one arm generating radial polarization light (thus producing longitudinal polarization under high numerical aperture focusing), the other arm controlling a linear polarization in the transverse plane. The amplitude ratio between the two arms can vary so as to get any linear polarized excitation in 3D at the focus of a high NA objective. This technique has been characterized by polarimetry imaging at the back focal plane of the focusing objective, and modeled theoretically. 3D polarized fluorescence microscopy is demonstrated on actin stress fibers in non-flat cells suspended on synthetic polymer structures forming supporting pillars, for which heterogeneous actin orientational order could be identified. This technique shows a great potential in structural investigations in 3D biological systems, such as cell spheroids and tissues.

9711-45, Session 7

Analysis of forward/backward second harmonic generation images reveals the nanoscale structure of collagen within bone and cartilage

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Collagen is a major structural protein in mammals that plays a central role in the architecture and function of a wide range of connective tissues. Studying collagen structure at the microscopic scale is therefore of considerable interest to understand tissue pathologies.

Here, we use Second Harmonic Generation (SHG) microscopy to obtain structural information about collagen I and II scaffold within human bone and cartilage. In particular, we performed Polarization-resolved SHG and analyzed the differences between forward and backward images. In the cartilage, the SHG signal is nearly uniform throughout the collagen meshwork, revealing a quasi-random distribution of fibrils in the focal volume. This fits with a function of entrapment in which collagen II provides a tensile force balance to pressure swelling during load carriage. In contrast, the variations of intensity observed in the bone suggest that the fibrils are packed into bundles with a constant orientation and polarity, indicating a more conventional fiber reinforcement role.

Finally, in cartilage, we observed an increase in the Forward/Backward ratio (F/B) from the surface to the bone. Coupling this result to numerical simulations, we demonstrated that the F/B follows the evolution of the fibrils diameter and provides information about the collagen structure at sub-resolution scale. This work illustrates the relevance of SHG microscopy to study the nanoscale architecture of tissues and presents a strong potential for a wide range of applications such as the investigation of remodeling processes, the study of fibrillation mechanisms in pathological tissues or the characterization of cell-collagen scaffold interactions.

9711-46, Session 8

Multispectral excitation based multiple fluorescent targets resolving in fluorescence molecular tomography

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Fluorescence molecular tomography (FMT) can visualize biological activities at cellular and molecular levels in vivo, and has been extensively used in drug delivery and tumor detection research of small animals. The ill-posedness of the FMT inverse problem makes it difficult to reconstruct and unmix multiple adjacent fluorescent targets that have different functional features but are labeled with the same fluorochrome. An algorithm based on independent component analysis (ICA) for multispectral excited FMT is proposed to unmix multiple fluorescent targets in this study. Fluorescent targets are excited by multispectral excitation, and the three-dimensional distribution of fluorescent yields under the excitation spectrum is reconstructed by an iterated Tikhonov regularization algorithm. Subsequently, multiple fluorescent targets are resolved from mixed fluorescence signals by employing ICA. Simulations were performed and the results demonstrate that multiple adjacent fluorescent targets can be unmixed if the number of excitation wavelengths is not smaller than that of fluorescent targets, which should have different concentrations.

Then phantom experiments were conducted on a noncontact, full-angle FMT system. The results demonstrate that the algorithm obtains both independent components that provide spatial information of different fluorescent targets and spectral courses that reflect variation trends of fluorescent yields along with the excitation spectrum. By using this method, it is possible to visualize the metabolism status of drugs in different structure organs, and quantitatively depict the variable trends of fluorescent yields of each functional organ under the excitation spectrum. This method may provide a pattern for tumor detection, drug delivery and treatment monitoring in vivo.
9711-47, Session 8

Robust organelle size extractions from elastic scattering measurements of single cells

Ashley E. Cannaday, Robert Draham, Andrew J. Berger, Univ. of Rochester (United States)

The goal of this project is to estimate non-nuclear organelle size distributions in single cells by measuring angular scattering patterns and fitting them with Mie theory. Simulations have indicated that the large relative size distribution of organelles (mean:width=2) leads to unstable Mie fits unless scattering is collected at polar angles less than 20 degrees. Our optical system has therefore been modified to collect angles down to 10 degrees. Initial validations will be performed on polystyrene bead populations whose size distributions resemble those of cell organelles. Unlike with the narrow bead distributions that are often used for calibration, we expect to see an order-of-magnitude improvement in the stability of the size estimates as the minimum angle decreases from 20 to 10 degrees. Scattering patterns will then be acquired and analyzed from single cells (EMT6 mouse cancer cells), both fixed and live, at multiple time points. Fixed cells, with no changes in organelle sizes over time, will be measured to determine the fluctuation level in estimated size distribution due to measurement imperfections alone. Subsequent measurements on live cells will determine whether there is a higher level of fluctuation that could be attributed to dynamic changes in organelle size. Studies on unperturbed cells are precursors to ones in which the effects of exogenous agents are monitored over time.

9711-48, Session 8

Development of a smartphone-based multispectral imaging system for mobile skin-care

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We built a mobile multi-spectral imaging system capable of being attached to a smartphone for quantitative detection and management of various skin lesions. For last decades, multispectral imaging has been widely utilized as one of the advanced quantitative optical imaging techniques for various biomedical applications. It has been particularly shown to be great potentials in the early detection of either malignant or nonmalignant skin lesions. Hence, many dermoscopes based on the technique have been already commercialized for use in the clinics. However, the dermoscopes are typically bulk and expensive and therefore may not be suited for mobile skin-care. Hence, we here built a mobile multispectral imaging system, easily attached to a smartphone, for quantitative mobile skin-care. This system consists of ten bandpass filters within the wavelength range of from 425 to 675 nm, polarizers, a magnifying lens, and an interface circuit, allowing synchronization between the multispectral imaging system and the attached smartphone via Bluetooth. An android application was also developed for the system control and spectral classification. To evaluate our developed system, acne regions of interest have been monitored while benzoyl peroxide treatment for 7 days using it. The area of acne regions was here quantitated by spectral classification. The changes in spectral signatures for the acne regions could have been successfully tracked in detail. These results demonstrated that our developed system offered continuous quantitative monitoring of skin lesions, suggesting the potential as a useful mobile skin-care device to detect and manage various skin lesions.

9711-49, Session 8

A method to compensate for the underestimation of collagen with polarized picrosirius red imaging in human artery atherosclerotic plaques

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Although picrosirius red (PSR) is known to be useful in quantifying collagen under polarized light (PL), commonly used linearly PL can result in an underestimation of collagen, as some of the fibers may appear dark if aligned with the transmission axis of the polarizers. To address this issue, a sample may be imaged with circularly PL at the expense of higher background intensity. However, the quality and alignment of the microscope illumination optics, polarizers and waveplates can still produce imaging variability with circular polarization. A simpler technique was tested that minimized PSR variability with linearly PL by acquiring images at multiple angles of slide rotation to create a composite image, permitting the optimal semi-quantitative visualization of collagen. Linear polarization imaging was performed on PSR stained artery sections from different cadaver hearts. By rotating the slide at 60° intervals, while maintaining illumination, polarization and exposure parameters, 6 images were acquired for each section and co-registered to create a composite image. Images from any of the 6 rotation positions consistently showed variation in PSR signal. The resulting composite image compensates for this variability, without loss of spatial resolution. Additionally, grayscale analysis show an increased intensity range of 15 – 50% with linearly polarized composite image over a circularly polarized image after background correction, indicating better SNR. Ongoing work will use the proposed technique as a reference method in the development of a near infrared spectroscopy algorithm in assessing collagen to detect vulnerable atherosclerotic plaques in vivo.

9711-50, Session 8

Intraoperative optical biopsy for brain tumors using spectro-lifetime properties of intrinsic fluorophores (Invited Paper)

Faridh Vasefi, David S. Kittle, Chirag G. Patil M.D., Ray M. Chu M.D., Adam N. Mamalek M.D., Keith L. Black M.D., Pramod V. Butte, Cedars-Sinai Medical Ctr. (United States)

We have intra-operatively shown a near real-time brain tissue optical biopsy using time-resolved fluorescence spectroscopy (TRFS) system. The clinically compatible TRFS system is able to distinguish between normal cortex, normal white matter, and brain tumor (low- and high-grade glioma) tissue types. The TRFS system contains the excitation light from laser (355nm, 400ps, 5 uJ/pulse) delivered to the fluorophores using a fiber probe. The same fiber collected fluorescence and coupled to a custom demuxer to split the spectral channels into 6 discrete wavelength bands from 360nm to 700 nm, a custom fiber delay unit to delay individual color bands before being combined onto the photomultiplier tube (MCP-PMT, 80 ps rise time), and a digitizer to transfer the fluorescence lifetime measurements to a computer. The system generates spectro-lifetime characteristic of biological tissues which can be used as a signature by machine training algorithm for tissue classification. Supervised learning algorithm using linear discriminant analysis (LDA) employs selected intrinsic fluorescence decay temporal points in 6 spectral bands to maximize statistical significance difference between training groups. Three classifiers have been implemented to distinguish between...
glioma, normal cortex, and normal white matter tissue types. Experiments conducted in eight patients undergoing craniotomy for tumor resection. The leave-one-out statistical analysis on in vivo human data obtained by TRFS measurements (N = 80) which were validated by histopathologic analysis (# of biopsies = 26) and or neuronavigation demonstrate that TRFS can obtain sensitivity, specificity, positive predictive value, and negative predictive value of 89%, 98%, 94%, and 96% respectively.

9711-51, Session 8

3D fabricated microoptic system for multispectral tissue fluorescence lifetime measurements

Luwei Zou, Univ. of Michigan-Dearborn (United States); Mohamad Mahmoud, Mehdi Fahs, Fadl Choughari, Kai Duan, Univ. of Michigan-Dearborn (United States); Joe F. Lo, Univ. of Michigan-Dearborn (United States)

Various forms of collagens comprises the main load-bearing components in biological tissues. Composition of these collagen types in tissue varies in clinical applications such as wound healing and cancer progression. These collagens have blue autofluorescence when excited by UV light, distinguishable by their unique fluorescent lifetimes across a range of emission wavelengths. Our system is based on free space optics with a common optical path for the excitation and the emission intensities to optimize coupling and increase signal. It is capable of spectral lifetime measurements by breaking up incoming intensity into three different bands via a series of longpass dichroics. The channels are separated by dichroics at 387nm, 409nm and 435nm, which correlates roughly to the collagen, elastin, and matrix crosslinking wavelengths seen in our previous results. To design the system, we used finite element modeling and ray tracing to analyze the effect of LED’s thermal strain on optical alignment. The optical mounts of the system is then 3D printed to 50 µm accuracy, critical for microoptic alignment and efficient coupling. Collagen fluorescence lifetimes was then quantified based on the phase shift and demodulation. The “tissue matrix spectroscopy gun,” was designed with portability for clinical applications in mind, and rapid prototyped by stereolithography 3D printing.

9711-52, Session 8

Quantitative image cytometry measurements of lipids, DNA, CD45 and cytokeratin for circulating tumor cell identification in a model system

Gregory Louis Futia, Lubna Qamar, Kian Behbakht, Emily A. Gibson, Univ. of Colorado Denver (United States)

Circulating tumor cell (CTC) identification has applications in both early detection and monitoring of solid cancers. The rarity of CTCs, expected at 1-10 CTCs per million nucleated blood cells (WBCs), requires identifying methods to be based around biomarkers with high sensitivity and specificity for accurate identification. Discovery of biomarkers with ever higher sensitivity and specificity to CTCs is desirable to potentially find more CTCs in more cancer patients thus increasing their clinical utility. Here, we investigate adding image cytometry measurements of lipids to the biomarker panel of DNA, Cytokeratin (CK), and CD45 commonly used to identify CTCs. We engineered a device for labeling suspended cell samples with fluorescent antibodies and dyes. We used it to prepare samples for 4 color confocal laser scanning microscopy. The total data acquired at high resolution from one sample is ~ 1.3 GB. We developed software to perform the automated segmentation of these images into regions of interest (ROIs) containing individual cells and quantified features including total signal, spatial second moment, spatial frequency second moment, and their product for each ROI. We performed a test of pure WBCs, cancer cell line MCF7 and mixed samples. We performed multivariable regressions and feature selection to produce combination features that are more sensitive and specific than any of the individual features alone. We also demonstrate that computation of spatial characteristics is better than intensity alone.

We compare the measured sensitivity and specificity to what is required for detecting small levels of CTCs in a human blood sample.
Correlated phosphorescence and fluorescence lifetime imaging for cell metabolism (Keynote Presentation)

Angelika C. Rueck, Jasmin Bryemayer, P. Schäfer, Bjorn von Einem, Christine A. F. von Arnim, Sviatiana Kalinina, Univ. Ulm (Germany)

Correlated imaging of phosphorescence and fluorescence lifetime parameters of metabolic markers is a challenge for direct investigating mechanisms related to cell metabolism and oxygen tension. A large variety of clinical phenotypes is associated with mitochondrial defects accomplished with changes in cell metabolism. In many cases the hypoxic microenvironment of cancer cells shifts metabolism from oxidative phosphorylation (OXPHOS) to anaerobic or aerobic glycolysis, a process known as “Warburg” effect. Also during stem cell differentiation a switch in cell metabolism is observed. A defective mitochondrial function associated with hypoxia has been invoked in many complex disorders such as type 2 diabetes, Alzheimers disease, cardiac ischemia/reperfusion injury, tissue inflammation and cancer.

Cellular responses to oxygen tension have been studied extensively, optical imaging techniques based on time correlated single photon counting (TCSPC) to detect the underlying metabolic mechanisms are therefore of prominent interest. They offer the possibility by inspecting fluorescence decay characteristics of intrinsic coenzymes to directly image metabolic pathways. Moreover oxygen tension can be determined by considering the phosphorescence lifetime of a phosphorescent probe. The combination of both fluorescence lifetime imaging (FLIM) of coenzymes like NADH and FAD and phosphorescence lifetime (PLIM) of phosphorescent dyes could provide valuable information about correlation of metabolic pathways and oxygen tension.

Within this presentation the basic mechanisms and relations of FLIM, PLIM and cell metabolism will be discussed and clinically relevant applications will be demonstrated. This includes investigations on Alzheimers related disease, tumour diagnosis as well as Sepsis control.

Depth-resolved incoherent and coherent wide-field high-content imaging (Keynote Presentation)

Peter T. C. So, Massachusetts Institute of Technology (United States)

Recent advances in depth-resolved wide-field imaging techniques have enabled many high throughput applications in biology and medicine. Depth resolved imaging of incoherent signals can be readily accomplished with structured light illumination or nonlinear temporal focusing. The integration of these high throughput systems with novel spectroscopic resolving elements further enable high-content information extraction. We will introduce a novel near common-path interferometer and demonstrate its uses in toxicology & cancer biology applications. The extension of incoherent depth-resolved wide-field imaging to coherent modality is non-trivial. Here, we will cover recent advances in wide-field 3D resolved mapping of refractive index, absorbance, and vibronic components in biological specimens.

Biomedical applications of SRS microscopy (Keynote Presentation)

Xiaoliang S. Xie, Harvard Univ. (United States)

Stimulated Raman scattering (SRS) microscopy is a label-free and noninvasive imaging technique using vibration spectroscopy as the contrast mechanism. Recent advances have allowed significant improvements in sensitivity, selectivity, robustness, and cost reduction, opening a wide range of biomedical applications. In particular, it provides instant tissue examination without the need of previous histological staining, and is best suited for imaging small metabolite molecules. An overview will be given to a variety of biomedical applications of SRS microscopy.

Label-free vibrational stark imaging of neuronal membrane potential (Invited Paper)

Ji-Xin Cheng, Purdue Univ. (United States)

Multisite measurement of membrane potential in neurons remains a challenge. Patch clamp techniques have limited number of simultaneous patches on a single cell. Current optical recording strategies utilize fluorescent labels, which have limitations including delivery, toxicity, and perturbation of membrane properties. We identified an intrinsic spectroscopic signature of membrane potential by hyperspectral stimulated Raman scattering imaging of live neurons. A significant decrease of stimulated Raman signal from CH3 stretch at 2930 wavenumber was found when the holding potential increased from negative 60 mV (resting potential) to 30 mV (depolarization). The space clamp effect was revealed by stimulated Raman mapping of the whole neuron during electrical stimulation. High-speed stimulated Raman imaging allowed direct visualization of instant depolarization of live neurons upon KCl puffing of brain slices, as confirmed by a simultaneous calcium response. The observed signature was found to be partially contributed by the sodium channels and is interpreted as a vibrational stark effect. These results collectively demonstrate the possibility of monitoring neural activities at multiple sites by label-free vibrational microscopy.

Phenotype classification of single cells using coherent Raman microscopy and RNA sequencing

Aaron M. Streets, Chen Cao, Xiannian Zhang, Yanyi Huang, Peking Univ. (China)

Phenotype classification of single cells reveals biological variation that is masked in ensemble measurement. This heterogeneity is found in gene and protein expression as well as in cell morphology. Many techniques are available to probe phenotypic heterogeneity at the single cell level, for example quantitative imaging and single-cell RNA sequencing, but it is difficult to perform multiple assays on the same single cell. In order to directly track correlation between morphology and gene expression at the single cell level, we developed a microfluidic platform for quantitative coherent Raman imaging and immediate RNA sequencing (RNA-Seq) of single cells. With this device we actively sort and trap cells for analysis.
with stimulated Raman scattering microscopy (SRS). The cells are then processed in parallel pipelines for lysis, and preparation of CDNA for high-throughput transcriptome sequencing. SRS microscopy offers three-dimensional imaging with chemical specificity for quantitative analysis of protein and lipid distribution in single cells. Meanwhile, the microfluidic platform facilitates single-cell manipulation, minimizes contamination, and furthermore, provides improved RNA-Seq detection sensitivity and measurement precision, which is necessary for differentiating biological variability from technical noise. By combining coherent Raman microscopy with RNA sequencing, we can better understand the relationship between cellular morphology and gene expression at the single-cell level.

9712-6, Session 2

Biological application of stimulated Raman scattering microscopy in modeling lipid dynamics (Invited Paper)

Yong Yu, Baylor College of Medicine (United States); Dan Fu, Univ. of Washington (United States); Harrison Liu, Univ. of California, San Francisco (United States); Xiaoliang S. Xie, Harvard Univ. (United States); Bo Huang, Univ. of California, San Francisco (United States); Meng Wang, Baylor College of Medicine (United States)

Lipid molecules are crucial for various cellular responses, and their misregulation results in such human diseases as metabolic disorders, neurodegenerative diseases, and cancers. As in proteins, the physiological and pathological activities of lipid molecules are tightly associated with their spatial distribution and temporal dynamics. The ability to track specific lipid molecules in vivo is essential for understanding their physiological impacts and regulatory mechanisms. Using stimulated Raman scattering (SRS) microscopy, we quantitatively imaged lipid distribution at the whole organism level in live Caenorhabditis elegans. Using hyperspectral SRS microscopy, we profiled lipid composition at the single lipid droplet level in living cells and revealed the changes of lipid composition in association with hepatic steatosis. Through incorporation of isotopic labeling/tracing into SRS microscopy, we directly visualized the temporal dynamics of specific lipid molecules during their transportation between cells and tissues. Taking the advantage of photohighlighting, we further conducted SRS-based genome-wide mutagenesis screens in C. elegans, and identified 58 genetic mutants that confer different phenotypes in lipid content and distribution. We mapped four genes into the Sma/Mab TGF-ß related pathway, and revealed that they confer different phenotypes in lipid content and distribution. We have successfully applied this technique to identify differences in lipid saturation distributions in selective C. elegans mutants and demonstrated that the technique is sufficiently sensitive to detect the effects of lipid metabolism altering drugs on wild type C. elegans.


9712-7, Session 2

In vivo lipid saturation study of C. elegans using quantitative broadband coherent anti-stokes Raman imaging

Bradley Littleton, Thomas Kavanagh, Yu Nie, Vincenzo Abbate, Peter Hylands, Stephen Sturzenbaum, David R. Richards, King’s College London (United Kingdom)

In vivo lipid saturation maps of microscopic nematodes (Caenorhabditis elegans) have been produced using our novel Spectral Interferometric Polarisation Coherent anti-Stokes Raman Scattering (SIP-CARS) imaging technique. This technique employs simple passive polarization optics and a balanced homodyne detection scheme to exploit symmetries in the CARS polarization response resulting in the complete cancellation of the non-resonant background (NRB) and real component of the CARS signal (with no prior or post assumptions as regards to their form). The remaining imaginary component of the CARS response is linear with analyte concentration and directly relatable to the spontaneous Raman spectrum [1]. Furthermore, the resonant CARS signal is interferometrically amplified by the non-resonant response, a necessity for rapid imaging at biologically relevant powers [2]. This technique permits acquisition of a broad NRB-free spectrum, in excess of 1800cm⁻¹, in a single exposure at each pixel. This allows simultaneous determination of lipid droplet saturation, from the fingerprint region, and lipid order, from the C-H stretch region from which maps can be readily constructed. Additionally exploiting the dispersive nature of our signal collection two-photon autofluorescence can be isolated and images subsequently produced.

We have successfully applied this technique to identify differences in lipid saturation distributions in selective C. elegans mutants and demonstrated that the technique is sufficiently sensitive to detect the effects of lipid metabolism altering drugs on wild type C. elegans.


9712-8, Session 2

Limitations and solutions for polarization resolved in-depth nonlinear vibrational imaging in biological tissues

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Quantifying lipid orientational order in biological tissues is of high interest for both biology and biomedical communities, for instance to access molecular scale information in the study of neurodegenerative diseases involving myelin sheath deficiencies. This information is gained by polarization analysis of nonlinear Coherent Raman Scattering emission signals, generally by tuning incident/detected polarizations. The high order of nonlinear light-matter interactions brings in particular a high level of detail on molecular orientational distributions within the focal spot of a microscope objective. Still limitations exist in this method. First, brain or spinal cord tissues are strongly scattering, which can alter light polarization. We show, using Coherent anti-Stokes Raman scattering (CARS) as a local probe for light depolarization, that polarization resolved CARS (pCARS) microscopy is robust with respect to depolarization, even at depths reaching tens of scattering mean free paths in the sample, providing that un-analyzed acquisition times required by polarization tuning. To address this issue, we introduce a novel scheme based on incident/detected circular polarizations that can adequately filter out molecular order properties without the need to tune any polarization. Using such scheme, we demonstrate how to optimally extract information on the orientational order of vibrational molecular bonds in complex biological samples using polarized CRS microscopy modalities.

9712-9, Session 3

Broadband CARS: instrumentation, quantitation, and application (Invited Paper)

Marcus T. Cicerone, Charles H. Camp Jr., National Institute of Standards and Technology (United States)

Coherent Raman imaging methods have been under development for almost 15 years. The field is beginning to mature, transitioning from a “new techniques” phase to an applications phase. I will discuss current capabilities
of broadband coherent anti-Stokes Raman scattering (BCARS) microscopy using optimized excitation paradigms, and provide a few examples of how broadband BCARS imaging has helped to answer (or raise) questions in investigations of tissues and small organisms. I will also discuss progress in processing BCARS spectra to make them independent of excitation profile or non-resonant response, and directly comparable to spontaneous Raman spectra. I will also discuss progress on a new approach to time-domain BCARS that promises to significantly simplify and speed BCARS data acquisition.

9712-10, Session 3

Intraoperative stimulated Raman scattering microscopy for guidance during brain tumor surgery

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Surgery is an essential aspect in the treatment of brain tumors. However, surgeons lack a reliable method for delineating tumor from normal brain during surgery. Here we describe the use of a fiber-laser based system for stimulated Raman scattering (SRS) microscopy as a label-free means of detecting brain tumor infiltration in 40 patients during brain tumor surgery. SRS microscopy delineates tumor from normal brain based on histologic and biochemical differences with clarity that parallels traditional hematoxylin and eosin staining. Distinctive histopathological features of primary and secondary brain tumors are readily apparent. The degree of brain tumor infiltration can be measured quantitatively and predicted via quantified SRS imaging data. These results demonstrate the potential of SRS microscopy as a clinical method for differentiating tumor from normal brain during surgery. Future work will focus on developing a surgical workflow and technical refinements to optimize the incorporation of SRS images into the clinical decision making process. By providing the surgeons with a means for rapid histologic assessment of the operative field, SRS microscopy may ultimately improve the safety and accuracy of brain tumor surgery.

9712-11, Session 3

Histological reconstruction, guided microdissection, and sequencing of cancer tissue using stimulated Raman scattering (SRS) microscopy

Tao Chen, Chen Cao, Biodynamic Optical Imaging Ctr. (China); Jianyuan Zhang, Peking Univ. School of Stomatology (China); Yanyi Huang, Biodynamic Optical Imaging Ctr. (China); Tiejun Li, Peking Univ. School of Stomatology (China)

In cancerous tissue, morphology of malignant cells exhibits abnormal features, including high karyoplasmic ratio, nuclear atypia. These abnormalities provide contrast that can help distinguish cancerous tissue from normal tissue. Hematoxylin and Eosin staining (HE staining) can reveal the morphology of cells in tissue slices by assigning different colors to nucleus and cytoplasm. HE staining is considered the gold standard in clinical cancer diagnosis. The multi-step chemical processes required for HE staining causes degradation of certain macromolecules, including RNA. This becomes a limitation when further biological information is desired from the sample like gene expression patterns. To preserve the RNA during imaging, we applied stimulated Raman scattering microscopy, a label-free multi-photon technique, to investigate the morphology of freshly sliced oral cancer tissue. Using multi-band vibration imaging, we were able to clearly distinguish various tissue types from one another. We then used in situ laser micro-dissection to isolate tissue fractions of interest containing roughly hundreds of cells. DNA and mRNA were successfully recovered from malignant and normal cells, as identified by SRS, and prepared for sequencing. Bioinformatic analysis showed a correlation between genome copy number variations and gene expression levels. Moreover, gene fusion was analyzed, and over 40 fusion pairs were identified. Among these pairs, more than ten had over ten copies each. Additionally, two fusion pairs were found to be associated with oncogenes. SRS based histological reconstruction enabled label-free tissue identification, and the acquisition of high quality genomic material for sequencing. This novel approach provides retention of spatial information in RNA sequencing studies, and hopefully can facilitate related biomedical research.

9712-12, Session 3

Stimulated Raman scattering imaging of metabolic activities of mammalian brain tissue (Invited Paper)

Fanghao Hu, Lu Wei, Michael R. Lamprecht, Barclay Morrison, Wei Min, Columbia Univ. (United States)

On the physiology level, brain activities comprise both relatively fast calcium or voltage signals and relatively slow metabolic conversion of small metabolites. While the former has been extensively probed by fluorescent techniques, visualizing cellular metabolism of small biomolecules (e.g., amino acids, nucleic acid, and fatty acids) has been a long-standing challenge. These chemical species either lack intrinsic imaging contrast or are easily prone to perturbation or inactivation with relatively bulky fluorescent labels. Novel imaging techniques that accomplish this goal would enable researchers the unprecedented ability to map out distributions and to follow dynamics of a wide variety of important small metabolites. Recently, our group has demonstrated imaging of small biomolecules in cell cultures. Here, we present our current progress in metabolic imaging of live rat brain hippocampal tissues, and apply this platform to study metabolic responses in a traumatic brain injury model. By coupling stimulated Raman scattering microscopy with integrated deuteron and alkyne labeled small metabolites, active metabolisms of protein, lipids and nucleic acids are visualized in both the dentate gyrus and CA regions of rat hippocampus tissues. Heterogeneous patterns of metabolic incorporation are observed for different metabolite species. By targeting at DNA synthesis, newly-generated neurons is highlighted at the single cell level and symmetric proliferating cell division is observed in the subgranular zone of dentate gyrus, where active neurogenesis occurs. Traumatic brain injury is known as the leading cause of disability. Compared to uninjured tissues, increased protein and lipid synthesis are found in rat hippocampal tissues undergoing mechanical stretch injury. Therefore, by achieving metabolic imaging of mammalian brain tissues with subcellular resolution and minimal perturbation, our method will allow the study of metabolic profiles in many neurologically crucial conditions.

9712-13, Session 3

Hyperspectral stimulated Raman scattering and multiphoton imaging for label-free colonic diseases diagnosis and characterization

Zi Wang, Wei Zheng, Jian Lin, Zhiwei Huang, National Univ. of Singapore (Singapore)

Histopathology examinations of H&E stained biopsied tissues is the golden standard for colonic diseases (e.g., polyps, adenoma, and adenocarcinoma) diagnosis. However, staining effect of sample and doctor’s expertise degree may greatly influence the diagnosis results. The information provided by the H&E stained sample is also limited to the morphological and PH information.
and no quantitative information is available. In this paper, we report the development of a unique multimodal nonlinear optical microscopy (i.e., hyperspectral stimulated Raman scattering (hSRS), second-harmonic generation (SHG), two-photon excitation fluorescence (TPEF)) platform for the diagnosis and characterization of colonic diseases. hSRS in both the fingerprint (800-1800 cm⁻¹) and high-wavenumber (2800-3600 cm⁻¹) regions allows us to discriminate different constituents with tiny difference in the Raman spectra. Independent component analysis (ICA) is applied to analyze the hSRS image to extract the distribution of proteins and lipids. The increase of proteins and reduction of lipids could be observed with the progress of colonic cancer. SHG shows the distribution of collagen, which is found to aggregate for adenocarcinoma. TPEF provides the cell morphological and can reflect the damage inside glands caused by the diseases. This work shows that the integrated hSRS and TPEF/SHG images can be an effective means for label-free diagnosis and characterization of gastric diseases at the cellular and molecular levels.

9712-14, Session 4

Biomedical lipid imaging in zebrafish with stimulated Raman scattering microscopy

Miriam J. Moester, Marjo J. den Broeder, Freek Ariese, Juliette Legler, Johannes F. de Boer, Vrie Univ. Amsterdam (Netherlands)

We show the potential of Stimulated Raman Scattering (SRS) microscopy for imaging in living organisms and cells. Our flexible SRS set up with a custom built detector allows shot noise limited detection over a broad range of biologically acceptable laser intensities. In SRS, energy is transferred from one color laser beam to another, provided the energy difference between them matches a molecular vibration of a molecule in focus. By applying amplitude modulation to one of the beams, modulation transfer can be measured. Combined with laser scanning microscopy, this technique allows for fast and sensitive imaging with sub-micrometer resolution. SRS microscopy provides a new, label-free research tool for investigating biomolecules in living organisms with many advantages compared to fluorescence staining, including no interference with biological function and no photobleaching. It is particularly suitable for imaging lipids in the C=H stretch region around 2900 cm⁻¹, where abundance of the relevant molecular bonds is high and Raman scattering cross-sections are large. Lipid metabolism in zebrafish is of particular interest as a model system for human healthy fat processing and disease. Storing excess energy as neutral lipid is an evolutionarily highly conserved characteristic. Vertebrates store lipid in adipocytes, a cell type specialized for storing fat in intracellular neutral lipid droplets. Using SRS microscopy, we investigate adipocytes in zebrafish exposed to different diets and certain toxic compounds known to have an effect on adipocyte development. Changes not only in adipocyte prevalence but also in other quantifiable parameters such as volume can be visualized with SRS microscopy.

9712-15, Session 4

Surface-sensitive coherent Raman scattering microscopy with total internal reflection illumination (Invited Paper)

Eric O. Potma, Alex Fast, Univ. of California, Irvine (United States); Christopher D. Syme, Univ. of Glasgow (United Kingdom)

We have developed a coherent Raman scattering microscope that combines total internal reflection illumination with surface plasmon resonance. The excitation geometry is based on an objective-type Kretschmann configuration, which allows widefield excitation of surface plasmon polariton modes in a thin gold film on a glass substrate. The surface plasmon fields enhance the excitation efficiency, enabling image acquisition at 10 frames/s. Since the evanescent field extends only over a length scale of ~100 nm, structures close the substrate surface are observed while bulk contributions are suppressed. We discuss the operational principles of this microscope in detail and point out its applications in cell biology.

9712-16, Session 4

Dispersion-based stimulated Raman scattering spectroscopy, holography, and optical coherence tomography

Francisco E. Robles, Martin C. Fischer, Warren S. Warren, Duke Univ. (United States)

Stimulated Raman scattering (SRS) enables fast, high resolution imaging of chemical constituents important to biological structures and functional processes. While this technology has shown remarkable potential, it is currently limited to point scans and can only probe a few Raman bands at a time. In this work we take a fundamentally different approach to detecting the small nonlinear signals based on dispersion effects that accompany the loss/gain processes in SRS. We use a modified pump-probe system (pulses with duration of ~0.5 ps and 75 fs, respectively) with interferometric detection in the Fourier-domain to demonstrate that the dispersive measurements are more robust to noise (e.g., laser noise) compared to conventional amplitude measurements, which in turn permits facile spectral and spatial multiplexing. Results show that it is possible to assess a broadband dispersion spectrum (currently limited to ~400 cm⁻¹) with a single laser shot or spectrometer acquisition (20-50 fs). For molecular imaging with broadband spectral information, we achieve spatial pixel rates of 2.5 kHz, and will discuss how this can be further improved to 20-50 kHz. We also combine SRS with optical coherence tomography (OCT) (molecular and structural information are rendered from the same data), which enables axial multiplexing by coherence gating and paves the way for volumetric biochemical imaging. The approach is tested on a thin water-and-oil phantom, a thick scattering polystyrene bead phantom, and thick freshly excised human adipose tissue. Finally, we will outline other opportunities for spatial multiplexing using wide-field holography and spectroscopic-OCT, which would massively parallelize the spatial and spectral information. The combination of dispersion-based SRS and phase imaging has the potential to enable faster wide-area and volumetric molecular imaging. Such methods would be valuable in a clinical setting for many applications.

9712-17, Session 4

Polarization modulated background-free hyperspectral stimulated Raman scattering microscopy

Marie-Andrée Houle, Institut National de la Recherche Scientifique (Canada); Marco Andreana, National Research Council Canada (Canada) and Univ. of Ottawa (Canada); Andrew Ridsdale, Douglas J. Moffatt, Rune Lausten, National Research Council Canada (Canada); François Légaré, Institut National de la Recherche Scientifique (Canada); Albert Stolow, National Research Council Canada (Canada) and Univ. of Ottawa (Canada)

Stimulated Raman Scattering (SRS) microscopy is a nonlinear microscopy technique based on Raman vibrational resonances determined by the frequency difference between Pump and Stokes laser pulses. Modulation of one laser beam transfers the modulation to the other, as either a gain in Stokes (SRS) or a loss in Pump power (SRL). SRS microscopy does not exhibit the four-wave mixing nonresonant background characteristic of CARS microscopy. However, other background signals due to two-photon
absorption, thermal lensing or cross-phase modulation (XPM) do reduce the detection sensitivity and can distort the hyperspectral scans. Phase sensitive lock-in detection can reduce contributions from two-photon absorption, which is out-of-phase for the SRG case. However, the background signal due to XPM, which can be in-phase with SRS, can reduce the detection sensitivity.

We present a novel polarization modulation (PM) scheme in SRS microscopy which greatly reduces the nonresonant XPM background, demonstrated here for the SRL case. Since many Raman vibrational transitions are parallel polarized, the SRS signal is maximum (minimum) when the polarizations of the pump and the Stokes beams are parallel (perpendicular). However, in both parallel and perpendicular Pump-Stokes geometries, XPM is non-zero in many media. Therefore, PM can remove the XPM background without significantly reducing the SRS signal. Our results show that the PM-SRS successfully removes the nonresonant signal due to XPM. High imaging contrast is observed, concomitant with high sensitivity at very low analyte concentrations and undistorted Raman spectra.

9712-18, Session 4

Resonant artefacts in broadband cars microspectroscopy

Bradley Littleton, Thomas Kavanagh, David R. Richards, King’s College London (United Kingdom)

Broadband coherent anti-Stokes Raman scattering (B-CARS) allows the acquisition of an entire vibrational spectrum in a single exposure, with millisecond exposure times and high signal-to-noise ratio in biological samples [1]. However, a full analysis of signal contributions shows that the cross-correlations and convolutions of broadband fields and coherences gives rise to significant resonant spectral artefacts, in addition to the well-known non-resonant background (NRB). These artefacts result in a sample-dependent modulation of vibrational line amplitudes, and contribute to the phase errors commonly seen in computational NRB removal techniques. Without accounting for these backgrounds broadband CARS does not give accurate concentration information.

We have used a spectral interferometry method that gives the imaginary third-order response of a medium (Spectral Interferometric Polarisation CARS [2]) to directly show the resonant background signal contributions. It has recently been shown that efficient B-CARS signal generation can be achieved using a hybrid 2-colour and 3-colour excitation scheme [1]: we find that, with certain conditions on the Stokes spectrum, resonant backgrounds can be avoided in the 2-colour (C-H stretch) spectral region, but not in the 3-colour (fingerprint) region. Artefacts in the fingerprint region arise from spectrally downshifted contributions from the C-H region; the correction to that, with certain conditions on the Stokes spectrum, resonant backgrounds can be avoided in the 2-colour (C-H stretch) spectral region, but not in the 3-colour (fingerprint) region. Artefacts in the fingerprint region arise from spectrally downshifted contributions from the C-H region; the correction to this fingerprint spectrum can therefore be calculated from the C-H region spectrum in a preprocessing step, allowing fully quantitative B-CARS measurements.


9712-19, Session 4

Electric field mapping and dosimetry by means of spontaneous and coherent Raman microspectroscopy

Erwan Capitaine, Christophe Louot, Claire Lefort, Dominique Pagnoux, Vincent Couderc, Philippe Leproux, XLIM Institut de Recherche (France)

We demonstrate the use of spontaneous and coherent Raman microspectroscopy for mapping and measuring the electric field in a biological sample at the microscopic scale. We first study the level of Raman signal for molecular vibrations of paraffin, whose orientation is fixed by applying a continuous electric field. To do so, a potential difference is set between two electrodes and the paraffin is placed between the latter. A polarization-resolved Raman microspectroscope is used to produce maps of the Raman scattering intensity of the paraffin molecular bonds for different orientations of the incident laser polarization vector. By this way, the mean orientation of each bond - and consequently the orientation of the electric field - can be determined in each pixel of the mapping. The experiment is then reproduced for other sets of electrodes with different geometries. The obtained electric field maps are compared to numerical simulations. The same experimental study is realized in the case of coherent anti-Stokes Raman scattering (CARS), which shows a higher sensitivity to the orientation of molecular bonds. The physical phenomena at the origin of this difference between spontaneous Raman and CARS are discussed. Finally the possibility of measuring the electric field intensity in a biological cell is introduced.

9712-20, Session 4

Synchronized and timing-stabilized pulse generation from a gain-switched laser diode for stimulated Raman scattering microscopy

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Stimulated Raman scattering (SRS) microscopy allows for high-speed label-free biomedical imaging. One of the remaining issues in SRS microscopy is to develop compact and easy-to-use laser sources that can generate two-color synchronized picosecond pulses. Recently, gain-switched laser diodes (GS-LDs) are regarded as compact light sources that can generate stable picosecond pulses. In fact, we previously demonstrated SRS imaging of polymer beads by using a GS-LD and a Ti:sapphire (Ti:S) laser, which were synchronized by driving the GS-LD with electrical pulses triggered by the photocurrent generated from Ti:S laser pulses. However, the timing of GS-LD pulses was unstable, and was affected by Ti:S laser’s power fluctuation. This is because the time required for the photocurrent to exceed the threshold value of the triggering circuit is dependent on the amplitude of the photocurrent. Here, we demonstrate a method for stabilizing the timing of GS-LD pulses. The photocurrent is launched to the triggering circuit, while its threshold value is varied according to the average photocurrent. We successfully suppressed the timing changes of the output waveform of the triggering circuit within -0.5 ps for the variation in Ti:S laser power of ~40 %. As a result, GS-LD pulses sufficiently overlapped with Ti:S laser pulses while we scanned the wavelength of the Ti:S laser pulses in the range of -10-nm. We believe that the presented technique is important for applying GS-LDs to SRS microscopy for realizing compact and practical laser sources.

9712-28, Session P5Un

Multiphoton fluorescence lifetime imaging of metabolic status in mesenchymal stem cell during differentiation

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The non-invasive imaging of cell metabolism within tissues to assess the efficacy of stem cell therapy and understanding the tissue development...
is of great interest. In this study we correlated metabolic trajectory of the mesenchymal stem cell (MSC) differentiation with changes in fluorescence lifetimes of free and bound forms of the reduced nicotinamide adenine dinucleotide (NADH), ratio unphosphorylated and phosphorylated forms of bound NADH, optical redox ratio of (NADH+FAD)/FAD using laser scanning microscopy combined with fluorescence lifetime imaging. Undifferentiated and adiogenically differentiated human MSCs were imaged with a Zeiss 710 microscope coupled to a FLIM system. The intrinsic fluorescence of NADH and FAD was excited at 750 nm and 900 nm respectively by a femtosecond Ti:sapphire laser. The data were analyzed with the commercially available SPCImage software.

The FLIM parameters of undifferentiated and adiogenically differentiated MSCs were obtained. The changes in the NADH fluorescence lifetime from the undifferentiated MSCs to the differentiated adipocytes were confirmed in a series of samples. The short and long NADH lifetime fluorescence, long lifetime contribution were increased in the differentiated cells. Also the lifetime contribution ratio of unphosphorylated and phosphorylated forms decrease in 2 times in adipocytes. Optical redox ratio of (NADH+FAD)/FAD decrease in adiogenically differentiated MSCs, that agreed well with the literature.

These results indicate switching pathway from glycolysis to oxidative phosphorylation, an increase metabolism and an activation of fatty acid biosynthesis during the differentiation process.

9712-58, Session PSun

Evaluation of collagen by second-harmonic generation microscopy support the heterogeneity of luminal breast cancer

Rodrigo de Andrade Natal, Vitor B. Pelegati, Caroline Bondarik, Luís O. Z. Sarian, Sophie F. M. Derchain, Carlos L. César, José Vassallo, Univ. Estadual de Campinas (Brazil)

Second harmonic generation (SHG) microscopy has provided a breakthrough in collagen research. In breast cancer (BC), collagen has been described as a potential prognostic biomarker. Despite luminal type BC has been associated with favorable prognosis and better response to treatment, some patients develop treatment resistance and more aggressive tumors, meaning that luminal type BC may be heterogeneous. Our aim is to evaluate the clinical value of assessing the quantity and organization of intratumoral (IT) and peritumoral (PT) collagen in luminal type BC. H&E stained slides from 34 patients with invasive ductal BC of the luminal A and B types underwent collagen analysis in an Inverted Zeiss LSM 780-NLO microscope. Collagen quantity and organization of IT and PT areas were compared using the t-test, and their relation with event-free survival (EFS) and overall survival (OS) using COX proportional hazards. Kaplan-Meier curves were used for survival analysis and the log-rank test to compare the curves. R was used as statistical program. PT collagen fibers were detected in larger quantity (p<0.001) and in a more organized distribution (p<0.001) than in IT areas. Further, COX proportional hazards showed that larger IT collagen fiber quantity was associated with more favorable EFS (p=0.004). Analyzing Kaplan-Meier curves (mean value), larger IT collagen fiber quantity was also associated with more favorable EFS (p= 0.017). Our results support the heterogeneity of luminal type BC, and that the assessment of intratumoral collagen fiber quantity is a potential prognostic marker for this tumor.

9712-59, Session PSun

In vivo imaging flow cytometry based on two-photon microscopy at kHz cross-sectional frame rate

Lingjie Kong, Meng Cui, Purdue Univ. (United States)

In vivo optical imaging of circulating cells provides the opportunity for biomedical researchers to visualize the morphology and dynamics of the cells trafficking in the vasculature system at high spatial resolution, but is usually limited by the imaging depth and speed. Laser scanning two-photon microscopy (TPM) is widely adopted for its superb imaging depth. However, for applications in flow cytometry where the cross-sections of vasculature should be imaged, the axial scanning speed of current TPM methods is far from optimal. We integrated an optical phase-locked ultrasound lens into a TPM and achieved microsecond-scale axial scanning, which enabled kHz cross-sectional frame rate. We demonstrate its applications in in vivo imaging flow cytometry, and show its ability in distinguishing cells and clusters, imaging cells of different morphologies, and counting ratio of cells with different fluorescent labeling. We expect its applications in the studies of circulating tumor cell detection, immunity cell migration mechanism, and stoke etc.

9712-60, Session PSun

Comparison of in vivo-ex vivo imaging of the microvasculature with 2-photon fluorescence microscopy

Joe Steinman, Univ. of Toronto (Canada) and The Hospital for Sick Children (Canada); Margaret Koletar, Sunnybrook Research Institute (Canada); Bojana Stefanovic, Sunnybrook Research Institute (Canada) and Univ. of Toronto (Canada); John G. Sled, Univ. of Toronto (Canada) and The Hospital for Sick Children (Canada)

This study evaluates ex vivo 2-Photon Fluorescence Microscopy of cleared samples for visualizing cortical vasculature, following perfusion with a FITC gel and clearing in fructose.

Four mice brains were imaged with in vivo 2PFM. The same regions imaged in vivo were imaged ex vivo. Vessels were segmented automatically in both images using an in-house developed algorithm that accounts for the anisotropic and spatially varying PSF ex vivo. Through non-linear warping, the ex vivo image and tracing were aligned to the in vivo image. The corresponding vessels were identified through a local search algorithm. This enabled comparison of identical vessels in vivo/ex vivo. A similar process was conducted on the in vivo tracing to determine the percentage of vessels perfused. Of all the vessels identified over the four brains in vivo, 98% were present ex vivo. The ex vivo estimate of the capillary diameter was 20% lower than that in vivo (3.2 ± 0.9 μm vs. 4.0 ± 1.1 μm). Furthermore, large diameter surface vessels attenuated in vivo signal from deeper cortical vessels by 43% (mean non-attenuated vessel signal 1600 ± 1000 vs. 900 ± 650 for attenuated vessels from 100-200 μm below the cortical surface), which does not occur ex vivo.

In summary, though vessel diameters shrink ex vivo, ex vivo imaging has reduced signal attenuation by surface vessels. Additionally, since imaging depths are only limited by the working distance of the microscope objective, ex vivo imaging is more suitable for imaging large portions of the brain.

9712-61, Session PSun

Four-wave mixing based light sources for real-world biomedical applications of coherent anti-stokes Raman scattering microscopy

Thomas Gottschall, Tobias Meyer, Michael Schmitt, Friedrich-Schiller-Univ. Jena (Germany); Jürgen Popp, Friedrich-Schiller-Univ. Jena (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany); Jens Limpert, Friedrich-Schiller-Univ. Jena (Germany); Andreas Tünnermann, Friedrich-Schiller-Univ. Jena (Germany) and Fraunhofer Institute for Applied Optics and Precision Engineering (Germany)
During the past decade coherent anti-Stokes Raman scattering (CARS) microscopy has evolved to one of the most powerful imaging techniques in the biomedical sciences, enabling the label-free visualization of the chemical composition of tissue in vivo and real time. While the acquisition of high-contrast images of single cells of large tissue sections enables a wide range of medical applications from routine diagnostics to surgical guidance, to date CARS imaging is employed in fundamental research only, essentially because the synchronized multiple wavelength pulsed laser sources required for CARS microscopy are large, expensive and demand regular maintenance. Laser sources based on optical fibers can overcome these limitations combining highest efficiency and peak powers with an excellent spatial beam profile and thermal stability. We re-evaluate the ideal laser parameters for CARS imaging and discuss the challenge that lies in a fiber based laser source for CARS. Four- wave mixing has been identified as the ideal conversion technique for this challenge and sparked the development of FWM based fiber laser sources. We recap the installments of these sources from unseeded all-fiber four-wave mixing setups and cw-seeded high resolution, low bandwidth laser sources. Finally, we take a look to widely tunable high peak-power FWM based fiber oscillator able to address all of the relevant Raman resonances while outperforming state-of-the-art bulk OPO systems. The combination of FWM based conversion coupled with all-fiber Yb-based fiber lasers are the ideal combination for turn-key laser concepts to be deployed for application of CARS microscopy in clinics.

Jen Lab Young Investigator Award

9712-62, Session PSun
Noninvasive visualization of pheomelanin using coherent Raman scattering microscopy
Hequn Wang, Massachusetts General Hospital (United States); Sam Osseiran, Massachusetts Institute of Technology (United States) and Harvard-MIT Health Sciences and Technology (HST) Program (United States); Elisabeth Roider, David E. Fisher, Massachusetts General Hospital (United States); Conor L. Evans, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Melanoma is the most deadly form of skin cancer with an increasing incidence rate. Recent studies suggest that pheomelanin (one of the two natural pigments of the skin) may play an important role in melanomagenesis. However, characterization of pheomelanin inside the skin has been hampered due to its poor visibility to human eyes and a lack of antibodies or stains. The goal is to develop a non-invasive, label-free, high resolution imaging system to image and characterize pheomelanin in vivo. Coherent Raman scattering (CRS) offers the ability to selectively tune into molecular vibrations and provide label-free, high-resolution, depth-resolved imaging in real time, which may be suitable for identifying pheomelanin in intact skin. A CRS system was designed and developed. The light source is an ultrafast laser system, which emits a fixed wavelength at 1040 nm as well as a tunable beam ranging from 680-1300 nm. The excitation light is then directed into a commercial confocal laser-scanning microscope. Here, we report CRS imaging and spectroscopic measurements focusing on synthetic pheomelanin, pheomelanin in cultured Si-RNA knockdown melanoma cells, melanocytes extracted from redhead mice, ex vivo and in vivo ear tissue from redhead mice. We also demonstrate the capability of CRS to identify pheomelanin inside amelanotic melanoma patient samples. Distinctive features on the acquired images were identified to serve as a potential cancer biomarker. We believe the developed approach has great potential to detect pheomelanin inside human skin and aid in early detection of melanoma precursors.

9712-63, Session PSun
The nature of multiphoton fluorescence from red blood cells
Ilyas Saytashev, Michigan State Univ. (United States); Michael Murphy, Wellman Ctr. for Photomedicine (United States); Sam Osseiran, Massachusetts Institute of Technology (United States); Dana M. Spence, Michigan State Univ. (United States); Conor L. Evans, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Marcos Dantus, Michigan State Univ. (United States)

We report on the nature of multiphoton excited fluorescence observed from human erythrocytes (red blood cells RBC’s) and their “ghosts” following 800nm sub-15 fs excitation. The detected optical signal is assigned as two-photon excited fluorescence from hemoglobin. Our findings are supported by wavelength-resolved fluorescence lifetime decay measurements using time-correlated single photon counting system from RBC’s, their ghosts as well as in vitro samples of various fluorophores including riboflavin, NADH, NAD(P)H, hemoglobin. We find that low-energy and short-duration pulses allow two-photon imaging of RBC’s, but longer more intense pulses lead to their destruction.

9712-64, Session PSun
Evaluation of oxidative stress in human skin exposed to common sun filters by two-photon excitation fluorescence (2PEF) and fluorescence lifetime imaging microscopy (FLIM)
Sam Osseiran, Yusuke Suita, Elisabeth Roider, Hequn Wang, David E. Fisher, Conor L. Evans, Massachusetts General Hospital (United States)

Skin cancer, including basal cell carcinoma, squamous cell carcinoma, and melanoma, is the most common form of cancer in North America. Paradoxically, in spite of the continually growing use of sunscreen products over the past decades, skin cancer incidence steadily remains on the rise. One potential explanation for this discrepancy involves the sun filters in sunscreens, which are responsible for blocking harmful ultraviolet radiation. It has been proposed that these agents may produce reactive oxygen species (ROS) at the site of application, thereby generating oxidative stress in skin that gives rise to genetic mutations, thus explaining the rising incidence of skin cancer. To test this hypothesis, five common chemical sun filters (avobenzone, octocrylene, homosalate, octisalate, and oxybenzone) as well as two physical sun filters (zinc oxide compounds) were exposed to human skin samples ex vivo, both with and without UV irradiation. Two-photon excitation fluorescence (2PEF) and fluorescence lifetime imaging microscopy (FLIM) of the skin samples were used to monitor levels of NADH and FAD, two key cofactors in cellular redox metabolism. The relative redox state of the skin was assessed based on the fluorescence intensities and lifetimes of both NADH and FAD. While the sun filters were indeed shown to have a protective effect from UV radiation, it was observed that they also generate oxidative stress in skin, even in the absence of UV light. These results suggest that sun filter induced ROS production requires more careful study, especially in how these reactive species impact the rise of skin cancer.
Two-photon endomicroscopic metabolic imaging via simultaneous autofluorescence lifetime and redox ratio measurement

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Two-photon fluorescence (2PF) microscopy of NADH and FAD redox states, and 2PF lifetime microscopy (FLIM) of free and protein-bound NADH have been proved valuable for metabolic status assessment and early dysplasia detection (such as precancer discrimination). The goal of undertaking redox and lifetime imaging using practical endoscopic devices,although promising to realize real-time prediction and even visualization of tissue histology in vivo, has been hindered by the lack of high-performance two-photon endomicroscopes. We herein report our newly-developed two-photon endomicroscopy system which features high signal collection efficiency and low inherent background, therefore allowing for simultaneous high-resolution two-photon FLIM of NADH and 2PF-intensity-based NADH/ FAD redox ratio measurement. The lifetime measurement is undertaken through the time-correlated single photon counting method, which not only achieves efficient utilization of the limited photon budget, but also enables real-time evaluation of the NADH composition (free or protein-bound) via phasor analysis of NADH decay dynamics. Experimental results showed that our endomicroscopic system could measure lifetime accurately with subcellular resolution (~0.7-µm lateral ~ 6.5 um axial) at a speed of ~3 frames/second on both stained and unstained biological samples. Moreover, cellular metabolic status and its change can be assessed in vivo on rodent models of hepatic ischemia-reperfusion injury without exogenous staining. In summary, our endomicroscope is well positioned to bridge the technical gap between bench-top multiphoton microscopy and flexible multiphoton endoscopy for realizing “optical histology”. Further investigation of other applications on animal models is underway.

In vivo multiphoton imaging measurement of blood-brain barrier permeability during early postnatal brain development in rats

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The blood-brain barrier (BBB) protects the central nervous system by its unique structure between the cerebral blood circulation and the delicate neural environment. But little is known about the BBB solute permeability during early stages of postnatal development in vivo. This study first applied multiphoton imaging to measure the BBB permeability of a brain of postnatal rat pups. Direct measurement of BBB permeability to TRITC-dextran 155 kD (an inert solute with molecular weight representative of humoral polypeptides) was conducted in two different brain regions: the primary motor cortex (MI) and the medial nucleus of the trapezoid body (MNTB) in the auditory brainstem. Immunohistochemistry combined with confocal imaging technique were also applied to quantify the changes of total vessel volume, and interactions between astrocyte end feet processes and endothelial cells between birth (P0) and P20. The results show that in the second postnatal week the BBB permeability decreased significantly in microvessels (diameter < 107m), whereas vascular structure and astrocyte end feet coverage of microvessels increased significantly in both MI and MNTB. Our studies provide the first high-resolution measurements of BBB permeability during development in vivo and suggest a unique relationship between BBB permeability, angiogenesis and astrocyte-endothelial cell interactions, independent of brain region.

Coherent Raman scattering microscopy for probing longitudinal molecular orientations using spirally polarized light

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Coherent Raman scattering (CRS) microscopy is a nonlinear Raman imaging technique that has attracted much interest for imaging tissue and cells because of its advantages such as high sensitivity and biochemical selectivity based on molecular vibrations. It has been proved that CRS microscopy can detect molecular orientation, for example, linearly and circularly polarized coherent anti-Stokes Raman scattering (CARS) can detect transversely orientated molecules; while longitudinally orientated molecules can be detected by radially polarized excitations. However, at the focus of a radially polarized light, besides the longitudinally polarized electric field, the transversely polarized electric field also excites the transversely oriented molecules, and hence deteriorates the specificity of detecting longitudinally orientated molecules. To solve this problem, we propose to use spirally polarized excitations, in which, the transversely polarized pump and Stokes fields are perpendicular to each other, which minimizes the CRS generation from transversely orientated molecules, but enhances the specificity for detecting longitudinally orientated molecules in the samples.

Monitoring and mapping intracellular concentrations of macromolecules by two-photon excited fluorescence lifetime imaging

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Fluorescence lifetime is a characteristic of a given fluorescent molecule and it can be used to quantify a lot of physiological parameters for its sensitivity to the microenvironment of the fluorophores. Moreover, fluorescence lifetime does not depend on dye concentration, photobleaching, light scattering and fluctuation of excitation light intensity. Therefore Fluorescence Lifetime Imaging (FLIM) is more robust than fluorescence intensity-based measurements and allows performing accurate cellular microenvironment analysis. When combined with two-photon excited fluorescence lifetime microscopy (TPEM), FLIM is given the advantages of deeper tissue penetration, efficient light detection, and reduced phototoxicity, etc. Molecular organization of a cell is dynamically transformed reflecting cellular physiological processes and pathologic developments or derived from interactions with drugs. To measure and monitor concentrations of macromolecules in a single cell would offer a unique tool for analysis of cellular processes. One of the most promising approaches for biomolecular concentration studies is measurements of local refractive indexes (RI) in the nuclear compartments using FLIM technique, which is based on an inverse quadratic relation between the fluorescence lifetime of a fluorophore and the local RI. In this paper, we exploited two-photon excited FLIM-based technique for the quantitative measurement and mapping of protein concentrations in the cell in real time. The protein density values in specific cellular compartments were determined and the dynamic changes of concentrations of proteins in subcellular domains over the time during the cell growth were recorded. The approach proposed here may have potential applications in cell biological research and early disease detection.
Observation of tendon repair in animal model using second-harmonic-generation microscopy

Eiji Hase, Takeo Minamikawa, Katsuya Sato, The Univ. of Tokushima (Japan); Mitsuhiro Takahashi, Takamatsu Red Cross Hospital (Japan); Takeshi Yasui, The Univ. of Tokushima (Japan)

Injury in tendons is a trauma difficult to recover the condition before injury. In previous researches, tensile test and staining method have been widely used to elucidate the mechanism of the repair process from the viewpoints of the mechanical property and the histological findings. However, since both methods are destructive and invasive, it is difficult to obtain both of them for the same sample. If both the mechanical property and the histological findings can be obtained from the same sample, one may obtain new findings regarding mechanisms of tendon repair process.

In this paper, we used second-harmonic-generation (SHG) microscopy, showing high selectivity and good image contrast to collagen molecules as well as high spatial resolution, optical three-dimensional sectioning, deep penetration, and without additional staining. Since SHG intensity sensitively reflects the structural maturity of collagen molecule and its aggregates, it will be a good indicator for the repairing degree of the ruptured tendon. From comparison of SHG images between the 4-weeks-repaired tendon and the sound tendon in the animal model, we confirmed that SHG intensity of the repaired tendon was significantly smaller than that of the sound tendon, indicating that the collagen structure in the repaired tendon is still immature. Furthermore, we performed both SHG imaging and the tensile test for the same sample, and confirmed a linear relationship between them. This result shows a potential of SHG light for an indicator of the tensile strength.

In situ quantitative evaluation of osteoblastic collagen synthesis under cyclic strain by using second-harmonic-generation microscope

Oki Matsubara, Eiji Hase, Takeo Minamikawa, Takeshi Yasui, Katsuya Sato, The Univ. of Tokushima (Japan)

In bone tissues, the metabolic activity, called to remodeling, is conducted by osteoblasts and osteoclasts. Although the osteoblastic remodeling activity is influenced by mechanical stimulus from their surroundings, it has been still unclear how mechanical stimulus affects collagen production by osteoblasts. Therefore, it is strongly required to investigate the characteristics of osteoblastic bone matrix production under the mechanical stimulus application for bone regenerative tissue engineering.

Conventionally, the staining method has been widely used to visualize collagen fiber in the tissue. However, the application of the staining method has been often limited to fixed cells due to its invasiveness. Recently, second-harmonic-generation (SHG) microscopy has attracted attentions for in situ visualization of collagen fiber because of less invasiveness, unstaining, and no fixation, as well as high spatial resolution and 3D imaging. Using SHG microscopy, one can track the temporal dynamics of collagen fiber during the cultured period of the sample. We applied cyclic stretch strain to osteoblasts (MC3T3-E1) by using originally developed cell stretching device. The amount of strain was set to 3%, 5% or 10% with same frequency 0.5Hz. Cells were seeded onto the silicone rubber chamber at a density of 10,000 cells/cm² and cultured in DMEM with 10% FBS, 1% P/S, 1% Ascorbic acid, 0.2% hydrocortisone and 2% Glycerophosphate. SHG imaging was carried out every 3 days. As a result, we confirmed from SHG image that the collagen production was enhanced by the cyclic stretch strain and stretch application term.

Imaging of three-photon induced fluorescence from graphene oxides on the biomimetic phantom

Seung Won Jun, Sang Min Park, Jin Soo Choi, Seok Hee Kang, Suck Won Hong, Chang-Seok Kim, Pusan National Univ. (Korea, Republic of)

We obtain two and three-photon induced fluorescence images of graphene oxides (GOs) on the biomimetic phantom to compare both cases. A spectral information and an emission intensity curve was measured by spectrometer and photo multiplier tube, respectively. GOs were synthesized by Hummer’s method that starts with graphite and sodium nitrate in sulfuric acid, then potassium permanganate and water is added and stirred, finally impurities were cleaned by phosphorus pentoxide. A custom-built multiphoton microscopy (MPM) was used to obtain two and three-photon induced fluorescence. In our custom-built MPM, laser sources of a Ti-sapphire tunable femtosecond pulse laser (680-1080 nm) and an optical parametric oscillator (1000-1600 nm) were used. A range of 750-850 nm wavelength was used for two-photon induced fluorescence form GOs and 1150-1250 nm wavelength was used for three-photon induced fluorescence. Three-photon induced fluorescence can enhance the deeper imaging depth, which is major issue on bioimaging, because of small light absorption and low light scattering of 1000-1350 nm wavelength range, known as optical window. A detrimentally photothermal effect on GOs can be reduced because a cube dependence of three-photon induced fluorescence on incident laser power is helpful to easily control a proper power of incident laser, furthermore low background and high contrast bioimaging can be achieved by greatly eliminated three-photon induced autofluorescence of sample. It was revealed that the three-photon induced fluorescence is suitable for bioimaging. Our results suggest that the three-photon induced fluorescence from GOs can pave the way for a potential application and a novel approach to bioimaging.

Multi-modal optical microscopy with multiphoton, second-harmonic generation and optical coherence microscopy using supercontinuum generation

Jaehun Kim, Daekeun Kim, Dankook Univ. (Korea, Republic of)

3D optical microscopies are widely used for diagnosing skin cancers since they can provide functional and morphological information in depth. However, single 3D imaging technique itself does not give enough information, and multimodal microscopy requires multiple light sources, which are not cost effective. In this presentation, multimodal optical microscopy with femtosecond pulsed laser is proposed for skin tissue analysis, which combines multiphoton microscopy (MPM), second harmonic generation (SHG) and spectral domain optical coherence microscopy (SD-OCT). In this microscope, femtosecond pulsed light generates both depth-resolved fluorescence and SHG images, and broad band light source induced by supercontinuum generation with photonic crystal fiber gives OCT images simultaneously. Its performance is also evaluated for coplanar image registration among different imaging techniques. Lastly, its potential for optical biopsy technique is discussed by evaluating multimodal images for ex-vivo human basal cell carcinoma.
9712-74, Session PSun

Observation of two-photon fluorescence spectrum in cellular and tissue structures
Sung-Ho Lee, Bo Ram Kim, Yumee Jeong, Hong-Gyu Ahn, Seung-Han Park, Yonsei Univ. (Korea, Republic of)

For the last decades, two-photon microscopy has been intensively utilized to visualize cellular and tissue structures with the help of fluorescent cell marker. However, in general, the total fluorescence light originated from the two-photon absorption has been collected to acquire biological images. In this talk, our newly developed two-photon spectroscopic microscopy (TPSM) will be presented. In particular, full spectrum of quantum dots and biological samples due to two-photon obtained by our TPSM will be also demonstrated.

9712-75, Session PSun

Real time imaging of live cell membrane using laser trapping, reflectance confocal microscopy, and multiphoton fluorescence microscopy
Yunxian Tian, Haishan Zeng, Shangyuan Feng, Yimei Huang, Yimei Huang, Jianhua Zhao, Eddie Shen, Wenbo Wang, BC Cancer Research Ctr. (Canada); Caigan Du, The Univ. of British Columbia (Canada)

Monitoring single cell morphology and dynamics is challenging in terms of imaging resolution and frame rate. Capturing plasma membrane dynamics in live cell imaging is especially critical due to its rapid rescaling speed. Here, we developed an integrated video rate multimodality microscopy system that is capable of acquiring both reflectance confocal microscope (RCM) image and two-photon fluorescence microscopy (TPM) image in real-time as well as performing Raman spectroscopy measurement of single living cell in culture. A 785 nm continuous wave (CW) diode laser was used for both trapping and Raman spectroscopy measurement of living suspended cell (LTRS - laser tweezers Raman spectroscopy). A wavelength tunable femtosecond (fs) Ti: Sapphire laser (715 nm – 950 nm) was used for both TPM imaging and RCM imaging of the trapped cell with submicron resolution. The system for the first time was tested in 3D imaging of HuT 78 human T lymphoma cells, in which the cell membrane and nucleotide distribution were visualized by TPM imaging under 900nm and 740nm fs laser excitation, respectively. Furthermore, we successfully observed the whole process of cell membrane rescaling (time duration 10 seconds, imaged at 15 frames/second) using this multimodality microscopy system after laser-induced cell membrane injury with varying laser power and exposure. This work demonstrated the potential usefulness of the integrated multimodality molecular imaging and spectroscopy system for analysis of some least understood fundamental cellular processes, such as plasma membrane repairing.

9712-76, Session PSun

Stimulated Raman imaging of newly synthesized proteome response under cellular stress
Lixue Shi, Lu Wei, Yihui Shen, Wei Min, Columbia Univ. (United States)

Protein homeostasis is of pivotal importance for cells to perform normal functions. The delicate balance between protein synthesis, degradation and aggregation is often broken in a wide variety of aging-related diseases including Huntington’s disease. However, it is rather challenging to study protein homeostasis in individual cells as it is technically difficult to probe the entire set of cellular proteins. Our recently developed technique of coupling stimulated Raman scattering (SRS) with metabolic incorporation of deuterated amino acids is a powerful way to monitor proteome response in individual cells under various conditions of cellular stress. In particular, we study how mutant Huntingtin protein perturbs proteome homeostasis by creating local aggregation of newly synthesized proteome. The correlation microscopy between fluorescence imaging of mutant Huntingtin morphology and SRS imaging of newly synthesized proteome offers new biological insights to the molecular nature of the Huntingtin toxicity.

9712-77, Session PSun

Second harmonic generation stokes ellipsometric microscopy for the imaging of the local tensors of collagen tissues
Ximeng You, Emma L. DeWalt, Paul D. Schmitt, Garth J. Simpson, Purdue Univ. (United States)

Polarization-dependent measurements provide rich and quantitative information on local structures of biological tissues including brain tissue, rat tail, corneas and skin tissues. Second harmonic generation (SHG) is particularly sensitive to polarization-dependent measurement due to its unique symmetry property. In the present work, nonlinear optical Stokes ellipsometric (NOSE) microscopy, or specifically second harmonic generation Stokes ellipsometric microscopy has been applied to the analysis of collagen rich tissues. NOSE is based on polarization-dependent SHG imaging. Fast modulation of the polarization state is achieved with an electro-optic modulator (EOM). Data analysis software packages have been developed for the recovery of parameters directly related to the sample including Jones tensors, sample orientation, and orientation-independent local frame tensors. For collagen, a set of three unique local-frame tensors are experimentally determined using both pooled analysis and pixel based analysis. An orientation map is also generated based on the global tensors recovered from the image. The experimental results are compared with bottom-up ab initio atomic modeling of the nonlinear optical (NLO) response of collagen triple helices, enabling visualization of differences in the degree of local order between different areas in the same image and between different tissue types.

9712-78, Session PSun

High speed wide field stimulated Raman scattering microscopy with an extreme high full well capacity camera and selective attenuation of background field
Yang-Hyo Kim, Massachusetts Institute of Technology (United States); Jeon Woong Kang, Ramachandra R. Dasari, Laser Biomedical Research Ctr. (United States) and Massachusetts Institute of Technology (United States); Shyamsunder Erramilli, Boston Univ. (United States); Peter T. C. So, Massachusetts Institute of Technology (United States)

Scanning stimulated Raman scattering (SRS) microscopy has demonstrated the feasibility of label-free imaging for biological application. However, scanning SRS microscope utilizes only a fraction of available laser power (<50mW) because of sample damage. The system is photon shot noise limited and the amount of photons that the system can handle determines the imaging speed with given sensitivity. We can increase the imaging speed by parallelizing the image acquisition, but there are critical difficulties to implement high speed wide-field SRS microscopy. SRS signal is detected as a small change of probe beam and the signal is a function of pump beam intensity. Pump beam must be spread out over the sample area for
wide field imaging and we need super high peak power laser to match the peak intensity with that of scanning SRS microscope. The original input beam acts as a large background for SRS signal, which causes another obstacle for wide field implementation. Conventional area type detectors like CCD or CMOS cameras have limited dynamic range which comes from limited electron well capacity of each pixel. Tens of µW level light saturates these detectors. We found an extreme high full well capacity camera (2M electrons) which is not available on the market yet. It can handle up to mW level, so only itself is not good enough. We suggest a selective attenuation of background field to enhance the dynamic range by orders of magnitude. SRS image of acetaminophen powder at 1648 cm⁻¹ would be presented for demonstration.

9712-79, Session PSun

**Novel pattern matching based fluorophore identification for advanced FLIM analysis**

Uwe Ortmann, Benedikt Kraemer, PicoQuant GmbH (Germany); Thomas Niehoerster, Anna Loeschberger, Julius-Maximilians-Univ. Würzburg (Germany); Volker Buschmann, Marcelle Koenig, Paja Reisch, Matthias Patting, Felix Koberling, PicoQuant GmbH (Germany); Ingo Gregor, Georg-August-Universität Göttingen (Germany); Sandra Orthaus-Müller, Olaf Schulz, Rainer Erdmann, PicoQuant GmbH (Germany)

The fluorescence lifetime of a fluorophore is an intrinsic parameter strongly depending on its photophysical properties and local environment. It can be determined via the widely used method FLIM (Fluorescence Lifetime Imaging). Here we present a novel FLIM image analysis tool called Pattern Matching that permits an unambiguous identification and separation of different populations (like fluorophores, background and autofluorescence) in each image pixel.

For this new approach, the fluorescence decay of each fluorescent contribution within a sample (e.g., background, autofluorescence) is acting as a fingerprint that is derived either from single labeled samples or from regions directly within the FLIM image with only one population. The Pattern Matching algorithm is searching for the best linear combination of these weighted reference patterns per pixel.

The new Pattern Matching analysis is very straightforward to apply and can be used for numerous applications. It allows to separate specific protein stainings from autofluorescence or background as well as the cross-talk-free analysis of multi-labeled samples. Furthermore, the typical lifetime patterns of cellular autofluorescence can be used, e.g., for discrimination between different cell types, tissue differentiation as well as cancer detection.

For samples containing specific populations with varying lifetime that reflect two different states (e.g., for sensor applications), the method allows to spatially determine differences in the local environment. In addition, the Pattern Matching is an excellent tool to identify the localization of FRET complexes in interaction studies in cells. We will show the enormous potential of the new Pattern Matching approach for FLIM image analysis.

9712-80, Session PSun

**Clustering and tracking of single membrane proteins in living bacteria by multi-dimensional microscopy**

Daniela Decker, Friedrich-Schiller-Univ. Jena (Germany); Gabrielle Deckers-Hebestreit, Univ. Osnabrück (Germany); Michael Börsch, Friedrich-Schiller-Univ. Jena (Germany)

Using confocal laser scanning microscopy and two-photon FLIM, 3D-STORM (Stochastic Optical Reconstruction Microscopy) as well as 3D-SIM (Structured Illumination Microscopy), we explore the spatial distribution and analyze the diffusion properties of bacterial FoF1-ATP synthases in living E. coli cells under different physiological conditions. FoF1-ATP synthases are membrane-embedded protein machines that catalyze the synthesis of adenosine triphosphate. We found aggregation of the FoF1-ATP synthases as well as freely diffusing membrane enzymes at different temperatures and growth conditions. For quantitative diffusion analysis, the limited size of the observation area in the membrane with a significant membrane curvature had to be considered. Because the surface coordinate system yielded different localization precision, we applied a sliding observation window approach to obtain one-dimensional diffusion coefficients of FoF1-ATP synthase in living E. coli cells.

9712-81, Session PSun

**Line-scanning two-photon microscope for in vivo imaging of brain activity**

Marie-Pierre Adam, European Lab. for Non-linear Spectroscopy (Italy); Domenico Alfieri, Light4Tech Firenze S.r.l. (Italy); Leonardo Sacconi, Francesco S. Pavone, European Lab. for Non-linear Spectroscopy (Italy)

Multi-photon microscopy allows imaging of thick and scattering tissues with a resolution approaching diffraction limit. In vivo two-photon imaging combined with fluorescent probes is nowadays extensively used for attaining critical insights into brain functionality and structural plasticity. However, functional studies can require fast acquisition that is not easily achievable by a point-by-point scanning technique as standard multi-photon microscopy. One possibility to perform faster imaging without compromising resolution is to scan an array of spots and detect them simultaneously on an array of detectors. Here, we propose an alternative design: scanning a line of spots and detecting on a wide field sensor through a rolling confocal slit. A diffractive optic element (DOE) is used to divide the laser beam in a line of 15 equally separated spots. The spots are scanned full speed, parallel to their axis by a first galvo mirror to create a line of light. The line is then scanned perpendicularly by a second galvo mirror to create the image. Detection is performed on a fast CMOS sensor and an electronic confocal slit following the excitation line is used to rejected part of the cross talk induced by tissue scattering. The capability of this new implementation has been investigated imaging brain activity of transgenic animals in which a subpopulation of neurons expresses a genetically encoded fluorescence calcium indicator (Thyl-GCaMP6).

9712-82, Session PSun

**Time-gated FLIM microscope for corneal metabolic imaging**

Susana Silva, Ana Batista, José Paulo Domingues, Univ. de Coimbra (Portugal); Maria João Quadrad, Univ. de Coimbra (Portugal) and Coimbra Hospital and Univ. Ctr. (Portugal); António Miguel Morgado, Univ. de Coimbra (Portugal)

Detecting corneal cells metabolic alterations may prove a valuable tool in the early diagnosis of corneal diseases. Nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) are autofluorescent metabolic co-factors that allow the assessment of metabolic changes through non-invasive optical methods. These co-factors exhibit double-exponential fluorescence decays, with well-separated short and lifetime components, which are related to their protein-bound and free-states. Corneal metabolism can be assessed by measuring the relative contributions of these two components.

For that purpose, we have developed a wide-field time-gated fluorescence lifetime microscope based on structured illumination and one-photon excitation to record FAD lifetime images from corneas. NADH imaging was not considered as its UV excitation peak is regarded as not safe for
in vivo measurements. The microscope relies on a pulsed blue diode laser (λ=443 nm) as excitation source, an ultra-high-speed gated image intensifier coupled to a CCD camera to acquire fluorescence signals and a Digital Micromirror Device (OMD) to implement the Structured Illumination technique. The system has a lateral resolution better than 2.4 μm, a field of view of 160 per 120 μm and an optical sectioning of 7.77 +/- 0.36 μm when used with a 40x, 0.75 NA, Water Immersion Objective.

With this setup we were able to measure FAD contributions from ex-vivo bovine corneas collected form a local slaughterhouse. Measurements were performed within different time intervals after animal sacrifice. Results were validated with a commercial Time-Correlated Single Photon Counting Microscope (TSCPC).

9712-83, Session PSun

**Higher harmonic generation microscopy of human brain tumors and temporal lobe epilepsy**

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Label-free intraoperative histopathology is a highly desirable tool for rapid spotting of malignant aberrations of brain cell densities during neurosurgeries.

High harmonic generation microscopy (HHGM) is a novel imaging technique in brain research providing non-invasive, label-free contrast of living, ex-vivo or post-mortem specimens with sub-cellular resolution, intrinsic depth sectioning and reduced phototoxicity. HHGM requires no external contrast agents and reveals tissue contrast provided by discontinuities, noncentrosymmetric molecular structures or autofluorescent organelles via generation of second and third optical harmonics and autofluorescence.

In our laboratory experiments HHGM of healthy ex-vivo human brain tissues reveals lipid-rich axonal networks of the white matter and extracellular matrix of the gray mater, neuronal cell layers in the cortex, sub-cellular details (nuclear envelopes, organelles) and vascularization (blood vessels, erythrocytes). HHGM of human tumor cases reveals key histological hallmarks of tumor-invaded brain areas: increased cellularity, malignant vascularization and nuclear pleomorphism. HHGM of human epilepsy cases reveals extensive neuronal loss and abundance of blood vessels in the atrophic areas of the sclerotic hippocampal tissues. HHGM images of healthy and diseased brain tissues are in good agreement with H&E histology slides.

Our goal is to implement the HHGM technique in a clinical tool – a biopptic needle for real-time optical biopsies of tumor margins and neuronal loss assessment during neurosurgeries, helping to spare healthy tissue, reduce unnecessary surgery and increase surgical success rate. We will present HHGM images of healthy and diseased brain tissues acquired both with a standard multiphoton objective and a multi-element GRIN lens needle objective.

9712-84, Session PSun

**FLIM data analysis of NADH and tryptophan autofluorescence in prostate cancer cells**

Meghan J. O’Melia, Horst K. Wallrabe, Zdenek Svindrych, Shagufta Rehman, Ammasi Periasamy, Univ. of Virginia (United States)

Fluorescence lifetime imaging microscopy (FLIM) is one of the sensitive techniques in the measurements of metabolic activity in living cells, tissues and animals. We used two- and three-photon fluorescence excitation together with time-correlated single photon counting (TCSPC) to acquire FLIM signals from the normal and prostate cancer cell lines. FLIM requires complex data fitting and analysis and we explored different ways to represent the data to connect to the biological interpretation. After non-linear least square fitting of the multi-photon TCSPC images using SPCImage software (Becker & Hickl), the photon and lifetime images were processed into intensity and lifetime histograms. This representation helped us in the interpretation of the data in cancer versus normal cells. The photon images generated in this software were used to create regions of interest within images. We developed ImageJ macros to selectively process the lifetime images in these regions of interest. Excel (Microsoft) plugins developed by us were used to process and analyze the resulting data. These macros provided massive data processing automation of prostate cancer cell FLIM data. In this report, we are able to demonstrate increased glycolytic activity in prostate cancer cells after glucose stimulation by analyzing NAD(P)H bound fraction (a2%) and efficiency of energy transfer (E%). The changes in Trp E% demonstrate quenching of tryptophan fluorescence. When cells were treated with Doxorubicin, a widely used chemotherapeutic agent in prostate cancer, a leftward shift was seen in the NAD(P)H a2% curves, indicating a decrease in cellular metabolism after treatment.

9712-85, Session PSun

**In-vivo dynamical analysis zonal difference of hepatobiliary metabolism in chronic hepatic diseases by multiphoton microscopy**

Chen-Yuan Dong, Chih-Ju Lin, National Taiwan Univ. (Taiwan); Hsuan-Shu Lee, National Taiwan Univ. Hospital (Taiwan)

In vivo dynamic analysis hepatobiliary metabolism was a novel subject to understand hepatic functions. The phenomenon of hepatobiliary metabolism was non-uniform in hepatic lobule, and liver diseases would cause hepatic functional variation. In this study, molecular probe of 6-carboxyfluorescein diacetate (6-CFDA) was hydrolyzed by intracellular esterase into fluorescent 6-carboxyfluorescein (6-CF) and 6-CF was excreted to canaliculi, whole 6-CFDA metabolism would investigated with two-photon fluorescence microscopy. Normalization of 6-CF metabolic curve, the mean results of liver fibrosis and fatty liver were similar to control liver. But perportal hepatocytes, hepatocellular 6-CF retain of fatty liver was about 30% of control and liver fibrosis. This result shows that the membrane transporter may increase in chronic liver diseases.

9712-21, Session 5

**Imaging of calcium transients in cultured neurons by TCSPC FLIM (Invited Paper)**

Wolfgang Becker, Becker & Hickl GmbH (Germany); Samuel Frere, Tel Aviv Univ. (Israel)
We present a technique that records transient changes in the concentration of free calcium in live neurons by TCSPC FLIM. The sample is incubated with a calcium-sensitive dye. To measure the temporal change in the calcium concentration the sample is periodically stimulated by an electrical signal and scanned at high image rate with a high-frequency pulsed laser beam. Single photons of the fluorescence light are detected, and a photon distribution over the coordinates of the scan, the arrival times of the photons after the excitation pulses, and the time after the stimulation pulses is built up. The result can be interpreted as a sequence of FLIM images for different times after the stimulation pulses. The signal-to-noise ratio only depends on the available photon rate and the total acquisition time, not on the speed of the sequence. The maximum resolution at which lifetime changes can be recorded is given by the frame rate of the scanner which is currently 38 ms. Faster changes can be recorded by line scanning. Transient lifetime effects can then be resolved at a resolution of about one millisecond.

9712-22, Session 5

**Binding of the immunomodulatory drug Bz-423 to mitochondrial FoF1-ATP synthase in living cells by FLIM-FRET and FRET acceptor photobleaching (Invited Paper)**

Ilka Starke, Friedrich-Schiller-Universität Jena (Germany); Gary D. Glick, Univ. of Michigan (United States); Michael Börsch, Friedrich-Schiller-Universität Jena (Germany)

Bz-423 is a promising new drug for treatment of autoimmune diseases. This small molecule binds to subunit OSCP of the mitochondrial enzyme FoF1-ATP synthase and modulates its catalytic activities. We are interested in the binding of Bz-423 action and how subunit rotation in FoF1-ATP synthase, i.e. the mechnochemical mechanism of the enzyme, is controlled by Bz-423. We selectively marked the enzyme by a fluorescent protein fusion and measured the binding of Cy5-labeled Bz-423 to mitochondrial FoF1-ATP synthase in living yeast cells by confocal time-resolved FRET, two-photon-FLIM as well as increased FRET donor fluorescence intensity after photobleaching of Cy5 with 640 nm using a Nikon superresolution microscope.

9712-23, Session 5

**Investigation of prostate cancer cells using NADH and Tryptophan as biomarker: multiphoton FLIM-FRET microscopy**

Shagufta Rehman, Meghan J. O'Melia, Univ. of Virginia (United States); Horst Wallrabe, Univ. of Virginia (United States); Zdenek Svindrych, Univ. of Virginia (United States); Dhyan Chandra, Roswell Park Cancer Institute (United States); Ammasi Periasamy, Univ. of Virginia (United States)

Fluorescence Lifetime Imaging (FLIM) can be used to understand the metabolic activity in cancer. Prostate cancer is one of the leading cancers in men in the USA. This research focuses on FLIM measurements of NADH and Tryptophan, used as biomarkers to understand the metabolic activity in prostate cancer cells. Two prostate cancers and one normal cell line were used for live-cell FLIM measurements on Zeiss 780 2P confocal microscope with SPCM FLIM board. Glucose uptake and glycolysis proceeds about ten times faster in cancer than in non-cancerous tissues. Therefore, we assessed the glycolytic activity in the prostate cancer in comparison to the normal cells upon glucose stimulation by analyzing the NADH and Trp lifetime distribution and efficiency of energy transfer (E%). Furthermore, we treated the prostate cancer cells with 1µM Doxorubicin, a commonly used anti-cancer chemotherapeutic. Increase in NADH a2%, an indicator of increased glycolysis and increased E% between Trp and NADH was seen upon glucose stimulation for 30 min. The magnitude of shift to the right for NADH a2% and E% distribution was higher in prostate cancer versus the normal cells. Upon treatment with Doxorubicin decrease in cellular metabolism was seen at 15 and 30 minutes. The histogram for NADH a2% post-treatment for prostate cancer cells showed a left shift compared to the untreated control suggesting decrease in glycolysis and metabolic activity opposite to what was observed after glucose stimulation. Hence, NADH and Trp lifetimes can be used biomarkers to understand metabolic activity in prostate cancer and upon chemotherapeutical interventions.

9712-24, Session 8

**Temporal binning of TCSPC data to improve exponential decay fits and improve imaging speed (Invited Paper)**

Alex J. Walsh, National Research Council (United States) and Air Force Research Lab. (United States); Melissa C. Skala, Vanderbilt Univ. (United States); Hope T. Beier, Air Force Research Lab. (United States)

Time-correlated single photon counting, TCSPC, remains the most robust method for fluorescence lifetime imaging using laser scanning microscopes. However, TCSPC is inherently slow due to the single photon per laser pulse acquisition limitation and the need for low fluorescence emission efficiency to avoid bias of measurement towards short lifetimes. Furthermore, thousands of photons per pixel are required for traditional instrument response deconvolution and fluorescence lifetime exponential decay estimation. Instrument response deconvolution and fluorescence exponential decay estimation can be performed in several ways including iterative least squares minimization and Laguerre estimation. This paper compares the limitations and accuracy of these fluorescence decay analysis techniques for single and double exponential decays across many data characteristics including various lifetime values, lifetime component values, signal-to-noise ratios, and number of photons detected. Furthermore, techniques to improve data fitting, including binning data temporally and spatially, are evaluated as methods to improve decay fits and reduce image acquisition time. Simulation results demonstrate lifetime measurements of fluorescent dyes validate that binning temporally to ~50 time bins instead of 256, improves decay curve signal-to-noise ratio, improves accuracy of fits for data with low contributions of the short lifetime component, and enables higher accuracy fitting for low photon count data. Such a technique reduces the required number of photons for accurate component estimation if lifetime values are known, such as for commercial fluorescent dyes and FRET experiments, and improves imaging speed 10-fold.

9712-25, Session 7

**Tunable PIE and synchronized gating detections by FastFLIM for quantitative microscopy measurements of fast dynamics of single molecules (Invited Paper)**

Yuansheng Sun, Ulas Coskun, Beniamino B. Barbieri, Shih-Chu Liao, ISS, Inc. (United States)

The cross talk between two fluorescent species causes problems in fluorescence microscopy imaging, especially for quantitative measurements such as co-localization, Förster resonance energy transfer (FRET), fluorescence cross correlation spectroscopy (FCCS) and etc. In laser scanning confocal microscopy, the lasers can be switched on and off by acousto-optical tunable filter (AOTF) in the microsecond scale for alternative line scanning to avoid the cross talk while minimizing the time
Conference 9712:
Multiphoton Microscopy in the Biomedical Sciences XVI

9712-26, Session 7

Ns-time resolution for multispecies STED-FLIM and artifact free STED-FCS (Invited Paper)

Felix Koberling, Paja Reisch, Rhys Dowler, Benedikt Kraemer, Sebastian Tannert, Matthias Patting, Marcelle Koenig, Rainer Erdmann, PicoQuant Gmbh (Germany)

Stimulated Emission Depletion (STED) Microscopy has evolved into a well-established method offering optical superresolution below 50 nm. Running both excitation and depletion lasers in picosecond pulsed modes allows for highest optical resolution as well as fully exploiting the photon arrival time information using time-resolved single photon counting (TCSPC).

Non-superresolved contributions can be easily dismissed through time-gated detection (gated STED) or a more detailed fluorescence decay analysis (FLIM-STEDE). This allows for a significantly improved imaging resolution. Furthermore, these methods allow for accurate separation of different fluorescent species, especially if subtle differences in the excitation and emission spectra as well as the fluorescence decay are taken into account in parallel. We will show some recent examples in superresolved multispecies imaging of cells and neurons to demonstrate the superiority of the time-resolved approach, especially since the variety of separable and STED-optimized fluorophores is still limited.

STEED can also be used to shrink the observation volume while studying the dynamics of diffusing species in Fluorescence Correlation Spectroscopy (FCS) to overcome averaging issues along long transit paths. An additional unique advantage of STED-FCS is that the observation spot diameter can be tuned in a gradual manner enabling, for example, the type of hindered diffusion to be determined in lipid membrane studies. Our completely pulselineated scheme allows via pulsed interleaved excitation (PIE) to check online in a straightforward way whether the STED laser has an influence on the investigated dynamics.

9712-27, Session 7

Live-tracking drug delivery in skin: multiphoton FLIM FRET

Hanna Thomsen, Johan Borglin, Danni Wang, Marica B. Ericson, Univ. of Gothenburg (Sweden)

Penetration and localization of a drug in skin is not abundantly clear from a fluorescent image alone. How can one be certain of how the particle penetrates and where it localizes? How can we know if a particle is taken up by cells or aggregates in the cytoplasm? Here, förster resonance energy transfer (FRET) is used as a method to track when and where nanoparticles designed for drug delivery are taken up by cells in skin. A FRET signal demonstrates energy transfer between a donor and acceptor molecule through non-radiative coupling and is highly dependent on the distance of the fluorophores from each other. The polymer encased nanoparticles thus demonstrate FRET signal only upon penetration of an in-tact particle. Fluorescence lifetime imaging microscopy (FLIM) is used to quantify the fluorescent signal seen in the images by matching the measured lifetime in various regions of the image to the lifetime of the FRET nanoparticles, allowing for highly specific tracking of drug delivery following percutaneous absorption. The particles are excited by two photon excitation in IR thus providing a platform for imaging in biomedical applications. Presented here is a novel nanoparticle compound allowing for potential simultaneous delivery of drugs through skin and live fluorescence lifetime tracking.

9712-29, Session 8

Multiphoton fluorescence lifetime microscopy quantifies in vivo tumor heterogeneity

Amy T. Shah, Kirsten E. Diggins, Alex J. Walsh, Jonathan M. Irish, Melissa C. Skala, Vanderbilt Univ. (United States)

Treatment-resistant subpopulations of cells in a tumor can enable relapse in cancer patients. Therefore, there is a need for techniques to assess tumor heterogeneity. Many anti-cancer treatments disrupt cellular metabolism, and measurements of metabolism can provide early markers of treatment response. In particular, the metabolic cofactors NAD(P)H and FAD exhibit autofluorescence. The optical redox ratio (fluorescence intensity of NAD(P)H divided by FAD) reports on the redox balance in the cell. The fluorescence lifetime of NAD(P)H and FAD reports on protein-binding activities. Multiphoton fluorescence lifetime microscopy (FLIM) can harness these intrinsic sources of contrast to provide cellular-resolution images of tumor metabolism and treatment response.

This study applies multiphoton FLIM in vivo to quantify cellular-level tumor heterogeneity. Mice with FaDu xenografts were administered cetuximab (antibody therapy) or cisplatin (chemotherapy). Two days post-treatment, multiphoton FLIM images were acquired in vivo and quantified on a per-cell basis. The NAD(P)H and FAD lifetimes decrease 2 days after cetuximab and cisplatin treatment (p<0.05), agreeing with decreased tumor sizes in mice after 9 days of treatment (p<0.05). Frequency histogram analysis identifies cell subpopulations, and a novel heterogeneity index quantifies cellular heterogeneity across treatment groups. Additionally, a dimensionality reduction technique (viSNE) preserves similarities based on multivariate optical measures (fluorescence intensity and lifetime parameters) across cells, enabling holistic visualization of cellular heterogeneity. These analyses indicate increased heterogeneity in cetuximab and cisplatin treatment groups compared with control. Ultimately, these techniques could be applied to characterize tumor heterogeneity, which could enable optimized treatment regimens for cancer patients.

9712-30, Session 8

STED FLIM: fluorescence lifetime imaging with 30nm resolution

Christian A. Wurm, Abberior GmbH (Germany); Wolfgang Becker, Becker & Hickl GmbH (Germany); Andreas Schöne, Abberior GmbH (Germany)

We present the seamless integration of optical super-resolution and fluorescence lifetime imaging based on multi-dimensional time correlated single photon counting (TCSPC) and stimulated emission-depletion (STED) in a commercial STED-FLIM microscope. The system maps the fluorescence intensity and lifetime at a spatial resolution of <30nm x 30nm in 2D images and <80 x 80 x 90nm in 3D image stacks. The system is able to separate the signals of several fluorophores by their fluorescence lifetimes, or to use the fluorescence lifetime as a probe function for the molecular environment. Three-channel STED measurements
Multiphoton Microscopy in the Biomedical Sciences XVI

9712-31, Session 8
A phasor approach analysis of multiphoton FLIM measurements recorded from three-dimensional Caco-2 models to detect changes in NAD(P)H characteristics

Pirmin Lakner, Universitätshospital Tübingen (Germany); Yvonne Möller, Monilola Olayioye, Univ. Stuttgart (Germany); Michael G. Monaghan, Universitätshospital Tübingen (Germany); Katja Schenke-Layland, Universitätshospital Tübingen (Germany) and Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik (Germany)

Fluorescence lifetime imaging microscopy (FLIM) is an approach to obtain very sensitive data regarding the endogenous fluorophores present in biological samples. The concise evaluation of FLIM data requires the use of robust mathematical algorithms. Multi exponential decay fitting is the standard and complex approach for calculating decay curves derived from FLIM. In this study, we developed a user-friendly phasor approach for analyzing FLIM data and tested the method on three-dimensional (3D) Caco-2 models of polarized epithelial cells in a Matrigel® environment. The models were left untreated, treated with heregulin, or with epidermal growth factor, which stimulates hyperproliferation. Autofluorescence from nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) in luminal Caco-2 cysts was stimulated by 2-photon laser excitation. FLIM data was recorded and analyzed with a standard multi exponential decay fitting approach and a phasor approach developed in this study. Using a phasor approach the distribution of emitted fluorophores, their lifetimes, and deviations were calculated with higher and faster precision, and with fewer initial conditions when compared to multi exponential decay fitting. The phasor approach simplified FLIM data analysis making it an interesting tool for non-experts in numerical data analysis. We observed that an increased proliferation led to a significant shift in the distribution of fluorescence lifetimes from protein bound to free NAD(P)H. Our data demonstrates that multiphoton FLIM analysis with the phasor approach is a novel method for the non-invasive analysis of 3D in vitro cell culture models qualifying this method for monitoring basic cellular features and the effect of external factors.

9712-32, Session 9
High-throughput 3D super-resolution imaging by volumetric parallel STED microscopy (Invited Paper)

Yi Xue, Christopher J. Rowlands, Peter T. C. So, Massachusetts Institute of Technology (United States)

STED super-resolution fluorescent microscopy is one of the methods overcoming diffraction limit to achieve sub-diffraction resolution. However, this method is a point-scanning technique which limited the imaging efficiency. Recently two-dimensional parallel STED is published, which largely improved the imaging speed. The technique uses uniform illumination as excitation light, while spots matrix illumination as depletion light. Our work further promoted the parallel STED microscopy to three-dimension by generating volumetric structure illumination pattern. The structure illumination pattern scans the specimen by relative phase modification of excitation beams. There are three major advantages of this method. First, the designed structure illumination pattern has higher energy efficiency, compared to 2D structure illumination. In 2D parallel STED microscopy, out of focus light of the excitation spots matrix is scattered and absorbed by the specimen. The energy was not only wasted but also led higher chance of phototoxicity for the specimen. Second, based on the simulation, this method refilled the “missing-cone” of k in the frequency domain, which means the excitation structure light achieved high axial resolution. Last, the method can detect the photons in the whole volume with high speed parallel scanning. Compared to single point-scanning 3D STED microscopy, this volumetric imaging speed increased significantly.

9712-33, Session 9
SearchLight: a freely available web-based quantitative spectral analysis tool (Invited Paper)

Prashant Prabhat, Michael Peet, Turan Erdogan, Semrock, Inc. (United States)

In order to design a fluorescence experiment, typically the spectra of a fluorophore and of a filter set are overlaid on a single graph and the spectral overlap is evaluated intuitively. However, in a typical fluorescence imaging system the fluorophores and optical filters are not the only wavelength dependent variables – even the excitation light sources have been changing. For example, LED Light Engines may have a significantly different spectral response compared to the traditional metal-halide lamps. Therefore, for a more accurate assessment of fluorophore-to-filter-set compatibility, all sources of spectral variation should be taken into account simultaneously. Additionally, intuitive or qualitative evaluation of many spectra does not necessarily provide a realistic assessment of the system performance.

“SearchLight” is a freely available web-based spectral plotting and analysis tool that can be used to address the need for accurate, quantitative spectral evaluation of fluorescence measurement systems. This tool is available at: http://searchlight.semrock.com/. Based on a detailed mathematical framework [1], SearchLight calculates signal, noise, and signal-to-noise ratio for multiple combinations of fluorophores, filter sets, light sources and detectors. SearchLight allows for qualitative and quantitative evaluation of the compatibility of filter sets with fluorophores, analysis of bleed-through, identification of optimized spectral edge locations for a set of filters under specific experimental conditions, and guidance regarding labeling protocols in multiplexing imaging assays. Entire SearchLight sessions can be shared with colleagues and collaborators and saved for future reference.


9712-34, Session 9
Recent developments in widely tunable and high peak power ultrafast laser sources and their adoption in biological imaging

Julien Klein, Spectra-Physics (United States)

Widely tunable ultrafast lasers have enabled a large number of biological imaging techniques including point scanning multiphoton excited fluorescence (MPEF), SHG/THG, stimulated Raman imaging. Tunable ultrafast lasers offer spectral agility, covering the entire relative transparency window in live tissue (700-1300nm) and flexibility with multi-color, synchronized outputs to support sophisticated label free techniques (e.g. stimulated Raman modalities).

More recently newly available high peak power lasers based on Ytterbium technology drive advances in two-photon light sheet, 3 photon excited fluorescence and holographic patterning for optogenetics photo-stimulation. These laser platforms offer a unique blend of compactness, ease of use and cost efficiency, and ideally complement tunable platforms based on
**9712-35, Session 9**

**Advanced femtosecond lasers enable new developments in non-linear imaging and functional studies in neuroscience, biology and medical applications**

Marco Arrigoni, Coherent, Inc. (United States); Darryl McCoy, Coherent Scotland Ltd. (United Kingdom)

In the last few years Multiphoton Excitation Microscopy witnessed a mutation from tool for imaging cellular structures in living animals deeper than other high-resolution techniques, into an instrument for monitoring functionality and even stimulating or inhibiting inter-cellular signaling. This paradigm shift has been enabled primarily by the development of genetically encoded probes like Ca indicators (GECI) and Opsins for optogenetics inhibition and stimulation. These developments will hopefully enable the understanding of how local network of hundreds or thousands of neurons operate in response to actual tasks or induced stimuli. Imaging, monitoring signals and activating neurons, all on a millisecond time scale, requires new laser tools providing a combination of wavelengths, higher powers and operating regimes different from the ones traditionally used for classic multiphoton imaging. The other key development in multiphoton techniques relates to potential diagnostic and clinical applications where non-linear imaging could provide all optical marker-free replacement of H&E techniques and even intra-operative guidance for procedures like cancer surgery. These developments will eventually drive the development of specialized laser sources where compact size, ease of use, beam delivery and cost are primary concerns. In this talk we will discuss recent laser product developments targeting the various applications of multiphoton imaging, as fiber lasers and other new type of lasers gradually gain popularity and their own space, side-by-side or as an alternative to conventional titanium sapphire femtosecond lasers.

**9712-36, Session 9**

**Airyscan detection combined with two-photon excitation improves imaging signal-to-noise and resolution in thick scattering samples**

Joseph Huff, Carl Zeiss Microscopy, LLC (United States); Ingo Kleppe, Carl Zeiss Microscopy GmbH (Germany)

The penetration depth of traditional confocal laser scanning microscopy is highly dependent on the scattering properties of the interrogated sample. For highly scattering samples the depth penetration of a laser scanning microscope can be extended beyond the depth provided by a confocal system by the utilization of pulsed NIR lasers for multiphoton excitation of fluorophores. The combination of improved depth penetration in scattering samples and improved live cell compatibility from utilizing red-shifted excitation has made multiphoton imaging the standard for neuroscience and life science fluorescence imaging. Recently multiphoton microscopy has been combined with image scanning based approaches to improve both the optical sectioning and spatial resolution performance. However, the current implementations of multiphoton image scanning systems suffer drawbacks associated with either limited penetration depths due to multi-focal spot cross talk or inherent speed limitations of using camera based detectors for point based illumination. Here we describe how the new Airyscan GaAsP-PMT detector is combined with multiphoton excitation allowing better depth penetration and optical sectioning than all previously reported multiphoton image scanning systems. We demonstrate Airyscan imaging in brain tissue at depths exceeding 300 μm from the surface.

**9712-37, Session 10**

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

Paul J. Campagnola, Karissa B. Tilbury, Kirby R. Campbell, Kevin W. Elieciiri, Mansih Patankar, Univ. of Wisconsin-Madison (United States)

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

**9712-38, Session 10**

**Volumetric imaging of oral epithelial neoplasia by MPM-SHGM: epithelial connective tissue interface**

Rahul Pal, Jinping Yang, Suimin Qiu, Vicente Resto, Susan McAmmon, Gracie Vargas, The Univ. of Texas Medical Branch (United States)

The majority of oral cancers are comprised of oral squamous cell carcinoma in which neoplastic epithelial cells invade across the epithelial connective tissue interface (ECTI). Invasion is preceded by a multi-component process including epithelial hyperproliferation, loss of cell polarity, and remodeling of the extracellular matrix. Multiphoton Autofluorescence Microscopy (MPAM) and Second Harmonic Generation Microscopy (SHGM) show promise for revealing indicators of neoplasia. In particular, volumetric imaging by these methods can reveal aspects of the 3D microstructure that are not possible by other methods and which could both further our understanding of neoplastic transformation and be explored for development of diagnostic approaches in this disease having only 55% 5-year survival rate. MPAM-SHGM were applied to reveal the 3D structure of the critical ECTI interface that plays an integral part toward invasion. Epithelial dysplasia was induced in an established hamster model. MPAM-
SHGM was applied to lesion sites, using 780 nm excitation (450-600 nm emission) for autofluorescence of cellular and extracellular components: 840 nm using 420 nm bandpass filter for SHG. The ECTI surface was identified as the interface at which SHG signal began following the epithelium and was modeled as a 3D surface using Matlab. ECTI surface area and cell features at sites of epithelial expansion where ECTI was altered were measured; imaged sites were biopsied and processed for histology.

ROC analysis using ECTI image metrics indicated the ability to delineate normal from neoplasia with high sensitivity and specificity and it is noteworthy that inflammation did not significantly alter diagnostic potential of MPAM-SHGM.

9712-39, Session 10

Characterization of human arterial tissue affected by atherosclerosis using multimodal nonlinear optical microscopy

Enrico Baria, European Lab. for Non-linear Spectroscopy (Italy); Riccardo Cicchi, European Lab. for Non-linear Spectroscopy (Italy) and Istituto Nazionale di Ottica (Italy); Matteo Rotellini, Daniela Massi, Univ. degli Studi di Firenze (Italy); Francesco S. Pavone, European Lab. for Non-linear Spectroscopy (Italy) and Istituto Nazionale di Ottica (Italy) and Istituto Nazionale di Ottica (Italy) and Univ. degli Studi di Firenze (Italy)

Atherosclerosis is a widespread cardiovascular disease caused by the deposition of lipids (such as cholesterol and triglycerides) on the inner arterial wall. The rupture of an atherosclerotic plaque, resulting in a thrombus, is one of the leading causes of death in the Western World. Preventive assessment of plaque vulnerability is therefore extremely important and can be performed by studying collagen organization and lipid composition in atherosclerotic arterial tissues. Routinely used diagnostic methods, such as histopathological examination, are limited to morphological analysis of the examined tissues, whereas an exhaustive characterization requires immune-histochemical examination and a morpho-functional approach. Instead, a label-free and non-invasive alternative is provided by nonlinear microscopy. In this study, we combined SHG and FLIM microscopy in order to characterize collagen organization and lipids in human carotid ex vivo tissues affected by atherosclerosis. SHG images, acquired from both normal arterial wall and different regions within atherosclerotic plaques, were processed through image pattern analysis methods (FFT, GLCM). The resulting information on collagen and cholesterol distribution and anisotropy, combined with collagen and lipids fluorescence lifetime measured from FLIM images, allowed characterization of carotid samples and discrimination of different tissue regions. The presented method can be applied for automated classification of atherosclerotic lesions and plaque vulnerability. Moreover, it lays the foundation for a potential in vivo diagnostic tool to be used in clinical setting.

9712-40, Session 10

Forward versus backward polarization-resolved SHG imaging of collagen structure in tissues

Claire Teulon, Ecole Polytechnique (France) and Consiglio Nazionale delle Ricerche - Inserm U1182 (France); Ivan Gusachenko, Ecole Polytechnique (France) and Consiglio Nazionale delle Ricerche - Inserm U1182 (France) and Okinawa Institute of Science and Technology Graduate Univ. (Japan); Gaël Latour, Ecole Polytechnique (France) and Consiglio Nazionale delle Ricerche - Inserm U1182 (France) and Univ. Paris-Sud 11 (France); Marie-Claire Schanne-Klein, Lab. d’Optique et Biosciences (France) and Consiglio Nazionale delle Ricerche - Inserm U1182 (France);

In situ visualization of fibrillar collagen in biological tissues is a major biomedical concern, whether to study collagen accumulation, impairment or disorder in numerous pathologies, or to understand structure of organs and guide tissue engineering. To this goal, second harmonic generation (SHG) has proven to be a powerful technique to visualize fibrillar collagen without any staining and with a good contrast. More information about collagen 3D distribution in tissues can be gained with polarization-resolved second-harmonic generation (P-SHG) microscopy. Two quantitative structural parameters are then measured: in-plane averaged orientation of fibrils in the field of view and SHG anisotropy, which is related to the molecular structure and the 3D orientation disorder inside the excitation volume (submicroscopic scale).

Nevertheless, observed tissues are heterogeneous and anisotropic media, and strong focusing is required for effective imaging. Light propagation in those media is therefore complex and not thoroughly understood yet, preventing accurate and reproducible measurements. Moreover, imaging can be performed through the sample (forwards) or backwards for in vivo imaging, but different measured anisotropy have been observed for those two modalities.

In this study, we performed advanced nonlinear optics studies to understand how this coherent SHG signal builds up. Theoretical analysis, vectorial numerical simulations and experiments in tendon and in cornea were implemented to determine how geometrical parameters affect second harmonic anisotropy in homogeneous media. We obtained an excellent agreement between simulations and experiments, showing that P-SHG measurements are highly sensitive to detection geometry.

Reference:

9712-41, Session 10

Impact of relative collagen fibril polarity in determining the signal intensity in second harmonic generation microscopy

Charles-André Couture, Stéphane Bancelin, Institut National de la Recherche Scientifique (Canada); Jarno N. Van der Kolk, Konstantin Popov, Univ. of Ottawa (Canada); Maxime Rivard, Katherine Légaré, Institut National de la Recherche Scientifique (Canada); Gabrielle Martel, Univ. de Montréal (Canada); Hélène Richard, Univ. of Ottawa (Canada); Cameron Brown, Univ. of Oxford (United Kingdom); Sheila Laverty, Univ. de Montréal (Canada); Lora Ramunno, Univ. of Ottawa (Canada); François Légaré, Institut National de la Recherche Scientifique (Canada)

In recent years, Second Harmonic Generation (SHG) microscopy has emerged as a powerful technique to image the architecture of non-centrosymmetric structures, such as collagen fibrils, in biological tissues. However, due to the coherence of SHG, the signal intensity depends not only on the density and the overall organization of harmonophores, but also on the relative polarity of the collagen fibrils within the focal volume. This last information, contain in the phase of the signal, is lost in standard SHG measurements.

Recently, Interferometric SHG (I-SHG) microscopy has been proposed to overcome this limitation by measuring the phase of the signals. In I-SHG microscopy, second harmonic is generated twice: first outside the microscope, to provide a reference SHG beam, and secondly within the sample. Varying the phase between these two interfering signals one can retrieve, pixel by pixel, the relative orientation of the second harmonic emitters.

In this work, we implemented I-SHG microscopy using femtosecond pulses and applied it to study the collagen meshwork in cartilage. The results are analyzed in regards of numerical simulations showing that the phase
measurements can be related to the local ratio of fibrils with opposite polarities. A comparison with polarization-resolved SHG highlights the role of both tissue organization and relative fibril polarity in determining the SHG signal intensity. This work illustrates how the complex architecture of non-centrosymmetric scatterers at the nanoscale, governs the coherent building of SHG within the focal volume and the observed features in SHG images.

9712-42, Session 11

Two-photon imaging during brain surgery: first clinical study using a certified multiphoton tomograph (Invited Paper)
Karsten König, Univ. des Saarlandes (Germany) and JenLab GmbH (Germany); Martin Weinigel, JenLab GmbH (Germany); Sven R. Kantelhardt, Alf Giese, Universitätsmedizin der Johannes Gutenberg-Univ. Mainz (Germany)

We report on the first preliminary intraoperative clinical multiphoton study based on two-photon label-free autofluorescence/SHG imaging during brain surgery. The clinical multiphoton tomograph MPTflex with its flexible mecha-optical arm was employed to perform in vivo histology with superior 3D resolution during surgery as well as fast analysis of tumor biopsies within the operation room.

The tomograph distinguishes autofluorescence (AF) signals from second harmonic generation (SHG) signals simultaneously. In addition, fluorescence lifetime imaging microscopy (FLIM) based on time-correlated single photon counting (TCSPC) technology offers additional information on the functional level of the intratissue fluorophores and their binding status. Interestingly, cancer tissue has a different mean lifetime than surrounding normal tissue. FLIM can therefore be used to detect tumor margins.

9712-43, Session 11

Biocompatibility of novel ultracompact femtosecond laser oscillators for multiphoton imaging (Invited Paper)
Aisada Uchugonova, Univ. des Saarlandes (Germany) and JenLab GmbH (Germany); Hans G. Brunig, JenLab GmbH (Germany); Tuan Li, FEMTOLASERS Produktions GmbH (Germany); Karsten König, Univ. des Saarlandes (Germany) and JenLab GmbH (Germany)

Femtosecond near infrared laser microscopes are widely used to perform high resolution 3D imaging of biological samples based on second harmonic generation (SHG) and non-resonant simultaneous absorption of two or more photons at GW/cm² intensities. However, high contrast imaging of living specimens without any destructive effect is limited to certain laser and exposure parameters with respect to the optical properties of the target.

We compared three different femtosecond lasers, including a novel ultra-compact ultrashort fiber laser, in the range of 15-180 fs and repetition rates of 50–300 MHz for optimal non-destructive two-photon autofluorescence imaging. In particular we determined the thresholds for the onset of photodamage effects such as impaired cell reproduction.

9712-44, Session 11

Multimodal imaging platform to study cancer progression in zebrafish
Angelika Unterhuber, Marco Andreana, Aart J. Verhoeef, Medizinische Univ. Wien (Austria); Martin Distel, St. Anna Kinderkrebsforschung e.V. (Austria); Alma del Carmen Fernandez Gonzalez, Technische Univ. Wien (Austria); René M. Werkemeister, Wolfgang Drexler, Medizinische Univ. Wien (Austria)

Zebrafish develop tumors that are histologically similar to human malignancies. In addition, zebrafish possess all of the major immune cell lineages found in humans and hematopoietic gene functions are conserved in zebrafish and vice versa, suggesting that findings in zebrafish will be directly translatable to humans. Furthermore zebrafish is a cost-effective model organism to perform drug screens. Tumor initiation stages in larval zebrafish embryos are accessible for high resolution nonlinear microscopy. We fuse high resolution OCT for fast volumetric screening with nonlinear optical imaging to monitor tumor progression and metastasis stages for intravital imaging of zebrafish in transmission and reflection mode. Our multimodal label-free nonlinear imaging platform is based on the spectral focusing of a compact and cost effective femtosecond Ti:sapphire laser with an inherently synchronized high power Yb fiber amplifier for hyperspectral multimodal CARS. CARS, SHG and THG imaging at 800 nm and 1050 nm are performed simultaneously down to 800 µm depths with localized chemical specificity at cellular levels to distinguish multiple molecules. THG provides information from the tumor borders and FWM as the non-resonant counterpart of CARS provides depth-resolved morphologic information helping in the co-registration with the wide-field OCT. The lipid content is significantly altered with tumor progression and changes in the collagen orientation occur mainly in the zebrafish tail. With the integration of fluorescence microscopy and the possibility to label macrophages, neutrophils, endothelial cells and T cells individually with mCherry, YFP, CFP and BFP we are able to benchmark our platform and to verify our findings.

9712-45, Session 11

Multimodal imaging with a nanosecond supercontinuum source
Claire Lefort, XLIM Institut de Recherche (France) and Univ. de Limoges (France) and Consiglio Nazionale delle Ricerche (France); Rodney P. O’Connor, XLIM Institut de Recherche (France); Véronique Blanquet, Fabienne Baraige, Unité de Génétique Moléculaire Animale (France); Vincent Tombelaine, LEUKOS (France); Philippe Leveau, Vincent Couderc, Philippe Leproux, XLIM Institut de Recherche (France)

Multimodal microscopy is a well-established technique for biological imaging of several kinds of targets. It is classically based on multiphoton processes allowing two means of contrast simultaneously: two-photon fluorescence (TPF) and second harmonic generation (SHG). Today, the quasi exclusive laser technology used in that aim is femtosecond titanium sapphire (Ti:Sa) laser. We experimentally demonstrate that a commercial nanosecond supercontinuum laser source (STM-250-VIS-IR-custom, Leukos, France; 1 ns, 600–2400 nm, 250 kHz, 1 W) allows to obtain the same kind of image quality in the case of both TPF and SHG, since it is properly filtered. The first set of images concerns the muscle of a mouse. It highlights the high contrast imaging of biological samples based on second harmonic generation (SHG) and non-resonant simultaneous absorption of two or more photons at GW/cm² intensities. However, high contrast imaging of living specimens without any destructive effect is limited to certain laser and exposure parameters with respect to the optical properties of the target.

We compared three different femtosecond lasers, including a novel ultra-compact ultrashort fiber laser, in the range of 15-180 fs and repetition rates of 50–300 MHz for optimal non-destructive two-photon autofluorescence imaging. In particular we determined the thresholds for the onset of photodamage effects such as impaired cell reproduction.
9712-46, Session 11

Coherence gated wavefront sensorless adaptive optics for two photon imaging

Yifan Jian, Michelle Cua, Simon Fraser Univ. (Canada); Stefano Bonora, IFN-CNR LUXOR Lab. (Italy); Edward N. Pugh Jr., Robert J. Zawadzki, Univ. of California, Davis (United States); Marinko V. Sarunic, Simon Fraser Univ. (Canada)

We present a novel system for adaptive optics two photon imaging. We utilize the bandwidth of the femtosecond excitation beam to perform coherence gated imaging (OCT) of the sample. The location of the focus is directly observable in the cross sectional OCT images, and adjusted to the desired depth plane. Next, using real time volumetric OCT, we perform Wavefront Sensorless Adaptive Optics (WSAO) aberration correction using a multi-element adaptive lens capable of correcting up to 4th order Zernike polynomials. The aberration correction is performed based on an image quality metric, for example intensity. The optimization time is limited only by the OCT acquisition rate, and takes ~30s. Following aberration correction, two photon fluorescence images are acquired, and compared to results without adaptive optics correction. This technique is promising for multiphoton imaging in multi-layered, scattering samples such as eye and brain, in which traditional wavefront sensing and guide-star sensorless adaptive optics approaches may not be suitable.

9712-47, Session 12

Large field of view in vivo multiphoton microscopy of human skin (Invited Paper)

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Clinical examination crucially relies on the ability to quickly examine large tissue areas and rapidly zoom in to regions of interest. Skin lesions often show irregularity in color and appearance in general, especially when they start to progress towards malignancy. Large field of view (FOV) and automatic translation of the imaging area are critical in the assessment of the entire lesion. Imaging of limited FOVs of the lesion can easily result in false negative diagnosis. We present a multiphoton microscope based on two-photon excited fluorescence and second-harmonic generation that images FOVs of about 1 mm² (without stitching adjacent FOVs) at speeds of 8 frames/second (512 x 512 pixels) with lateral and axial resolutions of 0.6 μm and 2 μm, respectively. The main novelty of this instrument is the design of the scan head, which includes a fast galvanometric scanner, relay optics, a beam expander and a high NA objective lens. We optimized the system based on the Olympus 25x, 1.05NA water immersion lens, that features a long working distance of 1 mm. Proper tailoring of the beam expander, which consists of the scan and tube lens elements, enables scaling of the FOV. The design criteria include a flat waveform of the beam, minimum field curvature, and suppressed spherical aberrations. All aberrations in focus are below the Marechal criterion of 0.07? rms for diffraction-limited performance. We demonstrate the practical utility of this microscope by in-vivo imaging of wide FOVs in normal human skin and of wound lesion margins in rat skin.

9712-48, Session 12

Design of a portable, wide field of view, GPU-accelerated multiphoton imaging system for real-time imaging of breast surgical specimens (Invited Paper)

Michael G. Giacomelli, Tadayuki Yoshitake, Osman O. Ahsen, Massachusetts Institute of Technology (United States); Lennart A. Husvogt, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); Yury Sheykin, Hilde Vardeh, Beth Israel Deaconess Medical Ctr. (United States); Jeffrey Brooker, Thorlabs, Inc. (United States); James L. Connolly, Beth Israel Deaconess Medical Ctr. (United States); Joachim Hornegger, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); Alex E. Cable, Thorlabs, Inc. (United States); James G. Fujimoto, Massachusetts Institute of Technology (United States)

Multiphoton microscopy is a promising method for performing intrasurgical evaluation of surgical specimen margins because of its molecular specificity and ability to optically section thick tissue. Previously, our group demonstrated high sensitivity and specificity for evaluating breast pathology post-operatively using multiphoton microscopy [1]. To facilitate clinical translation, we now describe the design and operation of a portable, inverted and wide field of view multiphoton system including microscope and femtosecond laser, operating on a 2.5 by 2 foot vibration-isolated cart suitable for operation in a clinic.

Combined with the new system, a new imaging protocol and a novel image processing algorithm are presented that render real-time virtual H&E histology images of fresh, unfixed tissue with dramatically improved imaging speed, contrast and correspondence with conventional H&E pathology as compared to previous methods. The combination of optimized scan optics and GPU-accelerated processing enable video rate, low latency virtual H&E histology of thick, unfixed tissue specimens at high resolution over a wide field of view. The system images a 2 mm x 2 mm field of view with 2048 x 2048 pixels at <1 μm transverse resolution and a 8 Hz frame rate. In addition, fast motorized actuation enables visualization of many-centimeter scale samples at surgically relevant time scales. We demonstrate the capability of the system by imaging human breast pathology in the clinic and demonstrating correspondence to conventional histopathology.


9712-49, Session 12

Compact fixed wavelength femtosecond oscillators as an add-on for tunable titanium sapphire lasers extend the range of applications towards multimodal imaging and optogenetics

Tommi Hakulinen, Spectra-Physics (Austria); Julien Klein, Spectra-Physics (United States)

In recent years multi-photon microscopy with fixed-wavelength at 1040 nm has raised increasing interest in life-sciences: Two-photon (2P) excitation of several dyes and fluorescent proteins (e.g. YFP, mFruit series) simultaneously with a single IR laser wavelength is feasible. Besides that SHG-, THG-imaging can be performed with 1040nm. Another way is a three-photon excitation of endogenous fluorophores present in cells such as NADH and FAD. The above mentioned methods can be combined for multimodal imaging and support all-optical physiological or optogenetics experiments for e.g. neuroscience.
A new approach is to extend the usability of existing tunable Titanium sapphire lasers by adding a fixed wavelength IR femtosecond oscillator. By that means a multitude of applications for multimodal imaging and optogenetics can be supported.

With this work we demonstrate that economical, small-footprint fixed-wavelength lasers can present an interesting add-on to tunable lasers that are commonly used in multiphoton microscopy.

9712-50, Session 12

Advanced multiphoton methods for in vitro and in vivo functional imaging of mouse retinal neurons
Noam Cohen, Adi Schejter, Nairouz Farah, Shy Shoham, Technion-Israel Institute of Technology (Israel)

Studying the responses of retinal ganglion cell (RGC) populations has major significance in vision research. Multiphoton imaging of optogenetic probes has recently become the leading approach for visualizing neural populations and has specific advantages for imaging retinal activity during visual stimulation, because it leads to reduced direct photoreceptor excitation. However, multiphoton retinal activity imaging is not straightforward: point-by-point scanning leads to repeated neural excitation while optical access through the rodent eye in vivo has proven highly challenging.

Here, we present two enabling optical designs for multiphoton imaging of responses to visual stimuli in mouse retinas expressing calcium indicators. First, we present an imaging solution based on Scanning Line Temporal Focusing (SLITE) for rapidly imaging neuronal activity in vitro. In this design, we scan a temporally focused line rather than a point, increasing the scan speed and reducing the impact of repeated excitation, while maintaining high optical sectioning. Second, we present the first in vivo demonstration of two-photon imaging of RGC activity in the mouse retina. To obtain these cellular resolution recordings we integrated an illumination path into a correction-free imaging system designed using an optical model of the mouse eye. This system can image at multiple depths using an electronically tunable lens integrated into its optical path.

The new optical designs presented here overcome a number of outstanding obstacles, allowing the study of rapid calcium- and potentially even voltage-indicator signals both in vitro and in vivo, thereby bringing us a step closer toward distributed monitoring of action potentials.

9712-51, Session 12

Widefield three-photon excitation: excitation of quantum dots and optogenetic opsins using 1300nm excitation
Christopher J. Rowlands, Demian Park, Kiryl Piatkevich, Oliver T. Bruns, Mounig Bawendi, Edward S. Boyd, Peter T. C. So, Massachusetts Institute of Technology (United States)

Three-photon excitation has been shown to improve the penetration depth of multiphoton excitation, by employing wavelengths with lower scattering cross-sections in biological tissue. In addition, higher-order multiphoton excitation serves to suppress the surface excitation that occurs due to the large incident power required for deep excitation. Nevertheless, all demonstrations to date have been for point-scanning techniques; while point-scanning is suitable for several forms of imaging, there are experiments that benefit from widefield excitation. These include (but are not limited to) stroboscopic illumination, optogenetic excitation of a cell, high-speed imaging and photodynamic therapy.

We demonstrate widefield three-photon excitation using temporal focusing to maintain z-sectioning. The instrument design will be described; it was constructed from off-the-shelf parts and requires little or no microscopy experience to operate. The excitation power is compatible with biological tissues.

We will demonstrate that quantum dots can be excited over fields of view that are hundreds of microns on a side, and that these dots can be imaged in a live mouse with a brain window. Finally, we will demonstrate that we can perform three-photon optogenetic stimulation of hippocampal neurons in a dish, despite the known difficulty of performing multiphoton excitation of an opsin.

9712-52, Session 13

Label-free NIR reflectance imaging as a complimentary tool for two-photon fluorescence microscopy (Invited Paper)
Anna Letizia Allegro Mascaro, European Lab. for Non-linear Spectroscopy (Italy) and Consiglio Nazionale delle Ricerche (Italy) and Istituto Nazionale di Ottica (Italy); Irene Costantini, Emilia Margoni, European Lab. for Non-linear Spectroscopy (Italy); Giulio Iannello, Alessandro Bria, Univ. Campus Bio-Medico (Italy); Leonardo Sacconi, European Lab. for Non-linear Spectroscopy (Italy) and Consiglio Nazionale delle Ricerche (Italy) and Istituto Nazionale di Ottica (Italy); Francesco S. Pavone, European Lab. for Non-linear Spectroscopy (Italy) and Univ. degli Studi di Firenze (Italy)

Two-photon imaging combined with targeted fluorescent indicators is extensively used for visualizing critical features of brain functionality and structural plasticity. Back-scattered photons from the NIR laser provide complimentary information without introducing any exogenous labelling. Here, we describe a versatile approach that, by collecting the reflected NIR light, provides structural details on the myelinated axons and blood vessels in the brain, both in fixed samples and in live animals. Indeed, by combining NIR reflectance and two-photon imaging of a slice of hippocampus from Thy1-GFPm mice, we show the presence of randomly oriented axons intermingled with sparsely fluorescent neuronal processes. The back-scattered photons guide the contextualization of the fluorescence structure within brain atlas thanks to the recognition of characteristic hippocampal structures. Label-free detection of axonal elongations over the layer 2/3 of mouse cortex under a cranial window was also possible in live brain. Finally, blood flow could be measured in vivo, thus validating label free NIR reflectance as a tool for monitoring hemodynamic fluctuations. The prospective versatility of this label-free technique complimentary to two-photon fluorescence microscopy is demonstrated in a mouse model of photothermal stroke in which the axonal degeneration and blood flow remodeling can be investigated simultaneously.

9712-53, Session 13

Brillouin imaging/sensing via time-resolved optical (BISTRO) measurements
Zhaoxiai Meng, Charles Ballman, Georgi I. Petrov, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Viscoelastic properties are essential for understanding biological function on cellular and tissue level. Brillouin microspectroscopy is a powerful tool for elasticity-specific non-invasive optical imaging. However, traditional approach using spontaneous Brillouin spectroscopy usually requires long acquisition time (> 100 ms), and its spectral quality is fundamentally limited by the spectrometer resolution. Nonlinear Brillouin spectroscopy, which is based on impulsive stimulation of acoustic phonons, coherently enhances the signal and allows time-dependent measurements permitting high spectral resolution and fidelity of Brillouin spectra. In this report, by
incorporating the concept of impulsive stimulated Brillouin scattering into microscopic measurements, we report a system for Brillouin Imaging and Sensing via Time-Resolved Optical (BISTRO) measurements. We employed a picosecond 532 nm or 1064 nm pulsed laser as a pump, and a 780 nm continuous-wave laser as a probe in a novel optical arrangement which allows microsecond-scale data acquisition. As a proof-of-principle, we demonstrated applications in the field of cell cytometry and microscopic imaging.

9712-54, Session 13
Multiphoton microscope driven by novel green laser pump
Dominik Marti, Anders K. Hansen, Mathias Christensen, Ole B. Jensen, Peter E. Andersen, DTU Risø Campus (Denmark)

We implemented a custom built diode based green laser source as a pump for the titanium sapphire (Ti:Sa) oscillator in a multiphoton microscopy setup. The green laser light at 532 nm is the result of sum frequency generation of two infrared laser diodes’ output, slightly distinct in their wavelengths, and exceeds 5 W in a nearly diffraction limited beam. This beam is then coupled into a Ti:Sa oscillator (Femtource Compact, Femtolasers GmbH) that in turn is the illumination source for a custom built multiphoton microscope. The diode based green laser can potentially be miniﬁed to a small footprint, making it very compact and versatile.

We characterized the oscillator’s output in terms of pulse length, spectrum, power and power stability, and compared these values to the ones obtained when pumping the same Ti:Sa oscillator with a Verdi V5 by Coherent. The Ti:Sa output parameters are equal in the two cases, apart from needing a 20% higher green pump power when pumping with our di-ode based laser to achieve the same output power as when pumping it with the Verdi V5.

We then used this system as the illumination source for a multiphoton microscope and compared images obtained when pumping the Ti:Sa oscillator with the two different green pump lasers. The images taken when pumping with our diode based green laser are at least of equal quality to the ones obtained when pumping the Ti:Sa oscillator with the Verdi V5. These results are encouraging and facilitate highly compact, high-quality nonlinear microscope systems.

9712-55, Session 13
Ratiometric detection of O2 sensing probe in tumor model via two photon microscopy
Calvin J. Yoon, Bumju Kim, Junhwa Lee, Seoyeon Bok, Joonhyuck Park, Seunghun Lee, Viet Hoan Le, Sungjee Kim, G-One Ahn, Ki Hean Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Hypoxia is one of the major characteristics of solid tumors and is linked with increased radiotherapy and drug resistance. Therefore, methods of pO2 monitoring in vivo have developed interest. For the purpose of detecting hypoxic regions in tissue and monitoring pO2 in vivo, RuQD nanoprobes were constructed by cowrapping oxygen-insensitive quantum dots (QD) with oxygen-sensitive ruthenium (Ru) in amphiphilic polymers. In this study, we have examined the ability of RuQD to detect changes in pO2 levels in vitro by ratiometric sensing using two photon microscopy. Two photon microscopy, optimal for deep tissue imaging, was used to detect the respective intensities of Ru and QD. Ratiometric sensing, which overcomes the instabilities of intensity-based imaging, showed that the Ru/ QD intensity ratio is greater in hypoxia than in normoxia due to the quenching of Ru phosphorescence by O2. In addition, ratiometric sensing of RuQD in spheroid and immunofluorescence detection via staining of hypoxic marker CA9, we have confirmed that ratiometric sensing of RuQD successfully detects pO2 changes and distribution. These results raise possibilities for the detection of hypoxic tumors in vivo as well as monitoring of pO2 levels.

9712-56, Session 13
A new method using multiphoton imaging and morphometric analysis for differentiating chromophobe renal cell carcinoma and oncocytoma kidney cancers
Binlin Wu, Southern Connecticut State Univ. (United States); Sushmita Mukherjee, Manu Jain, Weil Cornell Medical College (United States)

Distinguishing chromophobe renal cell carcinoma (chRCC) from oncocytoma on hematoxylin and eosin images can be difﬁcult and may require time-consuming ancillary procedures. Multiphoton microscopy (MPM) as an optical biopsy modality was used to rapidly generate images from ﬁxed unstained tissue sections, with sub-cellular histological resolution based on intrinsic tissue emissions. It may be an alternative rapid diagnostic tool to differentiate between chRCC and oncocytoma. Twenty-four unstained deparafﬁnized tissue sections of chRCC and oncocytoma (n=12 each) were imaged with MPM. Emission light which includes second harmonic generation (90nm) and autoﬂuorescence signal with wavelengths between 390 nm and 650 nm was recorded in multiple channels with different wavelength bands. Granular structure was observed in both chRCC and oncocytoma. Quantitative morphometric analysis was conducted to distinguish chRCC and oncocytoma. To perform the analysis, cytoplasm and granules in kidney cells were segmented from the images. Their area and ﬂuorescence intensity were found in different channels. Multiple features were measured to quantify the morphological and ﬂuorescence properties. Linear support vector machine (SVM) was used for classiﬁcation. Resubstitution validation, cross validation and receiver operating characteristic (ROC) curve are implemented to evaluate the efﬁcacy of the SVM classiﬁer. A wrapper feature algorithm was used to select the optimal features which provide the best predictive performance in separating the two tissue types (classes). Statistical measures such as sensitivity, speciﬁcity, accuracy and area under curve (AUC) of ROC are calculated to evaluate the efﬁcacy of the classiﬁcation. Over 80% accuracy was achieved as the predictive performance.

9712-57, Session 13
Quantitative structural markers of colorectal dysplasia progression in a cross sectional study of the APC/min mouse using label-free multiphoton microscopy
Sandra P. Prieto, Gage J. Greening, Cassandra Reed, Timothy J. Muldoon, Univ. of Arkansas (United States)

Label-free multiphoton imaging of tissue is of increasing interest, as advances have been made in endoscopic clinical application of multiphoton microscopy, such as second harmonic generation (SHG) scanning endoscopy monitoring of cervical collagen in mice [1]. We used C57BL/6J-APCmin mice as a model to investigate the progression of gastrointestinal dysplasia, as they are highly prone to develop spontaneous intestinal adenomas beginning around 5 weeks of age. We acquired label-free multiphoton images of ex-vivo murine colon and intestine, focusing on the collagen structure changes over time in mice ranging from 8 to 16 weeks of age. Age-matched wild-type mice were imaged for comparison. Series of images were acquired within the colonic and intestinal tissue at depth intervals of 20 microns from muscularis to the epithelium, up to a maximum depth of 180 microns. The imaging system comprised a two-photon laser tuned to 800nm wavelength excitation, and the SHG emission was filtered.

390 nm and 650 nm was recorded in multiple channels with different wavelength bands. Granular structure was observed in both chRCC and oncocytoma. Quantitative morphometric analysis was conducted to distinguish chRCC and oncocytoma. To perform the analysis, cytoplasm and granules in kidney cells were segmented from the images. Their area and fluorescence intensity were found in different channels. Multiple features were measured to quantify the morphological and fluorescence properties. Linear support vector machine (SVM) was used for classification. Resubstitution validation, cross validation and receiver operating characteristic (ROC) curve are implemented to evaluate the efficacy of the SVM classifier. A wrapper feature algorithm was used to select the optimal features which provide the best predictive performance in separating the two tissue types (classes). Statistical measures such as sensitivity, specificity, accuracy and area under curve (AUC) of ROC are calculated to evaluate the efficacy of the classification. Over 80% accuracy was achieved as the predictive performance.
with a 400/40nm bandpass filter before reaching the photomultiplier tube. Images were acquired at 30 frames per second, for 200 cumulative frames, with a field of view of 261um^2, and 40mW at sample. Image series were compared to histopathology H&E slides taken from both the precise imaged location and an adjacent location. Quantitative metrics for determining differences between wild-type and heterozygous collagen structure were applied, including measuring glandular size and circularity, and collagen orientation parameter [2].

Microscopy using source and detector arrays (Invited Paper)

Colin J. Sheppard, Marco Castello, Giuseppe Vicipiomi, Marti Duocastella, Alberto Diaspro, Istituto Italiano di Tecnologia (Italy)

There are basically two types of microscope, which we call conventional and scanning. The former type is a full-field imaging system. In the latter type, the object is illuminated with a probe beam, and a signal detected. We can generalize the probe to a patterned illumination. Similarly we can generalize the detection to a patterned detection. Combining these we get a range of different modalities: confocal microscopy, structured illumination (with full-field imaging), spinning disk (with multiple illumination points), and so on. The combination allows the spatial frequency bandwidth of the system to be doubled. In general we can record a four dimensional (4D) image of a 2D object (or a 6D image from a 3D object, using an acoustic tuneable lens). The optimum way to directly reconstruct the resulting image is by image scanning microscopy (ISM). But the 4D image is highly redundant, so deconvolution-based approaches are also relevant. ISM can be performed in fluorescence, bright field or interference microscopy. Several different implementations have been described, with associated advantages and disadvantages. In two-photon microscopy, the illumination and detection point spread functions are very different. This is also the case when using pupil filters or when there is a large Stokes shift.

Super-resolved nonlinear microscopy with spatial frequency modulated imaging

Keith Wernsing, Jeffrey J. Field, Randy A. Bartels, Scott R. Domingue, Colorado State Univ. (United States); Jeffrey A. Squier, Alyssa Allende Motz, Colorado School of Mines (United States); Dean H. Levi, Darius Kuciauskas, National Renewable Energy Lab. (United States); Jennifer DeLuca, Keith DeLuca, Colorado State Univ. (United States)

Spatial Frequency Modulated Imaging (SPIFI) is used in a nonlinear modality to produce resolution enhancements of 27, where is the nonlinearity of the optical response of the media. We demonstrate resolution enhancements along one spatial dimension in 2nd order (?2) nonlinear processes, achieving a 4x resolution enhancement beyond the diffraction limit. This holds for both second-harmonically-generated (SHG) light and two-photon-excited fluorescence (TPEF). In the former case multiphoton SPIFI (MP-SPIFI) becomes a robust super-resolved imaging technique for virtual-state signals, e.g., harmonically-generated light. In the latter case, MP-SPIFI places no additional requirements upon the fluorophore beyond those of conventional imaging; it does not require fluorophore photo-activation, saturation, or bleaching to resolve beyond the diffraction limit. Resolution enhancement in SPIFI works by projecting spatial frequency fringes onto the sample that lie beyond the cutoff frequency of the objective lens. A beam is focused to a line on a spinning disk modulator; the disk acts as a diffraction grating with a time-varying line density. A lens system collects the diffracted beams and causes them to reinterfere in the sample plane, creating a sequence of 1D intensity fringes where the spatial frequency increases with time. In linear SPIFI, images are encoded at 1x and 2x the diffraction limit. In two-photon MP-SPIFI, the nonlinearity drives additional spatial frequency content, encoding images up to 4x the diffraction limit in a single-shot image. Using pulsed near infrared excitation thus provides a pathway to nonlinear imaging at depth in scattering media while maintaining nearly confocal resolution.

Investigating the performance of reconstruction methods used in structured illumination microscopy as a function of the illumination pattern’s modulation frequency

Hasti Shabani, The Univ. of Memphis (United States); Emilio Sánchez-Ortega, Univ. de València (Spain); Chrysanthe Preza, The Univ. of Memphis (United States)

Surpassing the resolution of optical microscopy defined by the Abbe diffraction limit, while simultaneously achieving optical sectioning, is a
challenging problem particularly for live cell imaging of thick samples. Among a few developing techniques, structured illumination microscopy (SIM) addresses this challenge by imposing higher frequency information into the observable frequency band confined by the optical transfer function either doubling the spatial resolution or filling the missing cone based on the spatial frequency of the pattern. Reconstruction methods for SIM can be categorized into two main groups. The first group of methods decomposes the low and high frequency components from the recorded low-resolution images and then combines them to reach a high-resolution image. In contrast, the second group of methods relies on iterative optimization approaches to minimize the error between estimated and forward images. In this paper, we study the performance of both methods of SIM by simulating fluorescence microscopy images from different type of objects (ranging from simulated basic two-point sources to extended objects) to investigate their effectiveness on restoring various types of object’s power spectrum. Several aspects of these approaches, such as computational complexity and robustness to noise are compared when modulation frequency of the illumination pattern is changing from zero to the incoherent cut-off frequency of the imaging system. Our results show that increasing the amount of imposed information by using a randomly distributed pattern provides a low cost solution to obtaining resolution similar to that produced in confocal microscopy and other methods of structured illumination, without the requirement of complex or elaborate equipment, coherent light sources, or fluorescence. Further an LED is used rather than a laser to minimize speckling as well as increase the safety of the system in many applications.

9713-6, Session 2

Volumetric real-time wide-field microscopy with tunable acoustic gradient lens

Ting Hsuan Chen, Craig B. Arnold, Princeton Univ. (United States)

Three-dimensional real-time imaging is a fundamental challenge for understanding dynamic processes. In this presentation, we propose a new method based on a tunable acoustic gradient lens integrated in a simple optical system. By synchronizing a pulsed LED with a high-speed camera, we are able to resolve a volume of 2 millimeters and 2 millimeters with depth 1 millimeter in 7 microseconds. A simulation model of the optical system is provided and serves as a useful tool for designing the optical system for the desired aspect-ratio of the imaging volume. The ability to resolve a volume in microseconds opens the door to exploring the fundamental dynamics in micro scale.

9713-7, Session 2

Line-scan focal modulation microscopy: a comparison study

Nanguang Chen, Shilpa Pant, National Univ. of Singapore (Singapore)

Focal Modulation microscopy (FMM) is a novel imaging technique offering enhanced optical sectioning. FMM introduces spatiotemporal phase modulation in the illumination beam, resulting in intensity modulated excitation and emission light. As the background fluorescence excited by scattered photons are stationary, it is possible to differentiate it from the signal. Currently we are exploring a high-speed implementation of FMM. We have developed line scan focal modulated microscopy, which features parallel illumination and parallel detection. An imaging speed of 100 frames per second achieved with such a prototype. We have conducted a series of experiments to compare the performances of line-scan FMM, line-scan confocal microscopy, and point-scanning confocal microscopy. It is evident that line-scan FMM provides the best solution for a combination of high-speed, high contrast, and high spatial resolution.

9713-5, Session 1

Super-resolution through broadband random speckle patterns

Zachary Hoffman, Charles A. DiMarzio, Northeastern Univ. (United States)

Broadband structured illumination patterns are used to produce super-resolution images using a DMD in conjunction with an incoherent light source. By projecting patterns of varied spatial frequencies super-resolution can be achieved. This has been shown to work both in simulation and in experiment. Using the random pattern it can be shown that the total resolution enhancement is degraded slightly as a result of lower power at higher spatial frequencies. This is verified by comparing against a regular sinusoidal pattern that contain a discrete spatial frequency. Further, we explore the impact of resolution loss via the transmit and receive legs of the microscope. If the optical transfer function of each leg is known, an iterative scheme can be employed to further increase the resolution of the system. It is also shown that with a slight modification to this scheme, we can recover a fully sectioned image with an enhanced spatial resolution. Using a randomly distributed pattern provides a low cost solution to obtaining information similar to that produced in confocal microscopy and other methods of structured illumination, without the requirement of complex or elaborate equipment, coherent light sources, or fluorescence. Further an LED is used rather than a laser to minimize speckling as well as increase the safety of the system in many applications.

9713-8, Session 2

Speckle pattern analysis to provide displacement profile induced by acoustic radiation force

Ali Vakili, Northeastern Univ. (United States); Joseph L. Hollmann, ICFO - Institut de Ciències Fotòniques (Spain); Ray Glynn Holt, Boston Univ. (United States); Charles A. DiMarzio, Northeastern Univ. (United States)

Optical imaging in a turbid medium is limited because of the multiple scattering a photon undergoes while traveling through the medium. Therefore, optical imaging is unable to provide high resolution information deep in the medium. In the case of soft tissue, acoustic waves unlike light, can travel through the medium with negligible scattering. However, acoustic waves cannot provide medically relevant contrast. Hybrid solutions have been applied to use the benefits of both imaging methods.

One such method, acoustic radiation force (ARF) utilizes a focused, high intensity ultrasound beam to generate a force within an acoustically absorbing medium. This force causes the displacement of optical scatterers within the tissue. The amount of displacement is a function of the medium and the applied force. To monitor the displacement induced by the ARF, we have utilized speckle pattern analysis.
The speckle pattern is the result of interfering optical waves with different phases. As light travels through the medium, it undergoes several scattering events. Hence, it generates different scattering paths which depends on the location of the particles. Light waves that travel along these paths have different phases (different optical path lengths). ARF induces displacement to scatterers within the acoustic focal volume, and changes the optical path length. The result is a change in the speckle pattern. Results suggest that the average change in the speckle pattern measures the displacement of particles and can provide mechanical properties of the medium.

9713-9, Session 2

**Fluorescence microscopy with isotropic resolution using three objectives**

Thomas Huelsnitz, Peter Kner, The Univ. of Georgia (United States)

Widefield and confocal fluorescence microscopy using a single objective suffer from poor resolution and a strong anisotropy between the lateral and axial resolution. Coherently combining the excitation and emission from two coaxial objectives improves the axial resolution up to sevenfold. In ISM (widefield fluorescence imaging with two coaxial objectives) and 4Pi microscopy (confocal fluorescence imaging with two coaxial objectives), the axial resolution is dramatically improved but the lateral resolution is unchanged.

Here we investigate the coherent combination of three objectives to create a point spread function (PSF) that is isotropic with higher resolution in the plane of the objectives. We develop a theoretical framework for simulating the performance of interferometric imaging with three objectives in both confocal and widefield configurations. Using three identical objectives with a large working distance and 0.9 numerical aperture (NA), the full-width half maximum of the confocal PSF is 142 nm compared to the lateral FWHM of 274 nm for imaging with a single objective at a wavelength of 515 nm. In a confocal arrangement, the PSF is indeed isotropic, but in widefield imaging, the axial resolution suffers because the off-axis objectives must be corrected for oblique image planes.

9713-10, Session 3

**Coherent image reconstruction of incoherent contrast light**

Jeffrey J. Field, David G. Winters, Randy A. Bartels, Colorado State Univ. (United States)

3D microscopy with fluorescent light often requires serial data acquisition, restricting imaging speeds. Volumetric imaging of coherent light permits increases in frame rates by exploiting spatial phase to reconstruct a 3D image from a single measurement. Until now, coherent reconstruction techniques have been unavailable for incoherent contrast light, such as fluorescence. Here we introduce an imaging paradigm that encodes the phase of spatially-coherent illumination beams into a measurement of fluorescent light with a single-element detector. We show that coherent reconstruction methods, such as digital holography and diffraction tomography, can be directly applied to a measurement of spatially-incoherent light.

A spatially-coherent illumination beam is brought to a line focus on a modulator that imparts a time-dependent spatial frequency to the line focus. The beam diffracts into multiple beams propagating at varied angles with respect to the optic axis. A 4-f imaging system is employed to re-image the modulation plane onto a specimen, where the diffracted beams interfere to generate intensity fringes. The illumination pattern contains a unique spatial frequency, which is projected onto the object. The emitted phase difference between the illumination beams is also encoded into the photodetector signal. With both the amplitude and phase information, the fluorescence intensity signal can be numerically propagated to reconstruct a 2D image.

9713-11, Session 3

**Multimodal interferometric microscopy for label-free 3D imaging of live cells during flow**

Natan T. Shaked, Tel Aviv Univ. (Israel)

I present multimodal wide-field interferometric microscopy platform for label-free 3-D imaging of live cells during fast flow. Using holographic optical tweezers, multiple cells can be optically trapped and rapidly rotated on all axes, while acquiring an external off-axis wide-field interferometric module developed in our lab. The interferometric projections are rapidly processed into the 3-D refractive-index profile of the cells using a tomographic phase microscopy algorithms that take into consideration optical diffraction effects. The algorithms for the 3-D refractive-index reconstruction, and for calculating various morphological parameters that should serve for online sorting of cells, are efficiently implemented in a nearly real-time manner. The potential of this new high-throughput imaging technique is for label-free image analysis and sorting of cells during flow, to substitute current cell sorting devices, which are based on external labeling that eventually damages the cell sample.

9713-12, Session 3

**New approaches for the analysis of confluent cell layers with quantitative phase digital holographic microscopy**

Luisa Pohl, Mathias Kaiser, Steffi Ketelhut, Eva Doepker, Suzana Pereira, Jürgen Schnekenburger, Francisco Goycoolea, Björn Kemper, Westfälische Wilhelms-Univ. Münster (Germany)

Digital holographic microscopy (DHM) has been proven to be a versatile tool for high resolution non-destructive inspection of technical surfaces and minimally-invasive label-free live cell imaging in various application fields (see 1-3 and references therein). Analysis of living cells with DHM is typically performed utilizing quantitative phase imaging by detection of optical path length changes that are caused by specimens with a higher refractive index than the surrounding medium. For this reason, the analysis of confluent cell layers represents a challenge as the retrieved phase images do not contain sufficient information for the retrieval of absolute growth and morphology parameters like the cellular dry mass or the cell thickness. We present novel strategies for the analysis of confluent cell layers utilizing a histogram based-evaluation of quantitative DHM phase contrast images. The applicability of the proposed numerical procedures is demonstrated by the quantification of drug and flow induced cell morphology changes. The retrieved results show that the method is capable to detect cellular stress in confluent cell layers with high reliability.

**References**


 Imaging macroscopic targets hidden behind a scattering layer using a low-coherence and wide-field interferometry
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With the advancement of 3D display technology, 3D imaging of macroscopic objects has drawn much attention as they provide the contents to display. The most widely used imaging methods include a depth camera, which measures time of flight for the depth discrimination, and various structured illumination techniques. However, these existing methods have poor depth resolution, which makes imaging complicated structures a difficult task. In order to resolve this issue, we propose an imaging system based upon low-coherence interferometry and off-axis digital holographic imaging. By using light source with coherence length of 200 μm, we achieved the depth resolution of 100 μm. In order to map the macroscopic objects with this high axial resolution, we installed a pair of prisms in the reference beam path for the long-range scanning of the optical path length. Specifically, one prism was fixed in position, and the other prism was mounted on a translation stage and translated in parallel to the first prism. Due to the multiple internal reflections between the two prisms, the overall path length was elongated by a factor of 50. In this way, we could cover a depth range more than 1 meter. In addition, we employed multiple speckle illuminations and incoherent averaging of the acquired holographic images for reducing the specular reflections from the target surface. Using this newly developed system, we performed imaging targets with multiple different layers and demonstrated imaging targets hidden behind the scattering layers. The method was also applied to imaging targets located around the corner.

 Dual detection confocal microscopy: high-speed surface profiling without depth scanning
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We propose a new method for three-dimensional (3-D) imaging without depth scanning that we refer to as the dual-detection confocal microscopy (DDCM). Compared to conventional confocal microscopy, DDCM utilizes two pinholes of different sizes. DDCM generates two axial response curves which have different stiffness according to the pinhole diameters. The two axial response curves can draw the characteristic curve of the system which shows the relationship between the axial position of the sample and the intensity ratio. Utilizing the characteristic curve, the DDCM reconstructs a 3-D surface profile with a single 2-D scanning. The height of each pixel is calculated by the intensity ratio of the pixel and the intensity ratio curve. Since the height information can be obtained directly from the characteristic curve without depth scanning, a major advantage of DDCM over the conventional confocal microscopy is a speed. The 3-D surface profiling time is dramatically reduced. Furthermore, DDCM can measure 3-D images without the influence of the sample condition since the intensity ratio is independent of the quantum yield and reflectance. We present two methods to implement DDCM. The first system utilized two photo multiplier tubes (PMTs) and two pinholes of different diameters in the detection system. Another system utilized the digital micro-mirror device (DMD) as reconfigurable multi-pinholes. Here, we demonstrate the working principle of DDCM and the feasibility of the proposed methods.

 Optical axial scanning in structured illumination imaging enabled by a tunable lens
Taylor Hinsdale, Bilal H. Malik, Cory A. Olsovsky, Javier A. Jo, Kristen C. Maitland, Texas A&M Univ. (United States)

We present a volumetric imaging method for biological tissue that is free of mechanically scanning components. The optical sectioning in the system is obtained by structured illumination microscopy (SIM) with the depth of focus being varied by the use of an electronic tunable-focus lens (ETL). The performance of the axial scanning mechanism was evaluated and characterized in conjunction with SIM to ensure volumetric images could be recorded and reconstructed without significant losses in optical section thickness and lateral resolution over the full desired scan range. It was demonstrated that sub-cellular image resolutions were obtainable in both microsphere films and in ex vivo oral mucosa, spanning multiple cell layers, without significant losses in image quality. The mechanism proposed here has the ability to be integrated into any wide-field microscopy system to convert it into a three-dimensional imaging platform without the need for axial scanning of the sample or imaging optics. The ability to axially scan independent of mechanical movement also provides the opportunity for the development of endoscopic systems which can create volumetric images of tissue in vivo.

 Confocal fluorometer for diffusion tracking in 3D engineered tissue constructs
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There are many areas in healthcare diagnostics and screening where the lack of quantitative monitoring technologies is a major limitation. It is often impossible to develop diffusion theories for new pharmaceuticals, antibodies, cancer cells and liposomes without utilising 3D tissue constructs and tracking the diffusion in vitro in 3D. This monitoring must be in situ, non-invasive, real-time and economical.

By combining confocal fluorometry with 3D scanning it has been possible to achieve this required 3D measurement capability. A well plate containing the constructs is positioned relative to the measurement location with 1μm accuracy. Once positioned the meter illuminates the sample at 488nm, detects the returning 530nm signal, moves to the next location then repeats. In this way a full 3D map is built up. Whilst the data is collected it is buffered and then once the measurement is completed it is saved both as z-location image slices and CSV files. The operator therefore has access to visual images that provide immediate understanding of how the compound is moving and also the quantified data required for detailed analysis. The software combines the individual z-section slices to create a video either moving through the layers or allowing a 3D representation.

For an example oncology application, micro-particles diffusing in heterogeneous Type 1 collagen gel were assessed. A central region of the structure was seeded with fluorescently labelled microparticles and their movement assessed over 8 days. Deformation of the fibril meshwork was observed (for the first time) as the fluospheres moved throughout the medium.
Simultaneous fluorescence and high-resolution bright-field imaging with aberration-correction over a wide field-of-view with Fourier ptychographic microscopy

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We present a method to acquire both fluorescence and high-resolution bright-field images with correction for the spatially varying aberrations over a microscope's wide field-of-view (FOV). First, the procedure applies Fourier ptychographic microscopy (FPM) to retrieve the amplitude and phase of a sample, at a resolution that significantly exceeds the cutoff frequency of the microscope objective lens. At the same time, FPM algorithm is able to leverage on the redundancy within the set of acquired FPM bright-field images to estimate the microscope aberrations, which usually deteriorate in regions further away from the FOV's center. Second, the procedure acquires a raw wide-FOV fluorescence image within the same setup. Lack of moving parts allows us to use the FPM-estimated aberration map to computationally correct for the aberrations in the fluorescence image through deconvolution. Overlaying the aberration-corrected fluorescence image on top of the high-resolution bright-field image can be done with accurate spatial correspondence. This can provide means to identifying fluorescent regions of interest within the context of the sample's bright-field information. An experimental demonstration successfully improves the bright-field resolution of fixed, stained and fluorescently tagged HeLa cells by a factor of 4.9, and reduces the error caused by aberrations in a fluorescence image by 31%, over a field of view of 6.2 mm by 9.3 mm. For optimal deconvolution, we show the fluorescence image needs to have a signal-to-noise ratio of ~18.

The impact of absorption coefficient on polarimetric determination of berry phase based depth resolved characterization of biomedical scattering samples: a polarized Monte Carlo investigation

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The modulation of the state of polarization of photons due to scatter generates associated geometric phase that is being investigated as a means for decreasing the degree of uncertainty in back-projecting the paths traversed by photons detected in backscattered geometry. In our previous work, we established that polarimetrically detected Berry phase correlates with the mean photon penetration depth of the backscattered photons collected for image formation. In this work, we report on the impact of state-of-linear-polarization (SOLP) filtering on both the magnitude and population distributions of image forming detected photons as a function of the absorption coefficient of the scattering sample. The results, based on Berry phase tracking implemented Polarized Monte Carlo Code, indicate that sample absorption plays a significant role in the mean depth attained by the image forming backscattered detected photons.

Increasing spatial resolution in confocal Raman microscopy

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In the operating room, surgeons observe the surgical site in detail using the surgical microscopes. On the other hand, Optical Coherence Tomography (OCT) is a non-invasive optical imaging technique that shows cross-sectional images of biological tissue. Combination of OCT and surgical microscope would enable to observe the macroscopic image and the tomographic structures of surgical site at the same time. Full-field swept-source OCT (FF-SS-OCT) enables high-speed three-dimensional volumetric imaging since it does not require a scanning module that is needed in a point scanning OCT. However, A-scan time for one point is much longer than the point scanning OCT. Thus, the sample motion or vibration during the acquisition can affect the image quality significantly in FF-SS-OCT, compared to the point scanning OCT. The sample motion distorts the interference fringe pattern. In FF-SS-OCT acquisition, even a small motion can be magnified significantly, which is affected by the A-line acquisition rate and the frequency of the motion. The motion can be estimated by applying short-time Fourier transform on the A-line raw data, since the motion induces phase changes on interference pattern while sweeping the wavelengths. We can correct the image blur by correcting the motion-induced phase shift. Here, we present an integration of the surgical microscope with FF-SS-OCT and an algorithm to measure and correct the sample motion.

Application of linear-scale differential analysis in phase correlation method of image stitching

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The problem of sequent calculation of the value of spatial displacement (translation) of two adjacent frames having a common content is the basic one in the process of creation of panoramic digital images. The necessary condition for the calculation of the displacement value is to provide the presence of overlapping region. Thus it is evident that the bigger square of this region provides more reliable calculation of displacement value. On the other hand the increase of overlapping area leads to substantial increase in the time required to scan a ROI (Region Of Interest) and image processing that is highly undesirable for imaging systems operating in real time. In various fields of optical engineering such as thermal imaging, fluorescent or confocal microscopy the problem described above becomes more complicated because the source frames of digital images are often noisy, they have low contrast, low number of objects within field of view, the edges of said objects are blurred etc. The known methods of stitching become useless being applied to low quality images and cannot provide the
accurate calculation of displacement value with probability equal to 1. The aim of present work is to propose and investigate a robust method of constructing of panoramic images which does not consume much processing resources. The phase correlation (PC) method is taken as a basic one due to simplicity of its algorithm however it demonstrates high sensitivity to above-mentioned disturbing factors that strongly limits the possibilities of its practical application as it does not allow to find reliably the position of the correlation peak at noisy background. As conventional approach of noise removal in digital images is realized usually by means of low-pass filtering in frequency domain it is known that its effectiveness is not satisfactory in all cases. The algorithm of proposed method besides standard PC procedure includes the operation of pre-filtering of the original image in the spatial domain by a linear-scale differential analysis (LSDA) with subsequent thresholding of calculated values of intensity gradients to a predetermined level. Repeated multiple application of LSDA to the original image and reset to zero the image intensities which values are less than predetermined threshold significantly increases the SNR and accuracy of calculating of the displacement value of neighboring frames under stitching.

It is shown for artificially blurred (size of blur kernel was varied in the range from 1 to 5 pixels) images that proposed method allows to decrease in several times the square of overlapping region while the probability of successive stitching is kept to 1. It is shown also that method provides the reliable calculation of displacement value of stitching images corrupted by additive Gaussian noise having up to 20% level under frame overlapping index equal to 0.5. Modified PC method may be used in real-time imaging systems in the process of creation of panoramic digital images consisting in frames imposed by fatal degrading factors while it provides low-cost accurate calculation of frame displacement value under low overlapping condition.

9713-58, Session PMon

**Improving the lateral resolution of optical coherence tomography for imaging of skins**

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Qualitative and quantitative analysis of the skin is crucial for early detection of skin diseases, such as malignant melanomas and non-melanoma skin cancer. Although current optical imaging devices, such as confocal microscopy (CM), optical coherence microscopy (OCM) and multiphoton laser microscopy (MPLM), have very high lateral resolution (~sub-micrometer), their limited penetration depth (less than 0.2mm) prevents them detecting basal cell carcinomas (BCCs) at larger depth, not even to measure the thickness of BCCs in vivo. On the contrary, optical coherence tomography (OCT), as a non-invasive cross-sectional imaging technology, can provide larger imaging range (~2mm) than CM, OCM and MPLM with in vivo viewing. The only limitation of OCT to be widely applied to the skin research is the relatively low lateral resolution (5-15um). The traditional method to improve lateral resolution is to increase the numerical aperture (NA) at the expense of reducing the penetration depth, which is unacceptable for skin viewing at larger depth. Here, we present a superresolution method applied to OCT, using a series of slightly shifted C-scans, to improve lateral resolution without loss of penetration depth. Furthermore, compared with traditional median filter algorithm, the latest K-SVD denosing method is utilized to reduce noise as well as keeping the structure details. Finally, after deconvolution with a proper point spread function (PSF), we demonstrate this approach can significantly improve the quality of the 3D skin images, and has the potential to help researchers to detect and diagnose skin diseases correctly in vivo in the future.

9713-59, Session PMon

**Three dimensional measurement of cAMP gradients using hyperspectral confocal microscopy**

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Cyclic AMP (cAMP) is a ubiquitous second messenger known to differentially regulate many cellular functions over a wide range of timescales. Several lines of evidence have suggested that the distribution of cAMP within cells is not uniform, and that cAMP compartmentalization is largely responsible for signaling specificity within the cAMP signaling pathway. However, to date, no studies have experimentally measured three dimensional (3D) cAMP distributions within cells. Here, we used both 2D and 3D hyperspectral microscopy to visualize cAMP gradients in endothelial cells from the pulmonary microvasculature (PMVECs). cAMP levels were measured using a FRET-based cAMP sensor comprised of a cAMP binding domain from EPAC sandwiched between a donor and acceptor – Turquoise and Venus fluorescent proteins. Data were acquired using either a Nikon A1R spectral confocal microscope or custom spectral microscopy system. Analysis of 2D hyperspectral image stacks from a single confocal slice or from summed confocal slices (mathematical operation) or a widefield microscope (optical operation) indicated little or no cAMP gradients were formed within PMVECs under basal conditions or following agonist treatment. However, analysis of hyperspectral image stacks from 3D cellular geometries (z stacks) demonstrated marked cAMP gradients from the apical to basolateral membrane of PMVECs. These results strongly suggest that 2D imaging studies of cAMP compartmentalization – whether epifluorescence or confocal microscopy – may lead to erroneous conclusions about the existence of cAMP gradients, and that 3D studies are required to assess mechanisms of signaling specificity.

9713-60, Session PMon

**Three-dimensional dental scanning method based on structured illumination microscopy**

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Structured illumination microscopy was widely applied to investigate the three-dimensional structure of small objects. In this abstract, we present a three-dimensional scanning method which is based on structured illumination microscopy. By implementing a piezo motor stage and a liquid lens into the imaging setup, a fast and vibration-free scanning is achieved. We test the viability of the scanning method by imaging human teeth and acquire the point clouds (x, y, z coordinates) of the teeth. We expect our research will pave the way of the development of three-dimensional intraoral scanners.

9713-61, Session PMon

**The cubic protocol adapted for 3D imaging of the intact murine colon with light sheet microscopy**

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Following the CUBIC protocol we are currently capable of clearing intact murine colon tissue while maintaining the endogenously expressed
Hologram encoding strategies for non-Bayesian noise suppression in digital holography reconstructions and optical display

Vittorio Bianco, Pasquale Memmolo, Andrea Finizio, Melania Paturzo, Pietro Ferraro, Istituto di Scienze Applicata e Sistemi Intelligenti (Italy) and Consiglio Nazionale delle Ricerche (Italy)

Images from coherent laser sources are severely degraded by a mixture of speckle and incoherent additive noise. In Digital Holography, Bayesian approaches reduce the incoherent noise, but prior information are needed about the noise statistics. On the other hand, non-Bayesian techniques present the shortcomings of resolution loss or complex acquisition systems, required to record multiple uncorrelated holograms to be averaged. Non coherent methods successfully work on the image amplitude reconstruction, but these cannot preserve the coherence between amplitude and phase. What is highly required is a method able to directly work on the complex field, in order to yield a denoised hologram that can be propagated back and forth for optical display purposes by SLMs. Here we first propose a fast, non-Bayesian method which performs a numerical synthesis of a moving diffuser in order to reduce the noise without prior information. The technique is one-shot, as only one single hologram capture is required. Starting from a single acquisition, multiple uncorrelated reconstructions are provided by random sparse resampling masks, which can be incoherently averaged. Experiments show a significant improvement, close to the theoretical bound. Noteworthy, the resolution of the unprocessed image is preserved. We then move a step forward and we show a novel encoding formula allowing us to directly synthesize a denoised hologram which can be optically displayed with dramatically reduced noise. Starting from denoised holograms, we will show the most recent non coherent techniques to further suppress speckle artifacts, providing for the first time quasi-noise-free DH reconstructions.

Super-resolution optical microscopy by using dielectric microspheres and microwires

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Optical super-resolution imaging would have a profound impact on many areas, such as life and material sciences. We experimentally demonstrate that super-resolution imaging of specimens containing sub-diffraction-limited features is feasible by using dielectric microcylinders and microspheres placed in contact with the specimen to collect the sub-wavelength features of the specimen and transmit them to the far-field with magnification enabling three fold resolution enhancements. Through capillary force lithography followed by photopatterning, we fabricated monodisperse microwires with 5-10 μm diameters with refractive index ~1.3-1.6. We used such microwires and barium titanate glass microspheres (~2-100 μm diameters and refractive index ~1.9-2.1) to resolve sub-diffraction-limited features of various specimens. The achieved improvement in imaging resolution was measured based on measuring the imaging system's effective point-spread function through image deconvolution. We performed FDTD numerical modeling to study the super-resolution focusing properties of microspheres and microcylinders. Potential applications of this imaging technique include biological imaging, microfluidics, and nanophotonics applications for imaging individual cells and nanoparticles.
9713-22, Session 5

**EPIC microscopy generates high-speed 3D images of continuous structures without changing focus**

Carol J. Cogswell, Univ. of Colorado at Boulder (United States)

No Abstract Available

9713-23, Session 5

**Lensfree on-chip high-resolution imaging using two-way lighting, and its limitations**

Yasuhiro Adachi, Tokuhiko Tamaki, Motomura Hideto, Yoshihisa Kato, Panasonic Corp. (Japan)

When viewing a biological sample with an optical microscope, you can generally obtain a high-magnification image, depending on the lens, by performing a pixel shift through moving the image sensor before super-resolution by subsequent image processing. However, to obtain a high-resolution image, more images will be required for the super-resolution, and thus it is difficult to achieve real-time operation and the field-of-view (FOV) is not sufficiently wide.

The currently used digital holography has been developed to overcome this issue. In this technology, a sample is placed on the image sensor, which captures the interference fringe (hologram) to reconstruct a 3D high-resolution image in a computer. This technique ensures the feature of a wide FOV, whereas high resolution obtained by image processing, because it requires recursive calculation of light propagation and accurate computer specifications, cannot ensure real-time operation.

As a method to solve such problems, we have developed a new design theory of high-resolution imaging with suppressed computational costs: Lensfree on-chip high-resolution imaging using two-way lighting. In this technique, a high-resolution image is immediately obtained from low-resolution images by image processing after capturing the low-resolution images of the samples on an image sensor. Here, the collimated light is irradiated in two ways: from directly above the image sensor and from a position obliquely above the image sensor. This makes it possible to ensure a wide FOV, a deep depth of focus without the need for focus adjustment, and continuous expansion operation. We also discuss the limitations of such high resolution.

9713-24, Session 5

**Optically sectioned widefield fluorescence lifetime imaging endoscopy enabled by structured illumination**

Taylor Hinsdale, Bilal H. Malik, Jose Jesus Rico-Jimenez, Javier A. Jo, Kristen C. Maitland, Texas A&M Univ. (United States)

We present a wide-field fluorescence lifetime imaging (FLIM) system with optical sectioning by structured illumination microscopy (SIM). FLIM measurements were made using a time gated ICCD camera in conjunction with a pulsed nitrogen dye laser operating at 450 nm. Intensity images were acquired at multiple time delays from a trigger initiated by a laser pulse to create a wide-field FLIM image, which was then combined with three phase SIM to provide optical sectioning. Such a mechanism has the potential to increase the reliability and accuracy of the FLIM measurements by rejecting background intensity. SIM also provides the opportunity to create volumetric FLIM images with the incorporation of scanning mechanisms for the sample plane. We present multiple embodiments of such a system: one as a free space endoscope and the other as a fiber microendoscope enabled by the introduction of a fiber bundle. Finally, we demonstrate the efficacy of such an imaging system by imaging dyes embedded in a tissue phantom.

9713-25, Session 6

**Three-dimensional imaging using phase retrieval with two focus planes**

Tai Ilovitsh, Asaf Ilovitsh, Aryeh M. Weiss, Rinat Meir, Zeev Zalevsky, Bar-Ilan Univ. (Israel)

We present a technique for a full 3D imaging of gold-nanoparticles (GNPs) tagged cells using only two images, rather than many images per volume as is currently needed for 3D optical sectioning microscopy. The proposed approach is based on the Gerchberg-Saxton (GS) phase retrieval algorithm. The reconstructed field is free space propagated to all other focus planes using post processing, and the 2D z-stack is merged to create a 3D image of the sample with high fidelity. Because the phase retrieval is performed on nano particles, the regular ambiguities typical to the Gerchberg-Saxton algorithm, are eliminated.

Since the method requires the capturing of two images only, it can be suitable for 3D live cell imaging. In addition, the method can yield a specific volume mapping within the cell by targeting the GNPs into this specific volume.

The GNPs are excited by a laser illumination at a wavelength corresponding to their spectral absorption peak, and their scattering is being imaged at two different focus planes. The method is generic and applicable to all wavelengths, given GNPs with absorption peak that matches the laser’s wavelength. The proposed concept also has the main advantage of retrieving the phase information while being a non-interferometric configuration.

We believe that the proposed concept can be extremely applicable to the field of 3D biomedical imaging and it can provide valuable insights into cellular processes.

9713-26, Session 6

**Compressive sensing in reflectance confocal microscopy of skin images: a preliminary comparative study**

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Compressive Sensing-based technologies have shown potential to improve the efficiency of acquisition, manipulation, analysis and storage processes on signals and imagery with slight discernible loss in data performance. The CS framework is based on the reconstruction of signals that are presumed sparse in some domain, from a significantly small data collection of linear projections of the signal of interest. As a result, a solution to the underdetermined linear system resulting from this paradigm makes it possible to estimate the original signal with high accuracy. One common approach to solve the linear system is based on methods that minimize the L1-norm. Several fast algorithms have been developed for this purpose. This paper presents a study on the use of compressive sensing in high-resolution reflectance confocal microscopy (RCM) images of the skin. RCM offers a cell resolution level similar to that used in histology to identify cellular patterns for diagnosis of skin diseases. However, imaging of large areas (required for effective clinical evaluation) at such high-resolution can turn image capturing, processing and storage time consuming, presenting a limitation for use in clinical settings.

We present an analysis on the compression ratio that may allow for a simpler capturing approach while reconstructing the required cellular
resolution for clinical use. We provide a comparative study in compressive sensing and estimate its effectiveness in terms of compression ratio vs. image reconstruction accuracy. Preliminary results show that by using up to 25% of the original number of samples, cellular resolution may be reconstructed with high accuracy.

9713-27, Session 6

A superresolution algorithm in fluorescence microscopy with limited artefacts for conical diffraction microscopy

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Conical Diffraction Microscopy is a superresolution technique in fluorescence microscopy based on laser point scanning. The technique requires a high level processing, as the raw measurement consist in a set diffusion limited micro-images which contain superresolution information created by projection on the object, on each scanning point, of a series of patterns. One of the algorithms developed is based on an accelerated steepest descent formulation performing both Poisson denoising and resolution improvement under positivity and local resolution constraints. As commonly encountered in this kind of inverse problems, out-of-model, calibration errors, and bad SNR may contribute potential artefacts to the reconstruction in the form of distortions (out-of-focus, 3D object) and honeycombs (diffuse background/thick objects). To remove or reduce artefacts, we have developed a two steps approach: 1. Through a local analysis, in each micro-image, we evaluate the amount of light fitting the model and adapt the local resolution enhancement accordingly. 2. A noise analysis based on data perturbation enables the evaluation of the uncertainty due to low SNR regions. This local information is integrated in the algorithm in the form of local resolution constraints and provide results without or with limited artefacts in a variety of scenarios usually encountered in cellular imaging.

9713-28, Session 6

Fabrication of two-color annular hybrid wave plate for three-dimensional super-resolution microscopy

Hiroshi Kumagai, Kitasato Univ. (Japan); Yoshinori Iketaki, Olympus Corp. (Japan); Kornel Jahn, Nador Bokor, Budapest Univ. of Technology and Economics (Hungary)

In super-resolution microscopy, we use fluorescence depletion, where an erase beam quenches a molecule in the S1 state generated by a pump beam, and then prevents fluorescence from the S1 state. When a tight doughnut shaped erase beam with is focused on the dyed sample together with a Gaussian pump beam, the remaining fluorescence spot in the focal plane becomes smaller than the diffraction-limited size. Applying destructive interference to the erase beam, erase beam has a minute three-dimensional dark spot surrounded by the light near the focal region. Since this spot introduces fluorescence depletion along the optical axis as in the focal plane, we can achieve three-dimensional super-resolution microscopy. However, to overcome the diffraction limit, an extremely precise optical alignment is required for projecting the focused pump beam into the dark spot of the erase beam. To resolve this technical issue, we fabricated a two-color annular hybrid wave plate (TAHWP) by combining two multi-order wave quartz plates. Although the pump and erase beams co-axially pass through the plate; the pump beam retains its original Gaussian shape, while the erase beam undergoes destructive interference. Inserting the TAHWP into a commercial scanning laser microscope, a three-dimensional spherical fluorescence spot with a volume of (~100 nm)3 can be created. Beside eliminating alignment problems and yielding a compact setup, the TAHWP makes our proposed method very suitable for commercial microscope systems. In this study, we report about detailed fabrication procedure and three-dimensional image properties given by the TAHWP.

9713-29, Session 7

Development of a temporal multiplexed 3D beam-scanning Lissajous trajectory microscope for rapid, multimodal volumetric imaging

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A beam-scanning microscope based on Lissajous trajectory imaging is described for achieving streaming 3D imaging with continuous frame rates in the kHz regime. The Lissajous microscope utilizes two fast-scan resonant mirrors to direct the optical beam on a circuital trajectory through the field of view. 3D sectioning is achieved through temporal multiplexing by the use of optical delay lines in the beam path. Before recombining the delayed beams the convergence/divergence of the delayed line is slightly altered such that it will image a different focal plane in the sample space. To achieve frame-rates greater than video rate, the full Lissajous trajectory time-domain data is re-binned into sub-trajectories (partial, undersampled trajectories) resulting in many unsampled pixels per frame. A model-based image reconstruction (MBIR) 3D in-painting algorithm is then used to interpolate the missing data for the unsampled pixels to recover full images. The MBIR algorithm uses a maximum a posteriori estimation with a generalized Gaussian Markov random field prior model for image interpolation. Preliminary results demonstrate the acquisition of 4 simultaneous focal planes acquired with experimental frame rates of 1 kHz for the concurrent acquisition of laser transmittance and second harmonic generation (SHG). The use of a multi-channel, high-speed (500 MHz) digitizing card allows for acquisition of multiple data channels for multimodal imaging platforms with perfect image overlay. Combined with the MBIR in-painting algorithm, this instrument has the ability to generate video rate images across 6 total dimensions of space, time, and polarization for SHG, TPEF, and confocal reflective birefringence imaging.

9713-30, Session 7

Deformable mirror based remote focusing for fast three-dimensional microscopy

Mantas Zurauskas, Maria Frade Rodriguez, Martin J. Booth, Univ. of Oxford (United Kingdom)

We present a deformable mirror (DM) based remote focusing method for three-dimensional imaging in high-resolution microscopy systems. The method relies on using the DM for refocusing the imaging plane along the optical axis. A transport of intensity equation based phase retrieval method is used for direct wave front sensing during mirror training. The mirror training is performed before the imaging using low cost wave front sensing module, which consists of a camera and light emitting diode light source. During the training, the sets of mirror modes that allow focusing at different Z positions, are defined. The predefined modal arrays are used to drive the mirror during the imaging for continuous refocusing to monitor volumes or for observation of selected planes of interest. The mirror settling time, which is typically lower than a millisecond, limits the maximum refocusing speed. The remote focusing method and is compatible with further correction of sample induced aberrations. Some sample-induced aberrations, such as spherical arising due to refractive index mismatch between the sample and immersion medium can be corrected before the imaging. Further, quality
metric guided optimization is required for correcting the aberrations caused by the sample structure.

We will present examples two microscopy systems. First, we will show of how the technique is applied for fast volumetric imaging in Drosophila fruit fly brain with spatiotemporal focusing based two-photon microscopy. Second example will demonstrate how the remote focusing method can be used for cellular imaging in structured illumination system.

9713-31, Session 7

Design of adaptive objective lens for ultrabroad near infrared imaging
Gongpu Lan, Thomas F. Mauger, Guoqiang Li, The Ohio State Univ. (United States)

Adaptive objective lens with large dynamic optical depth scanning range for ultra broadband near infrared light imaging has wide applications in wide-field microscope, confocal microscope, optical coherence tomography (OCT), two-photon microscopy, etc. In this paper, the theory and design of the adaptive objective lens with an embedded tunable lens (e.g., a liquid lens) is presented. The guideline of the design is to have an extended depth range scanning range larger than the focal length zoom range, since this provides the advantage of keeping the magnification of the whole system, numerical aperture, field of view (FOV), and resolution more consistent. A systematic theory has been developed, for the first time to our acknowledgement, by inserting the varifocal lens in between a front and a back solid lens group. The designed objective has a compact size (10mm-diameter and 15mm-length), ultrabroad working bandwidth (760nm - 920nm), a large depth scanning range (7.36mm in air) — 1.533 times of focal length zoom range (4.8mm in air), and a FOV around 1mm × 1mm. Diffraction-limited performance can be achieved within this ultrabroad bandwidth through all the scanning depth (the resolution is 2.22 ìm - 2.81 ìm, calculated at the wavelength of 800nm with the NA of 0.214 - 0.171). The chromatic focal shift value is within the depth of focus (field). The chromatic difference in distortion is nearly zero and the maximum distortion is less than 0.05%. The methodology can be a good reference for design of other adaptive objective lenses.

9713-32, Session 7

Optical transfer function characterization using a weak diffuser
Gautam Gunjala, Aamod Shanker, Nick Antipa, Laura Waller, Univ. of California, Berkeley (United States)

We present a simple technique which uses a random phase object for single-shot characterization of an optical system’s phase transfer function. This technique is applied to the recovery of aberrations in a commercial microscope using a weak diffuser. Existing methods for aberation measurement typically involve holography, requiring complicated wavefront sensing optics or through-focus measurements with known test objects (e.g. pinholes, fluorescent beads) for pupil recovery from the measured wavefront [1,2]. Here, it is demonstrated that a weak diffuser can be used to recover the pupil of an imaging system in a single measurement, without exact knowledge of the diffuser’s surface. Due to its stochastic nature, the diffuser scatters light to a wide range of spatial frequencies, thus probing the entire pupil plane without the signal loss associated with pinhole based methods. A linear theory developed for weak objects predicts the spectrum of the measured speckle to depend directly on the pupil function. Numerical simulations of diffusers with varying strength confirm the validity of the theory and indicate sufficient conditions under which diffusers act as weak phase objects. Using index matching oils to modulate diffuser strength, experiments are shown to successfully recover aberrations from an optical system using both coherent and partially coherent illumination. [1] Hanser B.M, Gustafsson M.G.L, Agard D.A, Sedat J.W, Phase-retrieved pupil functions in wide-field fluorescence microscopy. J. Microsc. 216, 36–48. 2004 [2] Tyson R.K, Principles of adaptive optics. In Academic Press, 1991, London, UK:Academic Press

9713-33, Session 7

Confocal imaging with orthogonally polarized illumination beams
Bosanta R. Boruah, Ranjan Kalita, Indian Institute of Technology Guwahati (India)

Determining the orientation of fluorescent molecule in the sample being imaged can be very important in the imaging applications of biological samples. One method of determining the orientation of the molecules involves capturing two images of the sample, once using an X polarized beam and then using a Y polarized beam. This can be achieved by rotating a half wave plate in the path of the illumination beam. However any movement of a physical component in the beam path poses the threat of beam misalignment. In this work we develop a ferroelectric liquid crystal spatial light modulator based computer generated holography assembly. Using the assembly via a computer interface the user can programmably and quickly switch between two orthogonal polarizations of the illumination beam. Thus it is possible to conveniently obtain two images in an open frame confocal microscope with two mutually orthogonal polarizations of the illumination beam. The polarization switching is achieved without any physical movement of parts in the setup. Further the frame rate of the confocal microscope is high enough to produce multiple pairs of images in one second. In this paper we will provide a detail description of the confocal setup and present preliminary experimental results.

9713-66, Session 7

A linear algorithm for quantitative measure of corneal collagen fiber orientation using second harmonic generation microscopy
James McLean, Charles A. DiMarzio, Northeastern Univ. (United States)

No Abstract Available

9713-34, Session 8

Three-dimensional fluorescence imaging by stage-scanning oblique plane microscopy
Vincent Maioli, Frederik Görlitz, Sean Warren, Sunil Kumar, Paul M. W. French, George Chennell, Alessandro Sardini, David Carling, Imperial College London (United Kingdom); Frederike Alwes, Ecole Normale Supérieure de Lyon (France); Christopher W. Dunsby, Imperial College London (United Kingdom)

Oblique plane microscopy (OPM) is a light-sheet fluorescence microscopy technique that is implemented on a standard inverted microscope frame. OPM uses a single high numerical aperture microscope objective to both produce a tilted excitation light-sheet and to image the fluorescence emitted from the tilted plane back to the cameras. It is therefore compatible with conventional sample-mounting techniques such as microscope slides and multiwell plates. Four excitation laser lines and two high-speed sCMOS cameras with separate emission filters enable the simultaneous imaging of several fluorophores and spectral ratiometric FRET acquisitions. Previously, 3-D OPM imaging has been implemented by remote refocusing.
Here, a stage-scanning approach to 3-D OPM imaging is demonstrated - enabling three-dimensional multi-channel acquisition including of multwell plates - and the synchronization of the stage movement and camera acquisition will be described.

The ability of the stage-scanning system to image fields of view larger than the field of view of the primary microscope objective is demonstrated using fluorescently labelled limbs of crustaceans and its ability to perform time-lapse 3-D imaging over 12 hours is demonstrated using a sample of tumor spheroids with an acquisition time of 3 s for a typical spheroid providing 400x1280x1024 voxels per spheroid.

We also apply the system to spectral ratiometric Förster resonant energy transfer (FRET) measurements in tumor spheroids expressing a FRET biosensor and in a 96-well plate seeded with cell samples expressing varying concentrations of a FRETing and non-FRETing constructs.

9713-35, Session 8

Increasing the imaging depth through computational scattering correction

Benno Koberstein-Schwarz, Helmholtz Zentrum München GmbH (Germany) and Carl Zeiss AG (Germany); Lars Omlor, Tobias Schmitt-Manderbach, Timo Mappes, Carl Zeiss AG (Germany); Vasilis Ntziarchristos, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany)

Imaging depth is one of the most prominent limitations in light microscopy. The depth in which we are still able to resolve biological structures is limited by the scattering of light within the sample. We have developed an algorithm to compensate for the influence of scattering. The potential of algorithm is demonstrated on a 3D image stack of a zebrafish embryo captured with a selective plane illumination microscope (SPIM). With our algorithm we were able shift the point in depth, where scattering starts to blur the imaging and effect the image quality by around 30 µm. For the reconstruction the algorithm only uses information from within the image stack. Therefore the algorithm can be applied on the image data from every SPIM system without further hardware adaption. Also there is no need for multiple scans from different views to perform the reconstruction. The underlying model estimates the recorded image as a convolution between the distribution of fluorophores and a point spread function, which describes the blur due to scattering. Our algorithm performs a space-variant deconvolution on the image. To account for the increasing amount of scattering in deeper tissue, we introduce a new regularizer which models the increasing width of the point spread function in order to improve the image quality in the depth of the sample. Since the assumptions the algorithm is based on are not limited to SPIM images the algorithm should also be able to work on other imaging techniques which provide a 3D image volume.

9713-36, Session 8

Volumetric high resolution imaging of live cancer cell spheroids using light sheet fluorescence microscopy

Stylianos Psycharakis, Evangelos Liapis, Ilia Kyparissidis-Kokkinidis, Athanasios Zacharopoulos, Joseph Papamatheakis, Foundation for Research and Technology-Hellas (Greece); Jorge Ripoll, Univ. Carlos III de Madrid (Spain); Giannis Zacharakis, Foundation for Research and Technology-Hellas (Greece)

Tumor spheroids are in vitro cancer models of increasing interest for cancer evolution diagnostics and pharmaceutical intervention studies, as they more closely resemble real tumours compared to conventional monolayer cultures. However, optical imaging of tumour spheroids is technically challenging, since these are large and highly scattering specimens. Light Sheet Fluorescence Microscopy (LSFM) has the potential to overcome several of the challenges that prevent high resolution imaging of live tumor spheroids since it combines optical sectioning, with multi-angle and multispectral imaging.

In this study, we employed LSFM to investigate the response of live MDA-MB-231 breast cancer spheroids, stably expressing GFP, to the chemotherapeutic agent doxorubicin. Drag penetration into the spheroids was determined by direct imaging of doxorubicin’s inherent fluorescence. Moreover cell viability and necrosis were assessed by counterstaining the spheroids with the far-red nuclear dye Draq7.

The spheroids were imaged in our custom LSFM setup using two excitation wavelengths (488nm and 635nm) while detection was performed with appropriate bandpass emission filters. Each spheroid was imaged sequentially at 4 different projections (0o, 90o, 180o, and 270o). For each projection stacks of 45-65 optical sections were created for each fluorescent wavelength and maximum intensity projections/3D projections were produced using ImageJ.

The fluorescence distribution of Draq7 suggests that Doxorubicin was predominantly cytotoxic in the periphery of the spheroid, as the majority of dead cells were found to be there, while strong Draq7 fluorescence was detected in the center of larger spheroids, consistent with the presence of a necrotic core.

9713-37, Session 8

Light sheet polarimetric imaging

Thomas G. Brown, Univ. of Rochester (United States)

No Abstract Available

9713-63, Session 8

Volumetric retinal fluorescence imaging with extended depth of field microscope

Zengzhuo Li, The Ohio State Univ. (United States); Wei Li, National Eye Institute (United States); Guoqiang Li, The Ohio State Univ. (United States)

Wave-front coding has been proved to greatly extend the depth of field(DoF) for near-diffraction limited optical imaging systems. A bright field infinity-corrected transmitted light microscope is built and it adopts the wave-front coding concept by inserting a phase element designed with robust general polynomial function. The resulting extended depth of field(EDoF) system achieves an engineered point spread function that is much less sensitive to object depth variation than conventional systems and therefore 3D volumetric information can be obtained in a single frame with expanded tolerance of defocus. In Zemax simulation for a setup using 32X objective, NA=0,6, the EDoF microscope has DoF of 10µm whereas a conventional one has 0.75µm, indicating a 13 times increase. In experiment of a 60µm thick pig’s retina specimen, the DoF is extended over 50 times with 20X objective, NA=0.4. Retinal fluorescence images of the EDoF microscope using passive adaptive optical phase element illustrate a DoF of over 100µm and it is able to recover the volumetric fluorescence images that are almost identical to in-focus images after post processing. The image obtained from the EDoF microscope is also better in resolution and contrast, and the retinal structure is better defined. Hence, due to its high tolerance of defocus and fine restored image quality, EDoF optical systems have promising potential in consumer portable medical imaging devices where user’s ability to achieve focus is not optimal, and other medical imaging equipment so that achieving best focus is not a necessary.
Joint imaging and trapping by 2-color synthetic holography
Monika Ritsch-Marte, Medizinische Univ. Innsbruck (Austria)

We demonstrate an off-axis image plane digital holographic (DH) microscope capable of achieving full detector resolution and highly accurate phase measurement capability in a single shot operating mode. Single shot off-axis digital holograms are commonly processed using the Fourier transform method. The requirement of a finite filter size for selection of the off-axis term in Fourier space inherently limits the achievable resolution to much below the detector resolution in this approach. Typically overcoming this resolution limit requires a multi-frame phase shifting holography system that does not allow truly live imaging. Further since the photon resources have to be divided into multiple frames, there is a shot-noise penalty associated with the phase shifting approach. We present a novel non-linear optimization formalism for image recovery in DH that is capable of simultaneous accurate and full resolution phase imaging from single digital hologram frame. The effectiveness of this approach is described by means of: (1) experiments demonstrating sub-shot-noise phase measurement from a single low photon level hologram using classical light, and (2) simulations and experimental results on imaging of transparent phase objects such as Biological cells showing superior single shot resolution capability. Initial results on diagnostic studies using this microscope system are described. Our results suggest that for given photon resources and hardware costs, we achieve the best phase imaging resolution and noise performance.


High resolution image plane digital holographic microscopy
Mandeep Singh, Kedar B. Khare, Indian Institute of Technology Delhi (India)

We demonstrate an off-axis image plane digital holographic (DH) microscope capable of achieving full detector resolution and highly accurate phase measurement capability in a single shot operating mode. Single shot off-axis digital holograms are commonly processed using the Fourier transform method. The requirement of a finite filter size for selection of the off-axis term in Fourier space inherently limits the achievable resolution to much below the detector resolution in this approach. Typically overcoming this resolution limit requires a multi-frame phase shifting holography system that does not allow truly live imaging. Further since the photon resources have to be divided into multiple frames, there is a shot-noise penalty associated with the phase shifting approach. We present a novel non-linear optimization formalism for image recovery in DH that is capable of simultaneous accurate and full resolution phase imaging from single digital hologram frame. The effectiveness of this approach is described by means of: (1) experiments demonstrating sub-shot-noise phase measurement from a single low photon level hologram using classical light, and (2) simulations and experimental results on imaging of transparent phase objects such as Biological cells showing superior single shot resolution capability. Initial results on diagnostic studies using this microscope system are described. Our results suggest that for given photon resources and hardware costs, we achieve the best phase imaging resolution and noise performance.


Optimized numerical dynamic DIC by digital holography
Vittorio Bianco, Melania Paturzo, Valentina Marchesano, Pietro Ferraro, Istituto di Scienze applicata e Sistemi Intelligenti (Italy) and Consiglio Nazionale delle Ricerche (Italy)

Since its invention, Differential Image Contrast (DIC) imaging has been widely used for its feature to convert specimen phase gradients into intensity differences that can be appreciated by cameras or human eyes. Indeed, low amplitude contrast biological samples can be non-invasively investigated with enhanced quality, as the DIC imaging emphasizes the object boundaries, optically performing what is, from the mathematical point of view, a numerical derivative along a fixed direction. The best DIC direction depends on the object shape and orientation. However, the direction along which optical DIC is performed has to be chosen once, so that in the case of live moving samples optical DIC cannot be optimized. Here we show an optimized numerical DIC based on Digital Holography (DH) microscopy allowing to follow the 3D sample motion and to emphasize dynamically the contrast of each object portion. The flexible focusing capability typical of DH, along with autofocus criteria, allow to put in focus multiple objects displaced in various positions along the optical axis. Blind measures based on contrast metrics are performed on each image portion in order to build-up maps showing the best directions along which to perform numerical DIC. Differently from optical DIC, this is achieved with no need for special optics or complex setups. Thus, dynamic representations of floating samples are offered where the contrast of each portion of the objects is emphasized with an automatic blind algorithm able to follow their evolutions in time, providing an improved visualization movie of transparent specimens.

Dynamic photothermal interferometric phase microscopy
Nir A. Turko, Omry Blum, Natan T. Shaked, Tel Aviv Univ. (Israel)

We present our latest advances in highly dynamic photothermal interferometric phase microscopy for quantitative, selective contrast imaging. Specific cells are labeled with gold nanoparticles. When stimulating gold nanoparticles at their plasmon-peak wavelength, local increase of temperature occurs due to plasmon resonance. This causes rapid change of optical phase of the light beam interacting with the sample. These phase changes can be recorded by interferometric phase microscopy and analyzed to form a photothermal image of the binding sites of the nanoparticles in the cells. Furthermore, by increasing the excitation laser light, one can deplete certain cells at will. Usually, the analysis utilizes a Fourier transform, which is computational time consuming. This makes PT imaging not suitable for applications requiring dynamic imaging or quantitative analysis, such as for analyzing and sorting cells during their fast flow. To this goal, we have developed new algorithms, based on discrete Fourier transform variants, enabling fast analysis of photothermal signals from nanoparticles in live and highly dynamic cells. For the first time, video-rate photothermal signals are obtained, which forms the basis for real-time interferometric phase microscopy with molecular specificity. This technique holds great potential for using photothermal imaging in flow cytometry.

Swept-source holographic phase microscopy
Yizheng Zhu, Shichao Chen, Virginia Polytechnic Institute and State Univ. (United States)

Holographic phase microscopy has seen rapid growth in the past two decades. Numerous schemes have been proposed and commercial products are now available. Since most systems are laser based, speckle noise and other non-signal interference in the system have been problematic, limiting the technique’s phase sensitivity, image quality and the ability for accurate quantitative analysis. Low coherence source-based HPM have also been proposed to mitigate this issue, but often with increased system complexity and reduced implementation flexibility. Here, we demonstrate a swept-source HPM technique, which acquires on-axis holograms while continuously scanning the laser through a range of wavelengths. This technique is capable of identifying interference from various sources and effectively isolating sample interference, therefore minimizing unwanted signals and achieving high spatial and temporal sensitivity across the entire field of view. The ability of acquiring spectral interferograms for each pixel also make it possible to implement spectral shaping, which can further suppress interference side-lobes and improve sensitivity. Additionally, when coupled with a spectral modulation technique, such interference spectrum will permit spectroscopic measurement of phase-related properties of the sample. We will introduce the principle of the system, discuss its theoretical sensitivity bound, and present its application to phase imaging of live cells.
9713-43, Session 10

Integrated quantitative phase and polarization imaging using spectral multiplexing interferometry
Chengshuai Li, Yizheng Zhu, Virginia Polytechnic Institute and State Univ. (United States)

For imaging transparent, unstained biological specimens, optical phase is the primary intrinsic mechanism to enhance image contrast, as seen in techniques such as quantitative phase microscopy and differential interference contrast microscopy. Recently, polarized light microscopy has also received growing attention for its ability to reveal birefringence contrast of fine biological structures such as cell membrane/wall and mitotic/meiotic spindles. However, quantitative phase and birefringence imaging techniques utilize drastically different principles and optical setups, making it difficult to perform integrated multi-modality imaging.

In this presentation, we introduce a spectral multiplexing interferometry (SXI) method for integrated polarization distribution and topographic phase measurement with simple configuration and high sensitivity. With SXI, the retardation and orientation of sample birefringence are simultaneously encoded onto two spectral carrier waves, generated interferometrically by a birefringent crystal through polarization mixing. A single interference spectrum hence contains sufficient information for birefringence determination, eliminating the need for mechanical rotation or electrical modulation. In addition, with the simple insertion of a Nomarski prism, the same setup can acquire quantitative differential interference contrast images and therefore quantitative phase topography. The SXI approach can achieve both quantitative phase imaging and the corresponding birefringence imaging with high precision and sensitivity. The principle of the technique and its experimental validation on biological cells will be presented and discussed.

9713-44, Session 10

Multiplexed fluorescence and phase microscopy for simultaneous, single-camera, one-shot, multimodal imaging
Shwetadwip Chowdhury, Joseph A. Izatt, Duke Univ. (United States)

In the biological sciences, there is much emphasis on elucidating the functions of various biological components and processes. To do so, advances in general microscopy have yielded various imaging modalities to probe such processes under specific visualization and contrast requirements. Examples of modalities that are popularly integrated into conventional biological studies include fluorescent, dark-field, phase-contrast, and polarization-sensitive microscopies, with each modality offering unique insights into the biological function of the sample. Often times, however, a comprehensive understanding of biological phenomena requires the integration of the unique and separate visualizations of various modalities. Unfortunately, conventional microscopes typically support only one modality and rarely allow multiple modalities to be used in conjunction. Though high-end microscopes may support multimodal visualization, they often require either mechanical (and often manual) toggling, which obstruct real-time multimodal imaging, or simultaneous detection via multiple cameras, which dramatically increases the microscope’s cost. Here, we present a one-shot technique that allows multiple imaging channels, of potentially different modalities, to be simultaneously detected by a single camera. We experimentally demonstrate this method on transparent cells that have been tagged for F-actin and nuclear fluorescence. Our multimodal system consists of 2-channel fluorescence and 1-channel quantitative-phase (QP) imaging, and clearly demonstrates ability for simultaneous fluorescent and GP visualization. Though we experimentally verify our framework using dual fluorescent/QP imaging, we emphasize that our framework for single-shot, simultaneous single-camera detection is applicable to an arbitrary number of widefield imaging modalities so long as they fulfill criteria for Fourier spectra separation, SNR, and detector dynamic range.

9713-45, Session 10

A novel phase shifting structured illumination microscopy
Veena Singh, Vishesh Dubey, Azeem Ahmad, Gyanendra Singh, Dalip Singh Mehta, Indian Institute of Technology Delhi (India)

This paper describes a new and novel phase shifting technique for qualitative as well as quantitative measurement in microscopy. We have developed a phase shifting device which is robust, inexpensive and involves no mechanical movement. In this method, phase shifting is implemented using LED array, beam splitters and defocused projection of Ronchi grating. The light from the LEDs are made to fall on the beam splitters at spatially different locations. Due to variation in the geometrical distances of LEDs from the Ronchi grating and by sequentially illuminating the grating by switching on one LED at a time the m/2 phase shifted grating patterns are generated. The phase shifted structured patterns are projected onto the sample using microscopic objective lens. The phase shifted deformed patterns are recorded by a CCD camera. The initial alignment of the setup involves a simple procedure for the calibration and optimization of voltage for equal fringe width and intensity such that the phase shifted fringes are at equal phase difference. Three frame phase shifting algorithm is employed for the reconstruction of the phase map. The method described here is fully automated so that the phase shifted images are recorded just by switching of LEDs and has been used for the shape measurement of microscopic industrial and biological objects. The analysis of the phase shifted images provides qualitative as well as quantitative information about the sample. Thus, the method is simple, robust and low cost compared to PST devices commonly employed for phase shifting.

9713-46, Session 10

Label-free three dimensional reconstruction of biological samples
Sherazade Aknoun, PHASICS S.A. (France); Pierre Bon, Institut d’Optique Graduate School (France); Julien Savatier, Serge Monneret, Institut Fresnel (France) and Aix-Marseille Univ. (France); Benoit F. Wattellier, PHASICS S.A. (France)

We describe the use of spatially incoherent illumination combined with quantitative phase imaging (QPI) [1] to make tridimensional reconstruction of semi-transparent biological samples. Quantitative phase imaging is commonly used with coherent illumination for the relatively simple interpretation of the phase measurement. We propose to use spatially incoherent illumination which is known to increase lateral and axial resolution compared to classical coherent illumination. The goal is to image thick samples with intracellular resolution [2]. The 3D volume is imaged by axially scanning the sample with a quadri-wave lateral shearing interferometer used as a conventional camera while using spatially incoherent white-light illumination (native microscope halogen source) or NIR light. We use a non-modified inverted microscope equipped with a 2-axis piezo stage. A z-stack is recorded by objective translation along the optical axis.

The main advantages of this approach are its easy implementation, compared to the other state-of-the-art diffraction tomographic setups, and its speed which makes even label-free 3D living sample imaging possible. A deconvolution algorithm is used to compensate for the loss in contrast due to spatially incoherent illumination. This makes the tomographic volume phase values quantitative. Hence refractive index could be recovered from the optical slices.

We will present tomographic reconstruction of cells, thick fixed tissue of few tens of micrometers using white light, and the use of NIR light to reach deeper planes in the tissue.
Investigating the usage of point spread functions in point source and microsphere localization

Jerry Chao, Texas A&M Univ. (United States); Sripad Ram, Univ. of Texas at Dallas (United States); Elizabeth S. Ward, Texas A&M Health Science Ctr. (United States); Raimund J. Ober, Texas A&M Univ. (United States)

Using a point spread function (PSF) to localize a point-like object, such as a fluorescent molecule or microsphere, represents a common task in single molecule microscopy image data analysis. The localization may differ in purpose depending on the application or experiment, but a unifying theme is the importance of being able to closely recover the true location of the point-like object with high accuracy. We present two simulation studies, both relating to the performance of object localization via the maximum likelihood fitting of a PSF to the object’s image. In the first study, we investigate the integration of the PSF over an image pixel, which represents a critical part of the localization algorithm. Specifically, we explore how the fineness of the integration affects the performance of the object localization. To achieve high accuracy, we use a modified line spread function and find the use of too coarse a step size to produce location estimates that are far from the true location, especially when the images are acquired at relatively low magnifications. We also propose a method for selecting an appropriate step size. In the second study, we investigate the suitability of the common practice of using a PSF to localize a microsphere, despite the mismatch between the microsphere’s image and the fitted PSF. Using criteria based on the standard errors of the mean and variance, we find the method suitable for microspheres up to 1 μm and 100 nm in diameter, when the localization is performed, respectively, with and without the simultaneous estimation of the width of the PSF.

High-speed volumetric STED-like microscopy with focus extension

Kai-Ping Yang, Wei-Kuan Lin, Kuo-Jen Hsu, Shi-Wei Chu, National Taiwan Univ. (Taiwan)

Similar to stimulated emission depletion (STED) microscopy, suppression of scattering imaging (SUSI) microscopy is invented to achieve sub-diffraction-limit resolution by using a doughnut-shaped beam to suppress the scattering around a focal spot while leaving a central part active to emit scattering. Therefore, it sharpens the point spread function and overcomes the resolution limit. However, SUSI microscopy is based on confocal principle, so it can provide only a single-layer image at one frame of lateral scan. For high-speed observation of molecular dynamics over a three-dimensional tissue, SUSI is limited with slow axial translation of the objective or sample. Therefore, there are several possibilities to achieve high-speed three-dimensional volume imaging, including moving the objective with a piezoelectric material, acousto-optic deflectors (AODs) and axial extension of focus. However, the former two methods are based on partially sampling throughput a volume to increase speed, and thus also demand outstanding spatial stability to prevent motion artifact. Therefore, we chose to axially extend the focus with the aid of a curvature-variable lens. It not only allows high-speed volumetric imaging, but also relieves requirement of sample stability.

In this study, we combine SUSI microscopy with focus extension to achieve sub-diffraction-limit imaging for a complete three-dimensional volume within one second. Compared to conventional scanning based on stage translation, our technique provides two orders of magnitude enhancement in speed. It paves the way toward high-speed super-resolution imaging, and will be promising for volumetric imaging of molecular dynamics in biological tissues in vivo.

Spectral reconstruction strategies toward generalized-domain optical coherence tomography with a broadband source and a bucket detector

Pui-Chuen Hui, Néstor Uribe-Patarroyo, Martin Villiger, Brett E. Bouma, Harvard Medical School (United States)

One appealing aspect that compressive sensing offers is the possibility of retrieving a signal’s spectral information using a bucket detector and a characterized measurement matrix. Demonstrations of CS applied to optical coherence tomography (OCT) were performed, however, in the final signal-processing instead of the acquisition end. Here we propose a novel OCT system with a broadband superluminescent excitation and a bucket photodetector where the interferogram is obtained by spectral reconstruction. In particular, this system assumes the same interferometric setup as typical swept-source OCT systems except the excitation is replaced by a broadband source. The interferogram then passes through an off-the-shelf, fast tunable Fabry-Perot filter (FPF) of modest finesse whose free spectral range is designed to be much less than the excitation bandwidth. The spectral response is characterized a priori, before the filtered output is integrated by the photodetector. The spectral sampling measurement is repeated by altering the FPF’s resonant conditions multiple times through the cavity length. Having acquired the integrated photodetector values and the corresponding spectral filter functions, we reconstruct the original interferogram whose Fourier transform generates the tomogram. The sensitivity of this OCT technique is evaluated and compared using simulations with synthetic data. Moreover, B-scan reconstruction of the interferogram due to a fingertip was simulated using our scheme and the resultant image shows excellent reconstruction fidelity compared to the original OCT B-scan. These illustrations point towards a promising future of a new class of tomographic system which combines the respective strengths of swept-source and spectral-domain OCT.

Data driven 3D high resolution structure illuminated fluorescent microscopy based on Bayesian estimation

Hsi-Hsun Chen, Yuan Luo, National Taiwan Univ. (Taiwan); Vijay R. Singh, SMART-Singapore MIT Alliance for Research & Technology (Singapore)

Light induced fluorescent microscopy has long been developed to observe and understand the object at microscale, such as cellular sample. However, the transfer function of lense-based imaging system limits the resolution so that the fine and detailed structure of sample cannot be identified clearly. The techniques of resolution enhancement are fascinated to break the limit of resolution for objective given. In the past decades, the resolution enhancement imaging has been investigated through variety of strategies, including photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), stimulated emission depletion (STED), and structure illuminated microscopy (SIM). In those methods, only SIM can intrinsically improve the resolution limit for a system without taking the structure properties of object into account. In this paper, we develop a SIM associated with Bayesian estimation, furthermore, with optical sectioning capability rendered from HiLo processing, resulting the high resolution through 3D volume. This 3D SIM can provide the optical sectioning and resolution enhancement performance, and be robust to noise owing to the Data driven Bayesian estimation reconstruction proposed. For validating the 3D SIM, we show our simulation result of algorithm, and the experimental result demonstrating the 3D resolution enhancement.
9713-51, Session 11

**Nonlinear complex diffusion approaches based on a novel noise estimation for noise reduction in phase-resolved optical coherence tomography**

Shaoyan Xia, Yong Huang, Xiaodi Tan, Beijing Institute of Technology (China)

Partial differential equation (PDE)-based nonlinear diffusion processes have been widely used for image denoising. In the traditional nonlinear anisotropic diffusion denoising techniques, behavior of the diffusion depends highly on the gradient of image. However, it is difficult to get a good effect if we use these methods to reduce noise in optical coherence tomography images. Because background has the gradient that is very similar to regions of interest, so background noise will be mistaken for edge information and cannot be reduced. Therefore, nonlinear complex diffusion approaches using texture feature (NCDTF) for noise reduction in phase-resolved optical coherence tomography is proposed here, which uses texture feature in OCT images and structural OCT images to remove noise in phase-resolved OCT. Taking into account the fact that texture between background and signal region is different, which can be linked with diffusion coefficient of nonlinear complex diffusion model, we use NCDTF method to reduce noises of structure and phase images first. Then, we utilize OCT structure images to filter phase image in OCT. Finally, to validate our method, parameters such as image SNR, contrast-to-noise ratio (CNR), equivalent number of looks (ENL), and edge preservation were compared between our approach and median filter, Gaussian filter, wavelet filter, nonlinear complex diffusion filter (NCDF). Preliminary results demonstrate that NCDTF method is more effective than others in keeping edges and denoising for phase-resolved OCT.

9713-52, Session 12

**Modified K-factor image decomposition for three-dimensional super resolution microscopy**

Tali Ilovitsh, Aryeh M. Weiss, Bar-Ilan Univ. (Israel); Amihai Meiri, Carl G. Ebeling, The Univ. of Utah (United States); Aliza Amiel, Hila Katz, Batya Mannasse Green, Bar-Ilan Univ. (Israel) and Meir Medical Ctr. (Israel); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

This work presents a novel use of the nonlinear image decomposition technique called K-factor that reshapes the three-dimensional (3D) point spread function (PSF) of an XYZ image stack into a narrow Gaussian profile. The experimentally obtained PSF of a 2-stack raw data that is acquired by a widefield microscope has a much larger shape radius that is given by the Gibson and Lanni model. This shape increases the computational complexity associated with the localization routine, when used in localization microscopy techniques. Furthermore, due to its nature, this PSF spreads over a larger volume, making the problem of overlapping emitters detection more pronounced.

The ability to use Gaussian fitting with high accuracy on 3D data can facilitate the computational complexity, hence reduce the processing time required for the generation of the 3D superresolved image. In addition it allows the detection of overlapping PSFs and reduces the effects of the penetration of out of focus PSFs into in focused PSFs, therefore enables the increase in the activated fluorophore density by ~50%. The algorithm was tested both on simulated data and experimentally, where it yielded an increase in the localization accuracy by ~60% with compare to regular Gaussian fitting, and improved the minimal resolvable distance between overlapping PSFs by ~50%.

Since the proposed concept is generic and can be applied to any 3D microscope configuration, we believe that it can be extremely applicable to the field of 3D biomedical imaging, where the ability to track single fluorescent particles within a 3D cellular environment can provide valuable insights into cellular processes.

9713-53, Session 12

**Scale-up through sparse representation in chemical imaging of infected RBC cell components**

Nicolas Spegazzini, Rishikesh Pandey, Jeon Woong Kang, Massachusetts Institute of Technology (United States); Ishan Barman, Johns Hopkins Univ. (United States); Ramachandra R. Dasari, Peter T. C. So, Massachusetts Institute of Technology (United States)

The malaria disease in early detection during asexual blood stage-erythrocytic phase has been a long-standing obstacle. In this report scale-up resolution hyperspectral confocal Raman imaging coupled with independent target restoration microscopy can provide exhaustive multiplex information in spatial, multichannel spectral resolution and decompose the chemical fingerprinting information for each species considered in the study. This label-free molecular fingerprinting technique is used to visualize hemoglobin distribution in disambiguation way in a single infected red blood living cell with high resolution. We report the quantitative determination of chemical species and theirs for multicomponent system. Follow this way we observe major components in the red blood cell: hemoglobin, parasite’s food vacuole, hemozoin, and structural information from cytoskeleton protein component. This is a non-ambiguous information due to the spatial chemical imaging is resolved for each chemical component by this technique and corresponded unequivocally to the spectral multiplex molecular fingerprint. The quantitative multiplex scale-up resolution hyperspectral Raman imaging described here opens doors to early detection and monitoring cell-drug interactions and cell development with minimal perturbation of the biological system.

9713-54, Session 12

**A computational hyperspectral imaging technique**

Nasim Habibi, Mohammad Azari, The Univ. of North Carolina at Charlotte (United States); Mehrdad Abolbashari, Oponentics, Inc. (United States); Faramarz Farahi, The Univ. of North Carolina at Charlotte (United States)

A new computational hyperspectral imaging technique is introduced. In contrast to conventional spectral imaging methods, spectral and spatial resolutions are independent in the proposed technique and both can be independently improved. A system based on this technique computes the spectral information of whole field of view all at once and doesn’t require scanning across the surface of the object.

The system requires an imaging lens system, which is intentionally designed to have a large chromatic aberration, i.e. to be highly dispersive. The final spectral resolution of the system is determined by the amount of produced chromatic aberration. The spatial resolution, on the other hand, is determined by image quality of the lens system. Therefore, both spectral and spatial resolutions can be improved independently. Due to chromatic dispersion, a flat multi-color object in front of such imaging system wouldn’t have a unique image plane. Each point on the object would form a sharp image at a location determined by its wavelength content. If we can locate the sharp image(s) formed by every point on the object, then we have extracted the spectral information of the object. Since finding the location of sharp image is also the objective of 3D imaging problem, algorithms used for calculating the depth map of a 3D object can be used for our spectral imaging technique. Two of these algorithms are applied to images obtained...
from the designed lens system and the spectral information is computed. The results will be presented in this paper.

9713-55, Session 12

**A quantitative framework for the analysis of multimodal optical microscopy images**

Andrew J. Bower, Benjamin Chidester, Youbo Zhao, Marina Marjanovic, Eric J. Chaney, Minh N. Do, Stephen A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Integrated multimodal optical imaging platforms based on unique, yet complementary, contrast mechanisms have been developed and used successfully in a wide variety of biomedical applications, including many in vivo animal and human studies. While this multimodal approach has recently found more widespread use, thus far most applications of these techniques have been purely qualitative, ignoring the dense, multidimensional nature of the simultaneous, co-registered datasets acquired. Here, the basic framework and several applications of a quantitative model-based analysis method are presented. The analysis techniques developed attempt to provide a direct link between multimodal image contrast and physiological biomarkers that could be used to identify disease states and tissue components or detect important biological events. Specific applications of the developed analysis techniques include identification of tissue constituents in fixed tissue slices and classification of cell death mechanisms in a living engineered tissue sample. This work represents an important step towards the quantitative interpretation of this complex data critical to assessing the diagnostic power of integrated multimodal optical microscopy systems.
9714-1, Session 1

**Heterogeneity and restricted state selection in FRET with fluorescent proteins**

Angus J. Bain, Thomas S. Blacker, Michael R. Duchen, Univ. College London (United Kingdom)

Most fluorescent proteins exhibit multi-exponential fluorescence decays indicating the presence of a heterogeneous excited state population. In the analysis of FRET to and between fluorescent proteins it is convenient to assume that a single interaction pathway is involved. In recent work we have shown that this assumption does not hold and moreover that certain pathways can be highly constrained, leading to the potential misinterpretation of experimental data concerning protein-protein interactions. FRET and single photon absorption both obey the same global electric dipole selection rules but differ greatly in the mechanism of the acceptor photoselection. In an isotropic medium, single-photon excitation accesses all transition dipole moment orientations with an equal probability. However the FRET rate depends on the relative orientation of the donor and acceptor through the kappa squared orientation parameter. We show how time- and spectrally resolved fluorescence intensity and anisotropy decays of direct acceptor excitation combined with those for the interacting FRET pair can be used to identify restricted FRET state selection and to provide accurate measurements of protein-protein interaction dynamics.

9714-2, Session 1

**Optimizing enhanced green fluorescent proteins fused to membrane transporters for single-molecule FRET using a fast anti-Brownian electrokinetic trap**

Maria Dienerowitz, Mykhailo Ilchenko, Bertram Su, Friedrich-Schiller-Univ. Jena (Germany); Günter Mayer, Thomas Henkel, Leibniz-Institut für Photonische Technologien e.V. (Germany); Monika Düser, Nawid Zarrabi, ATINA Ingenieurbüro (Germany); Michael Börsch, Friedrich-Schiller-Univ. Jena (Germany)

Observation times of freely diffusing single molecules in solution are limited by the photophysics of the attached fluorescent markers and by a small observation volume in the femtoliter range that is required for a sufficient signal-to-background ratio. To extend diffusion-limited observation times through a confocal detection volume, A. E. Cohen and W. E. Moerner have invented and built microfluidic devices to actively counteract Brownian motion of single nanoparticles in electrokinetic traps (ABELtrap). Here we present a version of an ABELtrap with a laser focus pattern generated by electro-optical beam deflectors and controlled by a programmable FPGA chip. This ABELtrap could hold single fluorescent nanobeads for more than 100 seconds, increasing the observation times of a single nanoparticle by a factor of 10000. Different microfluidic designs were evaluated to enhance ABELtrapping performance. Because we are mainly interested in monitoring conformational changes of individual membrane transporters in real time, we record sequential distance changes between two specifically attached dyes using Förster resonance energy transfer (smFRET). Photophysics of different fluorophores used as FRET donors on FoF1-ATP synthase were measured in our ABELtrap and compared in order to determine the optimal dye for smFRET.

9714-3, Session 1

**Viscoelastic properties of the bacterial chromosome measured by fluorescence correlation spectroscopy**

Rudra P. Kafle, Molly R. Liebeskind, Jens-Christian D. Meiners, Univ. of Michigan (United States)

Mechanical quantities like the elasticity of cells are conventionally measured by directly probing them mechanically. This, however, is often impractical, and even impossible when subcellular structures inside living cells are concerned. We use a purely optical method instead: fluorescence correlation spectroscopy (FCS) is adapted to measure such mechanical quantities in chromosomal DNA in live E. Coli cells.

Intracellular FCS is an appealing technique because it is non-invasive, and it is now drawing increasing interest for the study of more complex systems like the dynamics of DNA or proteins in living cells and cell membranes. Unlike an ordinary dye solution, the dynamics of macromolecules like proteins or entangled DNA in crowded environments is often slow and subdiffusive in nature. This in turn can lead to artifacts from photobleaching and dye dissociation from the substrate molecule that can easily obscure the signature of the molecular dynamics of interest. We present methods to address these added complexities of live-cell FCS, and calculate viscoelastic moduli from the FCS data.

We compare the measured viscoelastic moduli of live cells with those that are ATP-depleted to stop all molecular motor action, and find substantial differences: As soon as active processes are stopped, the bacterial DNA appears to become stiffer and the surrounding intracellular medium more viscous. We discuss these measurements in the context of observations on other active gels, most importantly actin-myosin systems and the eukaryotic cytoskeleton.

9714-4, Session 2

**Study of the conformational dynamics of intrinsically disordered protein by PET-FCS**

Joerg Enderlein, Man Zhou, Qui Van, Ingo Gregor, Georg-August-Univ. Göttingen (Germany)

Intrinsically disordered proteins (IDP) form a large and functionally important class of proteins that lack an ordered three-dimensional structure. IDPs play an important role in cell signaling, transcription, or chromatin remodeling. The discovery of IDPs has challenged the traditional paradigm of protein structure which states that protein function depends on a well-defined three-dimensional structure.

Due to their high conformational flexibility and the lack of ordered secondary structure, it is challenging to study the flexible structure, dynamics and energetics of these proteins with conventional methods. In our work, we employ photoinduced electron transfer (PET) combined with fluorescence correlation spectroscopy (FCS) for studying the conformational dynamics of one specific class of IDPs: phenylalanine-glycine rich protein domains (FG repeats) which are dominant building blocks within the pore of nuclear pore complexes. Nuclear pore complexes are large protein assemblies that cross the nuclear envelope and form selective barrier, which regulate bidirectional exchange between nucleus and cytoplasm.
Photon-HDF5: open data format and computational tools for timestamp-based single-molecule fluorescence experiments
Antonino Ingargiola, Univ. of California, Los Angeles (United States); Ted A. Laurence, Lawrence Livermore National Lab. (United States); Shimon Weiss, Xavier Michalet, Univ. of California, Los Angeles (United States)

Archival of experimental data in public databases has increasingly become a requirement for most funding agencies and journals. These data-sharing policies have the potential to maximize data reuse, and to enable confirmatory as well as novel studies. However, the lack of standard data formats can severely hinder data reuse.

In photon-counting-based single-molecule fluorescence experiments, data is stored in a variety of vendor-specific or even setup-specific (custom) file formats, making data interchange prohibitively laborious, unless the same hardware-software combination is used. Moreover, the number of available techniques and setup configurations makes difficult to find a common standard.

To address this problem, we developed Photon-HDF5 (www.photon-hdf5.org), an open data format for timestamp-based single-molecule fluorescence experiments. Building on the solid foundation of HDF5, Photon-HDF5 provides a platform- and language-independent, easy-to-use file format that is self-describing and supports rich metadata. Photon-HDF5 supports different types of measurements by separating raw data (e.g., photon-timestamps, detectors, etc...) from measurement metadata. This approach allows representing several measurement types and setup configuration within the same core structure and makes possible extending the format in backward-compatible way.

Complementing the format specifications, we provide open source software to create and convert Photon-HDF5 files, together with code examples in multiple languages showing how to read Photon-HDF5 files.

Photon-HDF5 allows sharing data in a format suitable for long term archival, avoiding the effort to document custom binary formats and increasing interoperability with different analysis software.

We encourage participation of the single-molecule community to extend interoperability and to help defining future versions of Photon-HDF5.

Analyzing blinking effects in super resolution localization microscopy with single-photon SPAD imagers
Ivan Michel Antolovic, Technische Univ. Delft (Netherlands); Samuel Burri, Claudio E. Bruschini, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Ron A. Hoebe, Academisch Medisch Centrum (Netherlands); Edoardo Charbon, Technische Univ. Delft (Netherlands)

For many scientific applications, electron multiplying charge coupled devices (EMCCDs) have been the sensor of choice because of their high quantum efficiency and built-in electron amplification. Lately, many researchers introduced scientific complementary metal-oxide semiconductor (sCMOS) imagers in their instrumentation, so as to take advantage of faster readout and the absence of excess noise. Alternatively, single-photon avalanche diode (SPAD) imagers can provide even faster frame rates and zero readout noise.

SwissSPAD is a 1-bit S12x128 SPAD imager, one of the largest of its kind, featuring a frame duration of 6.4 ns. Additionally, a gating mechanism enables photosensitive windows as short as 5 ns with a skew better than 150 ps across the entire array. The SwissSPAD photon detection efficiency (PDE) uniformity is very high, thanks on one side to a photon-to-digital conversion and on the other to a reduced fraction of “hot pixels” or “screamers”, which would pollute the image with noise. A low native fill factor was recovered to a large extent using a microlens array, leading to a maximum PDE increase of 12%. This enabled us to detect single fluorophores, as required by ground state depletion followed by individual molecule return imaging microscopy (GSDIM). We show the first super resolution results obtained with a SPAD imager, with a localization uncertainty of 30 nm. The high timing resolution of 6.4 ns can be utilized to explore the dye’s photophysics or for dye optimization. We also present the methodology for the blinking analysis on experimental data.

Pile-up correction for high-throughput fluorescence lifetime imaging microscopy (FLIM)
Joerg Enderlein, Daja Ruhlandt, Anna Chithik, René Ebrecht, Fred S. Wouters, Ingo Gregor, Georg-August-Univ. Göttingen (Germany)

Fluorescence lifetime microscopy has become an important method of bioimaging, allowing not only to record intensity and spectral, but also lifetime information across an image. One of the most widely used methods of FLIM is based on Time-Correlated Single Photon Counting (TCSPC). In TCSPC, one determines this curve by exciting molecules with a periodic train of short laser pulses, and then measuring the time delay between the first recorded fluorescence photon after each exciting laser pulse. An important technical detail of TCSPC measurements is that the delay time between excitation laser pulses and resulting fluorescence photons are always measured between a laser pulse and the first fluorescence photon which is detected after that pulse. At high count rates, this leads to so-called pile-up: `early’ photons eclipse long-delay photons, resulting in heavily skewed TCSPC histograms. To avoid pile-up, a rule of thumb is to perform TCSPC measurements at photon count rates which are at least hundred times smaller than the laser-pulse excitation rate. The downside of this approach is that the fluorescence-photon count-rate is restricted to a value below one hundredth of the laser-pulse excitation-rate, reducing the overall speed with which a fluorescence signal can be measured. We present a new data evaluation method which provides pile-up corrected fluorescence decay estimates from TCSPC measurements at high count rates, and we demonstrate our method on FLIM of fluorescently labeled cells.

Carbocyanines in an RNA environment: experiment meets simulation
Richard Boerner, Fabio Steffen, Roland K. O. Sigel, Univ. Zürich (Switzerland)

The popularity of carbocyanine dyes in single molecule spectroscopy of nucleic acids is unbroken. Studying the dynamics of large RNA constructs and the binding kinetics such as the exon/intron binding site interaction of the group II intron in S. Cerevisiae have motivated a thorough photophysical characterization of the FRET pair Cy3/Cy5 in context of nucleic acids and RNA in particular. We showed that Mg2+ as a mediator of RNA-dye interactions enhances the cyanine fluorescence lifetime. The increasing window for depolarization as monitored by time-resolved anisotropy further revealed a dynamic equilibrium between free tumbling and stacking on the RNA backbone, with the stacked conformation preventing photomixing [4]. Tracking fluorescence mobility covalently bound to the RNA on an atomistic level by means of molecular dynamics [5] allow to disentangle different types of dye-dye and dye-RNA interactions. Our hybrid approach combining time-correlated single photon counting and computer simulations will benefit the interpretation of absolute distance measurement by smFRET.

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9714-9, Session 3

**PhotoGate: tracking single molecules in crowded environments (Invited Paper)**

Ahmet Yildiz, Univ. of California, Berkeley (United States)

Tracking single molecules inside cells reveals the dynamics of biological processes, including receptor trafficking, signaling and cargo transport. However, individual molecules often cannot be resolved inside cells due to their high density in the cellular environment. We developed a photobleaching gate assay, which controls the number of fluorescent particles in a region of interest by repeatedly photobleaching its boundary. Using this method, we tracked single particles at surface densities two orders of magnitude higher than the single-molecule detection limit. We observed lipid-induced dimerization of epidermal growth factor receptors (EGFR) on a live cell membrane. In addition, we tracked individual intraflagellar transport (IFT) trains along the length of a cilium and observed their remodeling at the ciliary tip.

9714-10, Session 3

**Single light-harvesting complexes: from detergent to a lipid membrane environment**

J. Michael Gruber, Vrije Univ. Amsterdam (Netherlands); Stefan Scheideelaar, J. Antoineknight Killian, Utrecht Univ. (Netherlands); Rienk van Grondelle, Vrije Univ. Amsterdam (Netherlands)

Most photosynthetic pigment-protein complexes are integral membrane proteins and thus have to be isolated in the presence of detergent. Unfortunately, detergents wash away any associated native lipids and can have a denaturing effect on proteins. It was recently shown that the styrene maleic anhydride (SMA) copolymer can be used to solubilize and isolate stable protein complexes with their native lipid environment into nanodisk particles [12]. In this study we investigate the photo-physics of single light-harvesting complexes II (LHCl II) in SMA nanodisks and compare them against detergent-isolated complexes. The amount of associated lipids and their composition is presented based on thin layer chromatography (TLC). We employ time-correlated single photon counting (TCSPC) to demonstrate an unquenched fluorescence lifetime of 3.5 ns in both samples and to investigate the quenching kinetics in correlation with the fluorescence intensity. The observed carotenoid triplet kinetics, together with the calculation of the relative absorption cross section, indicate the successful isolation of trimeric complexes, confirming the trimeric structure as a likely native configuration. The observed remarkable stability of LHCl II at room temperature in SMA nanodisks might be explained by a stabilizing effect of the co-purified lipids. The observed fluorescence intermittency (“blinking”) in detergent isolated complexes is retained in SMA nanodisks. This supports the hypothesis that blinking is present and may be involved in excess energy dissipation in native light-harvesting.


9714-11, Session 3

**Observing the proton-translocating motor of single FoF1-ATP synthase at work using an improved fluorescent protein mNeonGreen as novel FRET donor**

Thomas Heitkamp, Friedrich-Schiller-Univ. Jena (Germany); Gabriele Deckers-Hebestreit, Univ. Osnabrück (Germany); Michael Börsch, Friedrich-Schiller-Univ. Jena (Germany)

ATP is the universal chemical energy currency for cellular activities that is provided mainly by the membrane enzyme FoF1-ATP synthase in bacteria, chloroplasts and mitochondria. Over the last 15 years we have developed a variety of single-molecule FRET approaches to monitor catalytic action of individual bacterial enzymes in vitro. Catalysis is accompanied by subunit rotation within the enzyme. By specifically labeling rotating and static subunits in single enzymes we were able to observe three-stepped rotation in the F1 motor, ten-stepped rotation in the Fo motor, transient elastic deformation of the connected rotor subunits as well as the internal regulatory switch that prevents detrimental ATP hydrolysis by the enzyme. However, spatial and time resolution of motor activities were always related and limited by the photophysics of the FRET fluorophores which were used to report the stepwise distance changes between two marker positions within the single enzyme. Here we describe the novel FRET donor mNeonGreen with superior photophysical characteristics to our previous FRET donors EGFP or yeast-enhanced-GFP. Time-resolved single-molecule FRET allows to follow the individual steps of the Fo motor at different driving forces.

9714-12, Session 3

**Adhesion of living cells revealed by variable-angle total internal reflection fluorescence microscopy**

Marcelina Cardoso Dos Santos, Cyrille Vézy, Rodolphine Jaffiol, Univ. de Technologie Troyes (France)

Total Internal Reflection Fluorescence Microscopy (TIRFM) is a widespread technique to study cellular process occurring near the contact region with the glass substrate. In this field, determination of the accurate distance from the surface to the plasma membrane constitutes a crucial issue to investigate the physical basis of cellular adhesion process. However, quantitative interpretation of TIRF pictures regarding the distance z between a labeled membrane and the substrate is not trivial. Indeed, the contrast of TIRF images depends on several parameters more and less well known (local concentration of dyes, absorption cross section, angular emission pattern...). The strategy to get around this problem is to exploit a series of TIRF pictures recorded at different incident angles in evanescent regime. This technique called variable-angle TIRF microscopy (vTIRFM), allowing to map the membrane-substrate separation distance with a nanometric resolution (10-20 nm). vTIRFM was developed by Burmeister, Truskey and Reichert in the early 1990s with a prism-based TIRF setup [Journal of Microscopy 173, 39-51 (1994)]. We propose a more convenient prismless setup, which uses only a rotatable mirror to adjust precisely the laser beam on the back focal plane of the oil immersion objective (no azimuthal scanning is needed). The series of TIRF images permit us to calculate accurately membrane-substrate distances in each pixel. We demonstrate that vTIRFM are useful to quantify the adhesion of living cells for specific and unspecific membrane-surface interactions, achieved on various functionalized substrates with polymers (BSA, poly-L-lysin) or extracellular matrix proteins (collagen and fibronectin).

Development and application of 2-color live-cell STED nanoscopy (Invited Paper)
Edward S. Allgeyer, Francesca Bottanelli, Emil B. Krommann, Xiang Hao, Joerg Bewersdorff, Yale School of Medicine (United States)

Stimulated emission depletion (STED) microscopy has been established as an important technique for imaging below the diffraction limit facilitating new discoveries in an array of biological systems. In STED microscopy a “donut-shaped” laser focus is super-imposed upon the diffraction-limited focus of an excitation laser. The donut-shaped beam suppresses fluorescence in the periphery of the excitation spot, reducing the effective point spread function to a sub-diffraction size. However, the application of multicolor STED microscopy in living cells poses a number of challenges. Here we detail a novel STED system specifically designed for two-color STED applications. Our system employs FPGA-based gated detection and fast beam scanning to reduce pixel dwell time and photobleaching. We demonstrate the instrument’s capability with two-color continuous imaging of intracellular targets below the diffraction limit allowing observation of rare events within live-cells.

Super resolution imaging of HER2 gene amplification
Masaya Okada, Takuya Kubo, Kanako Masumoto, Shigeki Iwanaga, Sysmex Corp. (Japan)

HER2 positive breast cancer is currently inspected by detecting over expression of HER2 genes and/or HER2 proteins in a breast carcinoma. In the inspection, an analyte is classified into negative, middle or positive groups, according to the ratio between number of HER2 genes and CEP17 in a fluorescence in situ hybridization (FISH) image of the breast carcinoma. However, due to the limitation of spatial resolution in general fluorescence microscopy, it is difficult to distinguish HER2 genes in small distance, which further introduces inaccuracy into the classification of an analyte. To make HER2 genes counting in a breast carcinoma more accurate, a two dimensional super resolution fluorescence microscopy based on PALM/STROM was constructed. The spatial resolution of our super resolution microscopy was approximately 20 nm in lateral direction. By imaging a FISH-stained xenograft tissue section containing a formalin-fixed, paraffin-embedded human carcinoma cell line (Calu-3, 2R-75-1), or MCF-7 using the microscopy, the distribution of HER2 genes were observed more clearly, which enables us to count HER2 genes in the tissue section more precisely. Furthermore, Double-Helix PSF technique was applied to the super resolution microscopy to observe an analyte in three dimensions. By introducing an optical phase mask, a fluorescence molecule was imaged as two spots on a detector. Through measuring the rotation angle of the connection line between the two spots, which is related to the axial position of each fluorescence molecule, a three-dimensional image of HER2 genes distribution in a 4 um thickness breast carcinoma was reconstructed without stage scanning.

Multi-pulse pumping for far-field super-resolution imaging
Sebastian Requena, Texas Christian Univ. (United States); Sangram Raut, Texas Christian Univ. (United States) and Univ. of North Texas Health Science Ctr. at Fort Worth (United States); Hung Doan, Joseph D. Kimball, Texas Christian Univ. (United States); Rafał Fudala, Julian Borejdo, Ignacy Gryczynski, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); Yuri Strzhemechny, Zygmunt K. Gryczynski, Texas Christian Univ. (United States)

Recently, far-field optical imaging with a resolution significantly beyond diffraction limit has attracted tremendous attention allowing for high resolution imaging in living objects. Various methods have been proposed that are divided in to two basic approaches; deterministic super-resolution like STED or RESOLFT and stochastic super-resolution like PALM or STORM. We propose to achieve super-resolution in far-field fluorescence imaging by the use of controllable (on-demand) bursts of pulses that can change the fluorescence signal of long-lived component over one order of magnitude. We demonstrate that two beads, one labeled with a long-lived dye and another with a short-lived dye, separated by a distance lower than 100 nm can be easily resolved in a single experiment. The proposed method can be used to separate two biological structures in a cell by targeting them with two antibodies labeled with long-lived and short-lived fluorophores.

Fast and precise 3D fluorophore localization by gradient fitting
Hongqiang Ma, Jianquan Xu, Jingyi Jin, Ying Gao, Li Lan, Yang Liu, Univ. of Pittsburgh (United States)

Astigmatism imaging has been widely used to encode the fluorophore’s 3D position in single-particle tracking and super-resolution localization microscopy. Iterative Gaussian function fitting (GF) based algorithms are usually employed to reconstruct a standard 3D image, but the slow execution speed is an intrinsic disadvantage of the GF based methods. Hence, several single-iteration algorithms have been developed in the past few years to accelerate the execution speed while providing comparable accuracy to traditional multiple iterative Gaussian function fitting based algorithm. Unfortunately, these algorithms are mainly designed for 2D fitting of a circular PSF, and their accuracy for retrieving the 3D position is significantly compromised when the spatial distribution of fluorescent emission is not isotropic, such as astigmatism-based imaging with elliptical PSF. Here, we present a simple algebraic localization algorithm based on gradient fitting to decode the 3D subpixel position of the fluorophore with both superior localization accuracy and execution speed. This algebraic algorithm determines the center of the fluorescent emitter by finding the best-fit gradient direction distribution to the measured point spread function, and can retrieve the 3D subpixel position of the fluorophore in a single iteration. Through numerical simulation and experimental analysis, we demonstrate that our algorithm yields comparable localization precision to the multiple iterative GF fitting based method, while exhibits over two orders-of-magnitude faster execution speed. Our algorithm is a promising online analysis method for high-throughput 3D single-particle tracking and super-resolution localization microscopy, with a great potential for implementation in embedded devices and low-cost and portable microscopy using smart phones. Young Investigator best paper competition BO403

Generating 3D depletion distribution in an achromatic, single-channel, monolithic system
Clément Fallet, Arvid Lindberg, Gabriel Y. Sirat, Bioaxial (France)

Recent developments have shown that conical diffraction by a biaxial crystal can be used to create a perfect vortex beam for use in 2D STED microscopy. It has been shown that this concept can be extended and can also generate the depletion distributions used for 3D STED microscopy. A single beam
passes through a biaxial crystal that creates two co-propagating, co-localized beams; the first one is used for lateral depletion, and the other one for axial depletion. The two beams are cross-polarized and thus don’t interfere.

We will show here that the 3D distribution generation can be made achromatic, i.e. several depletion wavelengths can travel through a common path and still be shaped into the appropriate pattern by optimizing the geometry of the system. This system enables true one-channel 3D depletion at multiple wavelengths ranging from 580nm to 770nm, thus covering most of the conventional depletion wavelengths currently used. Preliminary results of depletion PSFs will be presented and the advantages and limitations of this system will be discussed as well as the experimental considerations required to successfully obtain the desired PSFs.

9714-19, Session 5
Nanopore integrated with Au cluster formation for single molecule analysis
Seong Soo Choi, Myoung Jin Park, Chul Hee Han, Tokutaro Yamaguchi, Sun Moon Univ. (Korea, Republic of); Sung In Kim, Kyoung Jin Park, Jung Ho Yoo, National Nanofab Ctr. (Korea, Republic of); Namkyoo Park, Seoul National Univ. (Korea, Republic of)

Au aperture shrinking phenomena on apex of pyramid were examined by 51 PA, 300 keV TEM electron beam irradiations. Pore shrinking and opening under TEM electron beam irradiations were examined dependent upon the electron beam current density. A pore diameter less than 5 nm was also drilled by using TEM in a focusing spot mode. We also investigated an Au cluster formation on the diffused membrane in room environment due to Ostwald ripening process. The fabricated nanopore on an Au pyramidal aperture with plasmonic effect can be utilized as single molecule optical nanosensor.

9714-20, Session 5
Custom field-of-view quantitative label-free microscopy by optofluidic space-time digital holography
Vittorio Bianco, Melania Paturzo, Valentina Marchesano, Pietro Ferraro, Istituto di Scienze applicata e Sistemi Intelligenti (Italy)

The study of biological specimens onboard compact Lab-on-a-Chip (LoC) platforms with embedded label-free, quantitative, 3D imaging functionalities is highly demanded for high-throughput rapid point-of-care diagnostics, especially in developing countries that lack of costly and adequate facilities. Here we introduce a novel imaging modality, named Space-Time Scanning Interferometry (STSI), which synthesizes space-time interferograms with intriguing features. Indeed, a single linear sensor array is sufficient to build up synthetic interferograms with unlimited Field of View (FoV) along the scanning direction and reduced noise, thus overcoming the trade-off existing between sample magnification and FoV. The STSI principle is well-suited to be applied in all the cases where the object motion is an intrinsic feature of the system, e.g. in case of microfluidics, so that the advantages of STSI have no cost associated with. Starting from these considerations, we applied the STSI method to in-flow on-chip microscopy of biological samples. Out-of-focus recordings are performed using a single line detector and polymeric micro-lenses embedded onboard chip, in order to synthesize a Space-Time Digital Hologram (STDH) carrying full-field, 3D information of the flowing samples. We discuss the method and prove that a STDH still maintains all the advantageous capabilities of DH microscopy. The throughput of the imaging system is dramatically increased as STDH provides unlimited FoV, refocussable imaging of multiple samples flowing inside a liquid volume with no need for hologram stitching. Thus, it is possible to move a huge step toward the integration of the imaging functionalities onboard chip for high-throughput rapid diagnostics.

9714-21, Session 5
Fourier-interpolation stochastic optical fluctuation imaging (fSOFI)
Joerg Enderlein, Simon C. Stein, Anja Huss, Dirk Hähnel, Ingo Gregor, Georg-August-Univ. Göttingen (Germany)

Stochastic Optical Fluctuation Imaging (SOFI) is a superresolution fluorescence microscopy technique which allows to enhance the spatial resolution of an image by evaluating the temporal fluctuations of blinking fluorescent emitters. SOFI is not based on the identification and localization of single molecules such as in the widely used Photoactivation Localization Microscopy (PALM) or Stochastic Optical Reconstruction Microscopy (STORM), but computes a superresolved image via temporal cumulants from a recorded movie. A technical challenge hereby is that, when directly applying the SOFI algorithm to a movie of raw images, the pixel size of the final SOFI image is the same as that of the original images, which becomes problematic when the final SOFI resolution is much smaller than this value. In the past, sophisticated cross-correlation schemes have been used for tackling this problem. Here, we present an alternative, exact, straightforward, and simple solution using an interpolation scheme based on Fourier transforms. We exemplify the method on simulated and experimental data.

9714-22, Session 6
Multicolor single-molecule imaging by spectral point-spread-function engineering
Yoav Shechtman, Lucien E. Weiss, Adam S. Backer, William E. Moerner, Stanford Univ. (United States)

Super-resolution localization microscopy has revolutionized the field of biological imaging over the past decade. Methods relying on sequential localization of single point emitters now enable imaging and tracking at ~10-20 nm resolution. Moreover, three-dimensional localization is made possible by various techniques, a prominent one being point-spread-function (PSF) engineering – namely, encoding the axial (z) position of an emitter in the shape of the microscope’s PSF. However, an outstanding challenge for current localization-microscopy methods is multicolor imaging - a task of the utmost importance for contextualizing biological data. Normally, multicolor imaging requires multiple cameras, segmented dedicated fields of view, or the use of sequential imaging. Here we demonstrate the encoding of an emitter’s spectral information (color), in addition to its 3D position, using PSF engineering. By designing special pupil-plane patterns that yield controllably different PSFs for different wavelengths with high efficiency, we encode the color of the emitter in the shape of its PSF, and are therefore able to perform simultaneous multicolor tracking and super-resolution imaging in a single optical path. To demonstrate the applicability of our method we use multicolor PSFs for super-resolution imaging and for multiple particle tracking.

The design scheme works by exploiting the chromatic dispersion of a liquid-crystal spatial light modulator (SLM), placed in the pupil plane of the microscope. Different wavelengths experience different optical phase delays when reflected by the SLM, and therefore the PSF is wavelength dependent. We use numerical optimization to find a single voltage-pattern that yields different desired phase delay patterns for different wavelengths.
9714-23, Session 6

Advanced pulse pattern generation and fine tuning for STED microscopy

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Stimulated Emission Depletion (STED) Microscopy has evolved into a well established method offering optical superresolution below 50 nm. Running both excitation and depletion lasers in picosecond pulsed modes allows for highest optical resolution as well as fully exploiting the photon arrival time information using time-resolved single photon counting (TCSPC). Non-supersolved contributions can be easily dismissed through time-gated detection or a more detailed fluorescence decay analysis. Furthermore, these methods allow for accurate separation of different fluorescent species.

We present here a new generation of our VisIR 765 “STED” depletion laser, featuring a pulse length and beam shape optimized for STED microscopy. The temporal overlap between excitation and STED laser pulses can be adapted specifically to different fluorescence lifetimes thanks to our fully computer controlled multichannel delay generator SOM-D. The SOM-D allows for the easy introduction of electronic delays between laser channels with time resolutions below 50 ps.

Pulsed interleaved excitation (PIE) patterns can also be realized with the SOM-D, allowing to cycle the illumination on the nanosecond timescale between STED and non-STED. This simultaneous probing is advantageous for the identification and elimination of unwanted STED induced processes. Examples from blinking and photobleaching in single molecule imaging as well as in fluorescence correlation spectroscopy (STED-FCS) will be given. This extended STED functionality along with improved data throughput are the latest extensions to the confocal microscope platform MicroTime 200 and will soon be available as upgrade for existing systems.

9714-24, Session 6

Video-rate super-resolution fluorescence microscopy using all-optical two-photon image scanning microscopy

Ingo Gregor, Martin Spiecker, Joerg Enderlein, Georg-August-Univ. Goettingen (Germany)

Since about 20 years Abbe’s resolution limit has been overcome in fluorescence microscopy by the first demonstration of STED microscopy (1). Later, methods based on single-molecule localization joined the field, such as PALM (2) and STORM (3). These methods use principles that operate beyond the diffraction of light. Increased spatial resolution can also be achieved by a class of methods exploiting excitation and detection modalities still bound to light diffraction like structured illumination microscopy (SIM) (4) and image scanning microscopy (ISM) (5). While not reaching the resolution of STED, PALM, and STORM, they do not require any special labels, sample conditions, or excitation power, and may be applied to any sample at any wavelength.

ISM was presented in a theoretical study by Sheppard (6) finding that it is able to double the resolution of a scanning confocal microscope. The first implementation needed several minutes to record one image. York et al. (7) had overcome this limitation by using a multifocal excitation scheme. We demonstrated an acquisition time of less than 1 s per image using a spinning-disc confocal microscope (CSD-ISM) (8). The drawback of these systems is that the images have to be processed after recording, so a huge amount of data has to be processed. Here, we present an all-optical implementation of ISM based on a resonant confocal laser-scanning microscope using two-photon excitation. The all-optical implementation means that the images are recorded directly with the full resolution and no post-processing is necessary. By this one can take the full advantage of the speed of the resonant laser-scanner providing a frame-rate of about 30 fps and a live view to the sample.

9714-25, Session 6

Restoration of STORM images from sparse subset of localizations

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To construct a Stochastic Optical Reconstruction Microscopy (STORM) image one should collect sufficient number of localized fluorophores to satisfy Nyquist criterion. This requirement limits time resolution of the method. In this work we propose a probabilistic approach to construct STORM images from a subset of localized fluorophores 3-4 times sparser than required from Nyquist criterion. Using a set of STORM images constructed from number of localizations sufficient for Nyquist criterion we derive a model which allows us to predict the probability for every location to be occupied by a fluorophore at the end of hypothetical acquisition, having as an input parameters distribution of already localized fluorophores in the proximity of this location. We show that probability map obtained from number of fluorophores 3-4 times less than required by Nyquist criterion may be used as superresolution image itself. Thus we are able to construct STORM image from a subset of localized fluorophores 3-4 times sparser than required from Nyquist criterion, proportionally decreasing STORM data acquisition time. This method may be used complementary with other approaches desired for increasing STORM time resolution.

9714-26, Session 7

Investigating the molecular basis of muscular dystrophy diseases by single molecule imaging in cells and live animal models (Invited Paper)

Anthony Fernandez, Ramunas Stanciauskas, Fabien Pinaud, The Univ. of Southern California (United States)

Muscular dystrophies constitute a diverse group of disorders characterized by mutations in genes encoding key sarcolemmal, cytoskeletal or nuclear membrane proteins. To gain new insight into the molecular pathogenesis of Emery-Dreifuss muscular dystrophy (EDMD) and Duchenne muscular dystrophy (DMD) at the nanoscale we used single molecule tracking and super-resolution optical microscopy in human cells and C. elegans animal models. The diffusional mobility and the spatial distribution of the nuclear membrane emerin and a variety of EDMD-associated emerin mutants were studied by single particle tracking PALM and STORM super-resolution imaging in rescued emerin-null cells. We identified different subpopulations of emerin associated with the endoplasmic reticulum, the outer or the inner nuclear membrane and show that emerin forms clusters at the nuclear envelope. We further show that the dynamic interactions of emerin with the inner nuclear lamin network and its clustering state are differentially impacted by mutations causing EDMD.

We also characterized the in situ biomolecular properties of dystrophin, a key structural protein of muscle cells that is mutated in DMD patients. Using split-fluorescent proteins and Complementation Activated Light Microscopy we image individual calcium channels at the muscle sarcolemma in living C. elegans worm models of DMD. Through diffusion and spatial pattern analyses, we show that dystrophin is a load-bearing apparatus and a tension transducer that modulates the confinement of calcium channels within sarcolemmal membrane nanodomains in response to varying muscle tonus. These single molecule imaging approaches provide new tools for biomedical research on muscular dystrophies at the cellular and animal levels.
Molecular orientational order imaging by polarized super resolution localization microscopy

Sophie Brasselet, Cesar A. Valades Cruz, Haitham A. Shaban, Nicolas Bertaux, Julien Savatier, Institut Fresnel (France)

While super-resolution microscopy can guide the interpretation of bio-imaging through morphological observation in biomolecular assemblies in vivo with unprecedented level of precision, it is still a challenge to provide fine structural information at the molecular scale. Understanding the structural organization of bio-molecules by measuring their orientation could bring considerable complementary information, related for instance to the relation between cell filaments organization and their mechanical properties involved in fundamental biological processes. In this work, we report a super-resolution polarization-resolved microscopy technique able to image molecular fluorophores' orientation in static and dynamic environments at single molecule level and nano-scale resolution. Using direct Stochastic Optical Reconstruction Microscopy (dSTORM) in combination with polarized detection, fluorescence anisotropy images can be reconstructed at a spatial resolution of 30nm. We show how this information can be used to extract the dynamical nature of the fluorescein orientational order and its statistical angular constraint, in filamentous biological structures. Based on a refined signal analysis technique which provides high accuracy in the estimation of molecular anisotropies, we report nano-scale orientational behaviors in actin stress fibers in fixed cells and in vitro structures such as amyloid fibrils and double stranded DNA. This method brings a superior level of information as compared to previously developed ensemble polarization dependent methods by adding a quantitative knowledge on the fluorophore orientational flexibility, a parameter that is determining in the interpretation of fluorescence polarization responses when investigating bio-molecular structural organization.

Novel 3D single marker switching microscope with isotropic resolution over large axial range

Haugen Grefe, Claudia Geisler, Alexander Egner, Laser-Lab. Göttingen e.V. (Germany)

To overcome Abbes diffraction limit in fluorescent microscopy two main methods established in the last years. The first, stimulated emission depletion (STED) microscopy, shrinks the possible area of fluorescent emission by saturated depletion of the excited state of fluorescent molecules. The size of the remaining fluorescent spot is far below Abbes limit. The other utilizes molecular switching events such that only a single fluorophore will emit at the same time within a diffraction limited volume. By localizing the center of the emerging point spread function (PSF) the position of the emitter can be determined with nanometer accuracy. Depending on the particular molecular transition used this single marker switching (SMS) technique is called photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), ground state depletion microscopy (GSDM) and so on.

While the expansion of STED in the third dimension (3D-STED, isoSTED [1]) is based on the same principle as in 2D, in SMS several techniques can be used to determine the labels axial position: The astigmatism and the double-helix PSF techniques unambiguously change the shape of the PSF depending on the labels axial-position, whereas in the biplane technique two axially displaced detection planes are used to enable unambiguous axial localization [2].

As none of these techniques results in isotropic localization accuracy, interferometric PALM (iPALM) utilizes detection through two opposing objective lenses in a 4Pi like geometry in order to overcome this limitation [3]. Theoretically iPALM provides a v2 fold increased lateral and about 6 fold increased axial localization accuracy [4]. Nevertheless it is restricted to layers not thicker than about 7/2. Taking into account the spherical shape of the wavefronts 4Pi-SMS extended that technique to a layer of ~17m thickness, placed anywhere within a stained cell, providing a resolution of about 6 nm in the axial and 8-22 nm in the lateral direction [5].

In line with this development, we realized a new 3D SMS microscope with improved axial performance. Our novel microscope allows an almost isotropic resolution within a several micron thick layer inside an extended sample. This is very suitable for imaging large sample volumes and renders axial scanning obsolete.

In contrast to previous setups there are no limitations due to the overall thickness of the sample like in iPALM and there is no need for any manipulations of the PSF (astigmatism, double-helix PSF) or challenging interference procedures (ipALM, 4Pi-SMS).


Screening photoswitching properties of synthesized BODIPY-based fluorophores for multispectral superresolution microscopy (MSSRM)

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Single-molecule localization microscopy (SMLM) utilizes photoswitchable fluorophores to detect biological entities with 10-20 nm resolution. Multispectral superresolution microscopy (MSSRM) extends SMLM functionality by improving its spectral resolution up to 5 fold facilitating imaging of multicomponent cellular structures or signaling pathways. Current commercial fluorophores are not ideal for MSSRM as they are not designed to photoswitch and do not adequately cover the visible and far-red spectral regions required for MSSRM imaging. To obtain optimal MSSRM spatial and spectral resolution, fluorophores with narrow emission spectra and controllable photoswitching properties are necessary. Herein, a library of BODIPY-based fluorophores was synthesized and characterized to create optimal photoswitchable fluorophores for MSSRM. BODIPY was chosen as the core structure as it is photostable, has high quantum yield, and controllable photoswitching. The BODIPY core was modified through the addition of various aromatic moieties, resulting in a spectrally diverse library. Photoswitching properties were characterized using a novel polyvinyl alcohol (PVA) based film methodology to isolate single molecules. The PVA film methodology enabled photoswitching assessment without the need for protein conjugation, greatly improving screening efficiency of the BODIPY library. Additionally, image buffer conditions were optimized for the BODIPY-based fluorophores through systematic testing of oxygen scavenger systems, redox components, and additives. Through screening the photoswitching properties of BODIPY-based compounds in PVA films with optimized imaging buffer we identified novel fluorophores well suited for SMLM and MSSRM.
Correlating structure and fluorescence dynamics of quantum dot clusters using super-resolution imaging

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Energy transfer in films of quantum dots plays a vital role for solar technology and in FRET studies. By studying clusters of QDs ~ 2-10 particles in size with single-molecule techniques (super-resolution imaging, confocal microscopy, and electron microscopy) the energy transfer process can be observed directly and localized. A dominant, smallest, particle in a cluster – the acceptor – functions as the primary emitter for the entire cluster as FRET rates exceed radiative recombination rates and excitons move primarily to the acceptor. In addition, the fluorescence blinking of individual QDs affects the behavior of the cluster. A modulated blinking of the cluster is observed because the blinking state of the acceptor does not fully control the emission state of the entire cluster. During periods of low intensity emission wavelengths shift towards smaller wavelengths – an indication the energy transfer occurs to a lower-bandgap particle and that the fluorescence is not completely quenched because of the acceptor state. This suggests that in larger networks such as films, local “hot spots” of recombination activity exist and that the efficiency across an entire film is not homogeneous due to the inherent distribution of bandgaps within a sample. Single-molecule FRET efficiencies can be investigated from a combination of the known structure of a cluster and defocused imaging which determine specific FRET rates instead of an ensemble average.

Superresolution imaging with enhanced axial section by STED structured illumination microscopy

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Structured illumination microscopy (SIM) is a wide field imaging technique with intrinsic optical sectioning by removing out-of-focus light. Its fast imaging speed and low phototoxicity make SIM the most compatible technique with live biological specimen. However, various technical constraints have limited the application of SIM in biological studies. Linear SIM improves lateral resolution by 2 fold but cannot achieve strong resolution enhancement along axial dimension. 3D SIM provides illumination modulation in both lateral and axial directions, resulting in resolution improvement in all spatial dimensions but cannot improved resolution beyond twice above diffraction limit. Existing nonlinear, structured illumination techniques, including Saturated SIM and Photo-switch SIM, combine nonlinear effect with structured illumination to obtain higher than 2-fold resolution enhancement in lateral dimensions but its axial resolution is still diffusion limited.

We are developing a new Nonlinear SIM approach that utilizes the Stimulated Emission Depletion effect to achieve 4-fold resolution enhancement. On top of its lateral resolution enhancement, STED-SIM also provides resolution enhancement along the axial dimension due to the strong axial confinement of the structured STED effect. The STED-SIM system uses low coherent light to generate a wide-field STED pattern, which creates a spatially structured STED effect. Due to the nonlinearity of STED effect, the contrast of the structured STED effect quickly deteriorates with defocus. This local confinement of structured nonlinear effect provides strong optical sectioning and therefore brings axial resolution improvement. The axial sectioning effect was simulated with parameters compatible to experiments and further validated using fluorescent beads samples. With its fast imaging speed, large field of view, theoretically unlimited resolution enhancement and 3D resolution the diffraction limit, STED-SIM is a promising super-resolution technique for imaging dynamic process in live biological samples.

Multi-color joint tagging localization nanoscopy with ultra-high density molecule tracking

Peng Xi, Zhiping Zeng, Xuanze Chen, Ning Huang, Yujie Sun, Peking Univ. (China)

Super-resolution microscopy plays important role to further our knowledge in the subcellular organelle structural and interaction. In localization-based nanoscopy, the temporal resolution is largely limited by the assumption of single molecule condition per focal area. To increase the imaging speed of localization super-resolution microscopy, parallel imaging can be employed. Here we proposed joint tagging protocol applicable to most types of fluctuation/blinking-based super-resolution techniques. Multiple types of quantum dots with distinguishable fluorescence spectra can be jointly labelled to the same subcellular structure. Taking advantage of the spectral separation, which leads to the QDs of each spectral channel closer to single-molecule state, accurate tracking of the individual molecules at high labeling density can be realized. When combining all the multiple channels, significantly enhanced labeling density can be yielded, as in previous single-channel single molecule detection the overlapping events are eliminated by the algorithm. Furthermore, single particle tracking with ultra-high density was achieved by introducing joint tagging scheme based on both PALM and super-resolution optical fluctuation imaging (SOFI) and imaging. Experimentally, we demonstrated that joint tagging combined with sptPALM enables simultaneously tracking massive individual lipid rafts spatially distributed far below optical diffraction limit in living cells.

Multi-scale imaging approach identifies novel roles for the scaffold protein IQGAP1 in epithelial cell development

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Stochastic optical reconstruction microscopy (STORM), has been developed on the characteristics of molecules that can switch between “off” and “on” state in response to the incident lights with the corresponding wavelengths. The spatial resolutions of these techniques are influenced by characteristics of these switchable molecules. The environment parameters, such as label density and imaging buffer, which can affect the switching properties of molecules, have been extensively discussed in former studies. Here, the different light powers were used to activate the same molecule in the same environment in order to obtain STORM imaging for evaluating the response of single molecule at different light power. These imaging results demonstrated that the incident light changes led to the changes of the molecule profiles, which is reliable to figure out suitable light parameters for improving STORM imaging experiments. In the following experiments, the optimized parameters have been applied for observing the changes of the cell cytoskeleton during C2C12 differentiation.
The precise sub-cellular spatial localization of multi-protein complexes is increasingly recognized as a key mechanism governing the organization of mammalian cells. Consequently, there is a need for novel microscopy techniques capable of investigating such sub-cellular architectures in comprehensive detail. Here, we applied a novel multiplexed STORM super-resolution microscopy technique, in combination with high-throughput immunofluorescence microscopy and live-cell imaging, to investigate the roles of the scaffold protein IQGAPI in epithelial cells. IQGAPI is known to orchestrate a wide range of biological processes, including intracellular signaling, cytoskeletal regulation, cell-cell adhesion, and protein trafficking, by forming distinct complexes with a number of known interaction partners, and recruiting these complexes to specific subcellular locations. Our results demonstrate that, in addition to supporting epithelial adherens junctions by associating with specialized cortical actin structures, IQGAPI plays a second role in which it controls the confinement of a unique, previously undocumented class of membranous compartments to the basal actin cortex. These largely immotile yet highly dynamic structures appear transiently as cells merge into clusters and establish of apical-basolateral (epithelial) polarity, and are identified as an intermediate compartment in the endocytic recycling pathways for cell junction complexes and cell surface receptors. Although these two functions of IQGAPI occur in parallel and largely independently of each other, they both support the maturation and maintenance of polarized epithelial cell architectures.

9714-34, Session PSun

**STED add-on for a standard time-resolved confocal microscope**

Felix Koberling, Marcelle Koenig, Rhys Dowler, Benedikt Kraemer, Sebastian Tannert, Matthias Patting, Rainer Erdmann, PicoQuant GmbH (Germany)

Overcoming the diffraction limit for fluorescence imaging has been shown to be crucial for addressing various relevant biological questions. Here, we show how superresolution, namely Stimulated Emission Depletion (STED), can be easily added to a standard confocal time-resolved fluorescence microscope, the MicroTime 200. An EASYDONut phaseplate converts the STED laser beam into the required donut-shaped focal spot while leaving the excitation beam unaffected. An alignment of the STED donut in respect to the excitation spot is not necessary since both, STED and excitation beam are delivered by the same optical single mode fiber. A resolution below 50 nm FWHM is achieved. Externally triggered pulsed lasers and confocal detection with time-correlated single photon counting (TCSPC) allow this system to take advantage of various excitation schemes like bunched excitation, pulsed interleaved excitation (PIE) and detection modalities such as gated STED. Combinations of STED with other time-resolved techniques such as FLIM (Fluorescence Lifetime Imaging Microscopy) or FLCS (Fluorescence Lifetime Correlation Spectroscopy) as well as investigations down to the single molecule level are feasible.

We present multi-label STED imaging exciting the labels with close-by excitation wavelengths in PIE mode in combination with just a single STED depletion wavelength. Small differences in absorption and emission spectra as well as in the fluorescence lifetimes can be utilized to create fluorescence patterns which act like a fingerprint. The labels are distinguished by applying fluorescence Pattern Matching analysis which takes into account the full spectral and temporal information. This method will be shown on double labeled biological cells.

9714-35, Session PSun

**Effortless adaptive optics correction enabling deep SMLM imaging**

Grégory Clouvel, Audrius Jasaitis Jr., Xavier Levecq, Imagine Optic SA (France)

Single molecule localization methods (SMLM) enable us to locate fluorescent molecules with nanometric precision. The quality of point spread function (PSF) strongly influences the accuracy of detections in these methods, therefore it is highly important to precisely correct for aberrations. Aberrations induced by optical setup and biological sample can be corrected using adaptive optics, for example high quality phase modulator such as deformable mirror. However the determination of aberrations remains a complicated task. Currently available image-based iterative algorithms, such as genetic, 3N etc [1, 2] typically require acquisition of 30-100 images, which is time consuming and also might bleach the sample. Moreover, the quality of correction in this case frequently depends on the user’s personal judgment. To simplify this process we adapted a new algorithm based on phase retrieval method [3]. The parameters, stability and performance of the image based algorithm for SMLM will be discussed. Current implementations of SMLM techniques are only efficient in the vicinity of the coverslip, like in total internal reflection (TIRF). Imaging deeper is perturbed mostly by spherical aberration, caused by refractive index mismatch between the sample and immersion oil of the objective. To enable SMLM imaging deep in the sample, here we propose a new method of PSF optimization on the surface of the coverslip using Phase Retrieval image-based iterative algorithm and correct for spherical aberration model using a linear depth dependence experimental model. To test the performance of this method for deep PALM imaging we constructed a model sample composed of fixed HeLa cells evenly distributed at different depth in agar. The performance of this imaging strategy will be discussed.


9714-36, Session PSun

**Super-resolved image acquisition with full-field localization based microscopy: theoretical analysis and evaluation**

Taehwang Son, Wonjui Lee, Donghyun Kim, Yonsei Univ. (Korea, Republic of)

We analyze and evaluate super-resolved image acquisition with full-field localization microscopy in which an individual signal sampled by localization may or may not be switched. For the analysis, Nyquist-Shannon sampling theorem based on ideal delta function was extended to sampling with unit pulse comb and surface-enhanced localized near-field that was numerically calculated with finite difference time domain. Sampling with unit pulse was investigated in Fourier domain where magnitude of baseband becomes larger than that of adjacent subband, i.e. aliasing effect is reduced owing to pulse width. Standard Lena image was employed as imaging target and a diffraction-limited optical system is assumed. A peak signal-to-noise ratio (PSNR) was introduced to evaluate the efficiency of image reconstruction quantitatively. When the target was sampled without switching by unit pulse as the sampling width and period are varied, PSNR increased eventually to 18.1 dB, which is the PSNR of a conventional diffraction-limited image. PSNR was found to increase with a longer pulse width due to reduced aliasing effect. When switching of individual sampling pulses was applied, blurry artifact outside the excited field is removed for each pulse and PSNR soared to 25.6 dB with a shortened pulse period, i.e. effective resolution of 72 nm is obtained, which can further be decreased. Width dependence disappears due to the lack of crosstalk between adjacent pulses. More realistic surface-enhanced localized near-field was applied, in which case the tendency was similar, but overall PSNR was reduced due to the sidemode of localized near-field.
Conference 9714: Single Molecule Spectroscopy and Superresolution Imaging IX

9714-37, Session PSun

Conventional fluorescence microscopy below the diffraction limit with simultaneous capture of two fluorophores

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To achieve resolution beneath the diffraction limit with a conventional microscope a standard cooled 1.4 mega-pixel fluorescence charge-coupled device (CCD) camera was used to simultaneously image closely adjacent paired quantum dots on a flat surface with emissions of 540 and 630 nm. Overlapping Airy discs were segregated according color using Matlab software. Noise reduction was performed using the Fourier transform of centroid of the image to filter and the image was reconstructed with inverse transform. The Cartesian coordinates of the centers of the point spread functions were calculated in all images. Histograms constructed from serial images fit well to Gaussian functions for resolving two quantum dots separated by as little as 10 nm in the x-y coordinates (R^2 values close to unity). The ANOVA test statistic for multiple pairs of quantum dots validated discrimination of inter-fluorophore distances that vary by 10 nm but not those below 6 nm (p<0.001). This was evident in the normalized probability distribution functions for pairs of fluorophores. This method is developed for and limited for x-y resolution of dilute fluorophores on a flat surface.

9714-38, Session PSun

A user-friendly two-color super-resolution localization microscope

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Localization microscopy, such as stochastic optical reconstruction microscopy or photoactivated localization microscopy, enables visualization of subcellular structures in nanometer scales, albeit with various caveats of possible crosstalk, unbalanced localization accuracies in different colors, or prolonged imaging time in multicolor imaging. We report a robust two-color method for super-resolution localization microscopy that adopts the two-dye combination of Alexa647 and Alexa750. With assistance of imaging buffer containing TCEP and COT, where TCEP works as the switching reagent and COT enhances photon emission by a factor of 2, both dyes have well-balanced switching dynamics with over 4,000 photons emitted per switching cycle under low to moderate excitation power (about 4kW/cm²). Our scheme enables peak-finding with accuracy down to 15nm on both channels. The matched duty cycles of dyes allow simultaneous capture of two channels on a single camera. The system is equipped with active sample locking that senses the sample movement from the cross-correlation of bright-field images then compensates with 1nm accuracy in real time. We have demonstrated that owning to the stable performance provided by the system, life scientists with minimal amount of training are capable of acquiring two-color super resolution images with high-quality in a wide range of samples including cell cultures, tissue sections and yeast cells.

9714-39, Session PSun

Simultaneous fluorescence imaging of multiple fluorophores using wide-field epi-fluorescence microscopy

Kwan Seob Park, Dong Uk Kim, Jooan Lee, Ki-Soo Chang, Korea Basic Science Institute (Korea, Republic of)

Simultaneous multicolor imaging is highly required in live cells imaging. For this purpose, we propose to apply the four-bucket technique to the epi-fluorescence microscopy. Multiple fluorophores are simultaneously excited by each laser with different wavelength and detected by a monochrome camera. To discriminate each fluorophore, intensities of lasers are modulated by each control signal, which has a same frequency but a different phase, generating intensity-modulated fluorescence signals. Modulation frequency for excitation sources is determined as one-fourth of the camera frame rate. From four frames of the camera, modulated fluorescence signals for each fluorophore are extracted by a four-bucket calculation. Detected fluorescence signals then, are discriminated after digital processing. Detailed procedures for digital processing are demonstrated. The experimental results verify that this technique can provide both simplicity of the wide-field epi-fluorescence microscopy for simultaneous multi-fluorescence imaging and high-contrast image.

9714-40, Session PSun

Accurate axial localization by conical diffraction beam shaping generating a dark-helix PSF

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We present here a new PSF-shaping technique using biaxial crystals to generate a highly z-dependent distribution in single molecule localization microscopy (SMLM). This distribution features two zeros of intensity that rotate together with defocus. This PSF features similarities to the double-helix introduced by Moerner and Pietsun and thus has been dubbed dark-helix since we track zeros of intensity. Preliminary numerical studies based on Cramer-Rao Lower Bound (CRLB) show that this PSF has the potential to obtain up to 20nm localization precision. This PSF can be easily generated by a very simple, monolithic add-on added in front of the detection camera. Additionally, the PSF remains of the approximate size of the Airy PSF, the x-y localization precision is not substantially affected and no trade-off is required. The x-y compactness of the PSF also enables theoretically a higher density of emitters than the double-helix which spreads on a larger scale.

Limiting factors for SMLM such as loss of photons, complexity and robustness will be discussed and considerations about the practical implementation of such techniques will be given.

9714-41, Session PSun

A bi-functional bolometer with sensitivity to IR radiation and hot air induced temperature variation

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ABSTRACT We have shown previously that doping a poly(4-vinyl pyridine)/pyridine gel with an ester group-containing polymer (e.g. poly(butyl methacrylate)) (polymer acids as additives) expands the wavelength range of the gel photoelectrical sensitivity from the uv into the infra-red.

Here we characterize the temporal response of the gel resistivity and demonstrate its ability to operate as a bi-functional bolometer. At constant room temperature, the bolometer can function as a rapidly responding IR detector. It can also respond to temperature variation produced by hot air, but with a much longer time constant.

The fractional change in resistance caused by IR irradiation at 1mm is R/RO = 0.13; the fractional change caused by hot air induced temperature variation - R/RO = 0.06/1°C. The relaxation rate of the IR response following switch-off is 260%/s. The temperature-induced relaxation curve could be fit to an exponential function with two time constants ~ t1=0.148s and t2=17.11s. Analysis of the relaxation of the photo-response was limited by the time resolution of the resistance measurements, i.e. 0.02s.
Polymer/liquid pyridine interactions are considered to be responsible for this interesting functionality of the polymer blend and they will be discussed.

References:

9714-42, Session PSun
Localization-based superresolution microscopy reveals tapering and asynchronous axonemal growths of primary cilia
Tony Yang, Yi-De Chen, Jung-Chi Liao, Academia Sinica (Taiwan)

Primary cilia are microtubule-based rod-shaped structures in cells essential for embryonic development and adult physiology mediating mechano-/photo-/chemo-sensations on diverse mammalian cell types. They contain an axoneme of 9 microtubule doublets supporting the slender ciliary structure and serving as tracks for molecular motor-based transport within cilia. Several distinct compartments have been identified along the ciliary axis. However, it is unclear how the axonemal structures are arranged along the axis and whether there is any architectural difference among different compartments. Single molecule localization-based superresolution fluorescence techniques such as direct stochastic optical reconstruction microscopy (dSTORM) offer a substantially higher spatial resolving power than conventional microscopy, allowing the studies of protein organization within a cilium. Here we used dSTORM to reveal subdiffractional contours of axonemes in primary cilia labeled with Alexa Flour 647 targeting acetylated α-tubulin. Statistical analysis of collective superresolution images shows that the width of a cilium, defined as the distance between two labeled boundaries, is reduced from the base to the tip, with a characteristic tapering profile along the ciliary axis. When promoting excess ciliary growth with lithium treatment, we found a further reduced width and zigzag axoneme along elongated primary cilia. Surprisingly, in some lengthened cilia, the distal segment appears to be merged into a single bundle or singlet microtubules, implying an asynchronous growth among 9-fold microtubule doublets. Together, our subdiffractional morphological analysis of primary cilia suggests that the axonemal architecture accommodates a narrower space toward the ciliary tip, with a potential asynchronous axonemal growth at the distal end.

9714-43, Session PSun
Quantitative protein labeling of the HER2 cancer signaling pathway for high-resolution microscopy
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Despite the development of promising targeted cancer therapeutics, many treatments have limited efficacy in patients over time. Often this is due to the ability of aberrant signaling pathways to circumvent drug-induced roadblocks. In most cases, this complex reorganization remains unresolved at the single-molecule level because of the inherent difficulty in visualizing individual proteins in situ. Multispectral superresolution microscopy (MSSRM), an extension of single-molecule superresolution microscopy (SRM) where up to 20 proteins in a single pathway may be tagged with distinct fluorophores and imaged at the nanoscale, provides a unique opportunity to obtain both structural and temporal single protein information while preserving spatial integrity in cells and tissues. However, MSSRM relies on the ability to site-specifically tag individual protein units with a fluorophore in a controlled manner. Few studies have quantified the efficiency, affinity and utility of different protein affinity tags (e.g. whole IgG, antibody fragments, nanobodies, affibodies, aptamers), particularly when multiple targets must be labeled within close proximity. In the current work, HER2, a transmembrane protein overexpressed in approximately 25% of all breast cancer, was used as a model system for quantitative protein labeling studies. By engineering HER2 monomers with genetically encoded fluorescent protein tags, we conducted co-localization studies to screen potential affinity tags for use in quantitative MSSRM. These techniques will be applied to other critical signaling pathway proteins, and offer the possibility for quantitative protein labeling in high-resolution microscopy including SRM and electron microscopy.

9714-44, Session PSun
Single-wavelength-controlled dynamic optical nanoimaging based on fluorescence molecular switches
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Photoswitchable fluorophores are promising in single molecule devices and optical nanoimaging, especially in single molecule photo-activated localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM). However, the scarcity of current photoswitchable fluorophores stimulates people to develop complicated optical system and processing software in accordance with limited photoswitchable fluorescent proteins and organic fluorophores. Previous efforts to develop synthetic photoswitchable fluorophores exhibit their promising potential in optical nanoimaging. Here we have designed and synthesized a new fluorescence molecular switch with reversible green emission, napthalimide-hexaarylbimidazole conjugate (NI-N-HABI), which exhibits fast thermal fading of photochromism, blue light-induced fluorescence quenching and successively spontaneous recovery. This new photoswitchable fluorophore enable that the red-edge wavelength of optical responsibility red-shift from current near-UV region to 500 nm. The relatively fast fading speed of NI-N-HABI and its sensitivity to longer blue light irradiation (400-500 nm) have simplified the utilization of two wavelength lasers to single wavelength. We successfully applied this new probe in single-wavelength-controlled optical nanoimaging for self-assembly and solvent annealing of amphiphilic block polymers, with 50 nm of optical resolution. Single-wavelength-controlled optical nanoimaging will facilitate nanoscale optical visualization for dynamic process of living and non-living systems both in theoretical method and experimental procedure.

9714-45, Session PSun
Novel plasmonic platform for ultra-sensitive detection and diagnostics
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In this work, we describe a plasmonic platform with silver fractals for metal enhanced fluorescence (MEF) measurements. When a dye containing surface was brought into contact with silver fractals, a significantly enhanced fluorescence signal from the dye was observed. Fluorescence...
enhancement was studied with the N-methyl-azadioxatriangulenium chloride salt (Me-ADOTA.Cl) in PVA films made from 0.2 % PVA (w/v) solution spin-coated on a clean glass coverslip. The Plasmonic Platforms (PP) was assembled by pressing together silver fractals on one glass slide and a separate glass coverslip spin-coated with a uniform Me-ADOTA.Cl in PVA film. In addition, we also tested the ADOTA labeled human serum albumin (HSA) deposited on a glass slide for potential PP bioassay applications. Using the new PP, we could achieve more than 20-fold fluorescence enhancement (bright spots) accompanied by decrease in fluorescence lifetime. The experimental results were used to calculate the extinction (excitation) enhancement factor (GA) and fluorescence radiative rate enhancements factor (GF). No change in emission spectrum was observed for a dye with and without contact with fractals. Our studies indicate that this type of PP can be a convenient approach for constructing assays utilizing metal enhanced fluorescence (MEF) without the need for depositing the material directly on metal structures platforms.
A point of care real time PCR platform based on silicon technology

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Since its invention in the early 90s, real-time PCR (Polymerase Chain Reaction) has become an indispensable tool in many fields of molecular diagnostics, including determination of viral or bacterial loads in clinical samples, identification and titer of germs in food, diagnosis of tumors, gene expression analysis, or forensic analyses. The key of its success is the ability to provide a quantitative result of target DNA sequence as it accumulates in the reaction solution (avoiding time-consuming post-PCR analysis) in a closed-system (reducing the risk of cross-contaminations) with enhanced accuracy and speed.

The miniaturization of real time PCR amplification systems are key to genetic point-of-care (POC) diagnostics to offer sample-in answer-out analysis. Actually, miniaturization typically enables shorter analysis times, reduces reagent consumption, minimizes risk of sample contamination, and often enhances assay performance. Often, it may result in inferior lower limit of detection due to smaller sample volume.

Here we present the genetic Real Time PCR PoC system based on micro machined silicon microchip able to address the quantitative and qualitative identification of multiple analytes of specific nucleic acids (DNA, RNA) sequences. Thanks to the integrated silicon temperature sensors and heaters, the chip allows a temperature control accuracy of ± 0.2 °C, heating rate of 15 °C/s and cooling rate of 8 °C/s. Additionally, a specific chip design enhances the optical fluorescent signal in combination with a smart detection software, so that an improvement of sensitivity of Log is achieved respect to the commercial tools.

The use of reverse iontophoresis based surface plasmon resonance for the development of a noninvasive real time transdermal biomarker sensor

Niraj K. Gupta, Yongsoon Hwang, Brent D. Cameron, The Univ. of Toledo (United States)

Recent developments in the identification of biomarkers offer a potential means to facilitate early disease detection, gauge treatment in drug therapy clinical trials, and to assess the impact of fatigue and/or stress as related to human physical and cognitive performance. For practical implementation, however, real-time sensing and quantification of such physiological biomarkers is preferred. Some key aspects in this process are continuous sample collection and real time detection. Traditionally, blood is considered the gold standard for samples but frequent phlebotomy is painful and inconvenient. Other sources like saliva and passive sweat cannot be precisely controlled and are affected by other limitations. Some of these can be addressed by reverse iontophoresis which is a noninvasive technique capable of facilitating controlled transport of biomolecules up to 20kDa in size across the skin barrier by passing a low level current between two dermal electrodes. The samples collected at the electrode site can be then be monitored at site or transported via a microfluidic channel towards a sensor. In the case reported here, the sensor is based on surface plasmon resonance (SPR), which is a label free, real time, and highly sensitive optical sensing technique. The real time SPR detection of targeted biomarkers is then achieved through the use of aptamer surface modification. In this experiment, extraction, detection and determination of orexin A, a stress related biomarker, is used for demonstration purposes.

Fluorescent detection of C - reactive protein from blood plasma on a 3D-printed device

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Bacterial infection and related toxins causes Sepsis which is the leading cause of mortality in hospitals. This infection can be quantified from blood plasma using C - reactive protein (CRP). Quantifying requires a 2-step process – removing interfering blood cells from the plasma and then capturing the protein of interest from the cell-free plasma. Researchers have previously reported on the use of 3D printing to isolate bacteria from a solution and then used complex magnetic fields to capture and image them. In this paper, the development and testing of a blood plasma protein detection kit consisting of 3D printed bead capture module is described.

Large (1.5 mm diameter) nylon beads were physically trapped in wells along a fluidic channel inside the bead capture module. The beads absorb Sepsis antigen (CRP) present in the blood plasma. Further addition of Primary (Monoclonal Anti-CRP G2) and Secondary Antibody tagged with fluorescent...
Point-of-care porphyria screening by fluorescence spectroscopy of blood plasma

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Porphyrias are rare genetic metabolic disorders that result from deficiencies of enzymes in the heme biosynthetic pathway. Each type of porphyria produces distinctive patterns of accumulations of intermediate compounds that may be found in erythrocytes, blood plasma, urine, and stool. Porphyrias can be classified as acute hepatic, hepatic cutaneous and erythropoietic cutaneous diseases. Acute hepatic porphyrias can lead to acute neurovisceral attacks, typically with abdominal pain, and may be fatal if diagnosis is delayed. Diagnosis requires a rapid screening test to allow for prompt identification and treatment of these patients. In this study, we compared an inexpensive custom-made fluorometer with a commercial fluorometer for porphyria detection from human blood plasma and blood plasma phantoms. In addition, we evaluated fluorescence spectroscopic methods to rapidly identify different types of porphyria.

We found that an inexpensive cuvette-based fluorometer with a 405nm laser diode for fluorescence excitation and a miniature CCD spectrometer (USB2000+, OceanOptics) could distinguish blood plasma from porphyria patients and healthy subjects. Blood plasma phantoms containing human serum albumin with added protoporphyrin-IX, coproporphyrin-III, and uroporphyrin-III, were used to compare different methods to distinguish the porphyrins spectroscopically. The spectral features of coproporphyrin and uroporphyrin were virtually identical but we found that acidification of the blood plasma phantoms with pH<2.0 induced a spectral shift in the excitation maxima of these porphyrins. Dual-wavelength excitation could then distinguish coproporphyrin and uroporphyrin.

In conclusion, the custom-made fluorometer potentially could provide a means for point-of-care screening for porphyria. In addition, methods to spectroscopically differentiate porphyrinas were evaluated.

Diffractive interference optical analyzer (DiOPTER)

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This report demonstrates a novel method for high-resolution refractometric measurements using, what we have termed as, a Diffractive Interference Optical Analyzer (DiOPTER). The setup consists of a laser, polarizer, a photo-lithographically patterned diffraction grating and Si-photodetectors.

The sensor is based on the differential response of diffracted orders to bulk as well as surface refractive index changes. The differential read-out suppresses signal drifts and enables time-resolved determination of refractive index changes in the sample cell. A remarkable feature of this device is that under appropriate conditions, the measurement sensitivity of the sensor can be enhanced by more than two orders of magnitude due to interference between multiply reflected diffracted orders. A noise-equivalent limit of detection (LoD) of 6x10^-7 RIU was achieved with scope for further improvement. This detection methodology is experimentally straightforward and can be used across a wide array of applications, ranging from detecting changes in surface adsorbates via binding reactions to estimating refractive index (and hence concentration) variations in bulk samples. An exciting prospect of this technique is the potential integration of this device to smartphones using a simple interface based on transmission mode configuration. In a transmission configuration, using low-cost PDMS based sensors, we were able to achieve an LoD of 4x10^-4 RIU which is sufficient to explore several applications in food quality testing and related fields. We are envisioning the future of this platform as a personal handheld optical analyzer for applications ranging from environmental sensing to healthcare and quality testing of food products.
Return to Contents

Conference 9715: Optical Diagnostics and Sensing XVI: Toward Point-of-Care Diagnostics

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9715-10, Session 3
Surface enhanced Raman spectroscopy as a point-of-care diagnostic for infection in wound effluent

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One of the clinical challenges with combat-related injuries is the prevalence of bacterial infection. Current methods of identifying bacterial infection rely on culturing microbes from patient material and performing biochemical tests, which can take 2-3 days. Surface Enhanced Raman Spectroscopy (SERS) is a powerful vibrational spectroscopy technique that allows for highly sensitive detection of analytes adsorbed onto specially prepared metal surfaces. Here, we use SERS to assess the presence of bacteria in wound effluent samples taken directly from patients. Citrate reduced silver colloid solution was prepared and pipetted onto gold slides. Wound effluent was pipetted and dried onto each nanoparticle spot and SERS spectra were collected. SERS spectra were obtained for both uninfected and infected wound effluent samples, in which the infected samples comprised of 10 different species including Staphylococcus aureus, Enterococcus faecalis, Enterobacter cloacae and Acinetobacter species. In order to quantify the magnitude of signal enhancement, SERS spectra were compared to the Raman spectra of effluent samples. While little to no Raman signal was detected in the standard Raman spectra of the samples, SERS spectra showed clear bands, achieving up to 102 fold enhancement. This study demonstrates an important step towards developing SERS as a powerful and rapid tool for detecting bacterial infections in complex clinical samples. Future studies will include exploring methods of enhancing signal of bacteria in effluent with centrifugation and filtration based techniques along with developing and validating classification models for bacterial identification using SERS of effluent spectra.

9715-9, Session 2
Low-cost computing and network communication for a point-of-care device to perform a 3-part leukocyte differential

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Point-of-care approaches for 3-part leukocyte differentials (granulocyte, monocyte, and lymphocyte) are essential not only to reduce cost but to provide faster results in low resource areas. Traditionally, this differential is performed within a panel of tests, called a complete blood count (CBC), using a hematology analyzer which conventionally relies on flow cytometry, electrical impedance, or a combination of both. Recent developments in lab-on-a-chip devices have shown promise in reducing the size and reagents used, relating to a decrease in overall cost [1]. Furthermore, smartphone diagnostic approaches have shown much promise in the area of point-of-care diagnostics, but the relatively high per-unit cost may limit their utility in some settings [2]. We present here a method to reduce computing cost of a simple high-throughput epi-fluorescence imaging system using a Raspberry Pi (single-board computer, <$40) to perform a 3-part leukocyte differential comparable to results from a hematology analyzer. This system uses a USB color camera in conjunction with a leukocyte-selective vital dye (acridine orange) in order to determine leukocyte count and differential from a low volume (<40 microliters) of blood obtained via fingerstick. Additionally, the system utilizes a “cloud-based” approach to send image data from the Raspberry Pi to a main server and receive results back to the user, exporting

the bulk of the computational requirements.


9715-11, Session 3
Raman spectroscopy for predicting wound healing outcome: towards in vivo application

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Combat wounds are sometimes confounded by healing complications that are not as prevalent in civilian wounds due to their high energy etiology. Some complications of wound healing include: delayed healing, in which wound healing takes significantly longer than the average hospital stay; and dehiscence, where a surgically closed wound reopens after closure. It is necessary to develop techniques that can be used in vivo to assess and predict wound healing point-of-care so that clinicians can decide the best way to make informed clinical decisions regarding their patient’s healing. Raman spectroscopy is a perfect candidate for predicting wound
In this study, we examined an optimal lattice based on nanoimprint lithography using cyclo-olefin polymer to improve the sensitivity for measuring drug concentration in tear fluid. This is photonics crystal which is one of the nanophotonics based device was fabricated. Target is Sodium Phenobarbital which is an anticonvulsant agent. We show the effectiveness of Surface Enhanced Raman Spectroscopy of tear fluid with soft substrate for point-of-care therapeutic drug monitoring. For use in clinical practice, we challenge the further improvement of the sensitivity to drug solution down to 0.01mM for detecting a small amount of drug secreted in tear fluids.

9715-14, Session 4

Investigation of surface enhanced Raman spectroscopy for hemozoin detection in single malaria parasites of ring stage

Keren Chen, Aoli Xiong, Peter Preiser, Quan Liu, Nanyang Technological Univ. (Singapore)

Drugs developed for malaria treatment generally inhibit the formation of hemozoin and facilitate toxic free heme stacking to kill malaria parasites. Therefore, monitoring hemozoin within malaria parasites is important in malaria drug development. Raman spectroscopy has been used to detect hemozoin crystals within vacuoles of parasites in the trophozoite stage.

However, hemozoin detection from parasites in the ring stage is much more challenging due to the small amount of hemozoin. Surface enhanced Raman spectroscopy (SERS) has been explored to detect hemozoin in the ring stage in the literature, in which aggregated hemozoin from the entire sample, not hemozoin in a single parasite was detected. In this study, we will review the development of multiple SERS techniques for malaria diagnosis that our group has developed in the past a few years. Then we will report a new method of SERS for hemozoin detection in single Plasmodium falciparum in the ring stage. In this method, silver nanoparticles were synthesized within the parasites after the lysis of erythrocytes’ membranes. The Raman spectra of hemozoin were acquired from parasites with silver nanoparticles synthesized inside and those without for comparison. The results confirmed the feasibility of detecting hemozoin crystals within single parasites in the ring stage using our method. This method can have significant impact in drug development.

9715-15, Session 4

An embedded point-of-care malaria screening device for low-resource regions

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In this article we propose a point-of-care screening device for the detection and identification of malaria parasite, Plasmodium vivax, Plasmodium malaria, Plasmodium oval and Plasmodium falciparum with a time frame of 15-20 minute. In our device we can provide 97-98% sensitivity for each species as we are using traditional staining methods for detecting the parasites. In addition, as we are also quantifying the parasites, it is possible to provide an accurate estimate about the malarial stage of the patient.

The image processing approach increases the total numbers of samples screened by reducing interventions of trained pathologists. This helps in reducing the delays in screening process arising from increased number of potential cases based on seasonal and local variations. The same reduces...
9715-16, Session 4
Whole-animal imaging of bacterial infection using endoscopic excitation of ?-lactamase (BlaC)-specific fluorogenic probe

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Tuberculosis, caused by Mycobacterium tuberculosis (Mtb), remains one of the most frequent causes of death worldwide. The slow growth rate of Mtb limits progress toward understanding tuberculosis including diagnosis of infections and evaluating therapeutic efficacy. Development of near-infrared (NIR) ?-lactamase (BlaC)-specific fluorogenic substrate has made a significant breakthrough in the whole-animal imaging to detect Mtb infection. The reporter enzyme fluorescence (REF) system using a BlaC-specific fluorogenic substrate has improved the detection sensitivity in whole-animal optical imaging down to ~100 colony forming units (CFU) of bacteria, about 100-fold improvement over recombinant strains. However, improvement of detection sensitivity is strongly needed for clinical diagnosis of early stage infection at greater tissue depth. In order to improve detection sensitivity, we have integrated a fiber-based microendoscope into a whole-animal imaging system to transmit the excitation light from the fiber bundle to the fluorescent target directly and measure fluorescent level using BlaC-specific REF substrate in the mouse lung. REF substrate, CNIR800, was delivered via aerosol route to the pulmonary infected mice with M. bovis BCG strain at 24 hours post-infection and groups of mice were imaged at 1-4 days post-administration of the substrate using the integrated imaging system. We observed as low as ~100 CFU of bacteria in the mouse lung using our integrated imaging system with BlaC-specific substrate. Integration of these technologies has great promise for improved detection sensitivity allowing pre-clinical imaging for evaluation of new therapeutic agents.

9715-17, Session 4
Non-invasive detection of iron deficiency by fluorescence spectroscopic quantitation of erythrocyte zinc protoporphyrin in the lower lip

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Iron deficiency affects more individuals than any other health problem, especially children, women of childbearing age and pregnant women, in both developed and developing countries. At present, detection of iron deficiency requires obtaining a blood sample and subsequent laboratory analysis, limiting the availability of screening in resource-limited settings. We aim to use optical fiber probe fluorescence spectroscopy of the lower lip to measure the erythrocyte zinc protoporphyrin (eZnPP)/heme ratio, an established indicator of iron deficiency, without the need for a blood sample. Fluorescence spectroscopic measurements were carried out on women after childbirth at the Perinatal Center, Klinikum der Universität München. This patient group was chosen because during pregnancy, women often become iron deficient with an elevated eZnPP/heme ratio. A custom made fiber-based fluorescence spectrometer with 425nm and 407nm excitation was used to acquire tissue fluorescence spectra in about one minute. From the same subjects, residual blood was used to quantify the eZnPP using a reference HPLC method, as well as other iron deficiency indicators. We found that dual-wavelength excitation allows for elimination of more than 90% of the tissue fluorescence background. Spectral fitting is then applied to quantitatively extract the ZnPP fluorescence from the remaining background. For measurements where a sufficient amount of blood was present in the probed tissue volume, a close correlation with the reference HPLC measurements was found. This fluorescence method potentially allows for non-invasive, point-of-care screening for iron deficiency in resource-limited settings.
magnitude smaller than current automated instruments. This method uses less than 1 microliter of blood, and less than 5 microliters of body fluids to make its measurements, making it highly compatible with finger-stick style collections, as well as appropriate for small animals such as laboratory mice where larger volume blood collections are dangerous to the animal’s health.

9715-37, Session PMon

Thermography: a potential tool in detecting exercise induced muscle damage (EIMD)
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Exercise induced muscle damage (EIMD), is usually experienced in humans that have been physically inactive for prolonged periods of time, and begin with sudden training trials, or can also occur in athletes who train over their normal limits. EIMD has gained a considerable amount of interest amongst researchers and scientists in exercise physiology, sports, and rehabilitation fields and there have been various published studies exploring this painful phenomenon in respect to its principal mechanisms. Nonetheless, it is not an easy condition to quantify, as there is a wide amount of variability between the measurement tools and methods that can be used. Thermography has been used successfully as a research detection tool in medicine for the last 6 decades. Nevertheless, very limited work has been reported on EIMD and changes in skin temperature. The main purpose of this research was to assess changes in EIMD using this safe and non-invasive advanced imaging technique. For this reason, thermographic images of the rectus femoris muscle were taken before, as well as immediately post and 24, 48 and 72 hours after an acute bout of eccentric exercise (5 sets of 15 maximum repetitions), on males and females (20-30 year old) that participated in the study. Eccentric exercise was performed with the one lower extremity, whereas the other lower extremity served as control. The results showed that DOMS and creatine kinase increased significantly 24 hours and remained elevated up to 72 hours post-exercise. Torque decreased significantly 24 hours and remained elevated up to 72 hours post-exercise. Changes in Temperature were also recorded post exercise.

9715-39, Session PMon

Long range non-contact imaging photoplethysmography
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Non-contact, imaging photoplethysmography uses photo-optical sensors to measure variations in light absorption caused by blood volume pulsations to assess cardiopulmonary parameters including pulse rate, pulse rate variability, and respiration rate. Recently, researchers have studied the applications and methodology of imaging photoplethysmography. Basic research has examined some of the variables affecting the data quality and accuracy of imaging photoplethysmography including: signal processing, imager parameters (e.g. frame rate and resolution), lighting conditions, subject motion, and subject skin tone. This technology may be beneficial for long term or continuous monitoring, where contact measurements may be harmful (e.g. skin sensitivities), or where covert or unobtrusive measurements are desirable. Using previously validated signal processing methods, we examined the effects of imager to subject distance on one-minute, windowed estimates of pulse rate. High resolution video was collected using an enthusiast-grade, mirrorless, digital camera equipped with a fully manual, super-telephoto lens at distances of 25, 50, and 100 meters from stationary subjects with simultaneous contact measurements of electrocardiography, photoplethysmography, and respiratory effort. By comparison, previous studies have usually been conducted with imager to subject distances only up to a few meters. At distances of 25, 50, and 100 meters, mean absolute error for one-minute, windowed, pulse rate estimates (compared to those derived from gold-standard electrocardiography) were 1.8, 1.5, and 4.0 beats per minute, respectively. It appears that imager to subject distance itself has relatively little effect on measurement accuracy, and image resolving power and quality image formation are most principally responsible for pulse rate measurement accuracy.

9715-40, Session PMon

Biomedical imaging with wearable smart eyeglasses
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Imaging of the eye’s fundus has been used for many diverse applications. In addition to eye’s pathology, it is possible to observe non-invasively its vascular system. Indirectly, they eye is a window to the brain. The fundus has been analyzed for detecting chemical poisoning (e.g. cyanide), hypertension and Alzheimer’s disease. Furthermore eyes movement is strongly related to the mind status and it has been used for studying attention and personal taste. Data for these applications have to be acquired continuously and without interfering with the user.

Here we present a system based on a Confocal Laser Scanning Ophthalmoscope (CSLO). A transflective hologram is used to separate the path of the scanning beam from the user’s field of view. The light is brought to the display by an optical fiber and the optical response by the fundus is also collected by an optical fiber.

For testing the proof of concept, the system has been realized as a table top set-up. Resolution and contrast have been analyzed and compared with commercial CSLO performance.

9715-41, Session PMon

Low-dose intrathecal fluorescein for diagnosis of cerebrospinal fluid rhinorrhea using the scanning fiber endoscope in the human nasal cavities
Vivian W. Hou, Calvin G. Davis, Greg E. Davis, Eric J. Seibel, Univ. of Washington (United States)

Intrathecal fluorescein (ITF) enhances detection of cerebrospinal fluid rhinorrhea (CSFR). Clinically administered doses fall in the range of 0.1ml to 0.5ml of 5% to 10% fluorescein (~10-3M to ~10-2M). Though uncommon, significant morbidities associated with high doses of fluorescein have been reported. High concentrations are necessary for white light visual assessment; in contrast, fluorescent imaging enhances signal contrast and requires lower ITF concentrations for visualization. The ultrathin and flexible, multimodal scanning fiber endoscope (SFE) can visualize nanomolar concentrations of fluorescein as pseudocolor over reflectance, video-rate imaging. The application of the SFE for CSFR detection was assessed in a cadaver study.

Briefly, 10µM (10-5M) fluorescein, 100X-1000X less than the standard clinical dose, was injected intra-cranially into the epidural space through an orbital roof puncture. The resulting rhinorrhea was assessed with a conventional, rigid ENT scope and second with the SFE in both full color reflectance and multimodal fluorescent imaging mode.

Neither system could visualize the 10µM ITF during white light imaging however the nanomolar sensitive SFE visualized the rhinorrhea during fluorescent imaging. Despite the low concentration used, a target-to-
background ratio of 5.6 ± 2.7 was achieved.

To demonstrate SFE guidance of CSFR detection and repair, de-identified patient computed tomography (CT) scans were used to generate 3D printed phantoms. Cases were selected for unique anatomical features and overall clinical difficulty as determined by an experienced ENT clinician (GED). The sensitivity and minimally invasive nature of the SFE provide a unique platform for enhancing diagnosis and monitoring interventions in surgical endoscopic approaches.

9715-42, Session PMon

Non-contact measurement of pulse wave velocity using RGB cameras

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Non-contact measurement of pulse wave velocity (PWV) using red, green, blue (RGB) digital color images is proposed. Generally, PWV is used as the index of arteriosclerosis.

The change in blood volume is based on the change in the color information and is estimated by combining multiple regression analysis (MRA) with a Monte Carlo simulation (MCS) model of the transit of light in human skin. At first, RGB values is transformed into Commission Internationale de l’Éclairage XYZ (CIE XYZ) tristimulus values using a first transform matrix that was calculated in advance. Next, skin chromophore concentrations – i.e., the melanin concentration, the oxygenated blood concentration, and deoxygenated blood concentration – represent the light absorption coefficients of the skin tissue. Thus, using various combinations of these three concentration values, diffuse reflectance spectra of the skin’s surface is calculated by use of MCS. Second transformation matrix is calculated by MRA, wherein the skin chromophore concentrations are the response variables and the XYZ values are the predictor variables. Using two matrices, RGB values can be transformed into blood volume (=the oxygenated blood concentration + deoxygenated blood concentration).

After two pulse waves in human skin is measured using RGB cameras, PWV is calculated by use of the pulse transit time, which is calculated from these waves, and the distance between two-measurement points.

9715-43, Session PMon

Dramatic reduction of tracer concentration in renal function monitoring through time-resolved fluorescence detection

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For evaluation of potential nephrotoxicity of newly developed drugs, preclinical measurements of renal function using the glomerular filtration rate (GFR) as indicator are highly relevant. Fluorescent markers (e.g. FITC-Sinistrin) can be used to achieve highly accurate and reliable values using percutaneous measurements with intelligent patches [1, 2]. However, high costs for the marker substance demand for a more sensitive detection technology.

We developed a fiber-based system that records a continuous photon stream for assessing the GFR. A lightweight measurement head is connected via an optical fiber to awake animals in order to minimize motion artifacts. Pulsed laser excitation combined with time-correlated single photon counting (TCSPC) allows for extracting information from the fluorescence decay pattern. This strategy is able to distinguish between background (mainly characterized by autofluorescence) and marker signal by applying statistical unmixing of measured decay curves. Remaining artifacts are removed by a pattern recognition approach [3]. Thereby, a sensitivity that is higher by three orders of magnitude than compared to the intelligent patch method is achieved, while being able to completely eliminate artifacts and background signal.

The high sensitivity of this new approach may become especially interesting for long term observations, in which the marker dose applied becomes the major cost factor.

9715-44, Session PMon

Performance testing of a mid-infrared spectroscopic system for clinical chemistry applications utilising an ultra-broadband tunable EC-QCL radiation source

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Mid-infrared (MIR) spectroscopy is a valuable analytical method for patient monitoring within point-of-care diagnostics. For implementation, quantum cascade lasers (QCL) appear to be most suited regarding miniaturization, complexity and eventually also costs. External cavity (EC) - QCLs offer broad tuning ranges and recently, ultra-broadly tunable systems covering a spectral range from 1920 to 780 cm-1 became commercially available. Using such a system, spectra were recorded with a 100 µm pathlength cell and glucose concentrations between 50 and 100 mg/dL using thermoelectrically cooled MCT-detectors. Exploratory factor analysis with tools from the domain of pattern recognition (PCA, LDA, matrix rotation) was used, providing four significantly contributing factors. The glucose signal only ranks third when regarding its contribution to the total variance. Short-term drift possibly related to thermal effects causes most of the variance, even though running the laser with only half of its maximum duty cycle of 5%. Long-term drift due to varying water vapour absorption lines causes twice as much variance as the glucose signal. Based on the a-posteriori obtained scores, an estimate of the achievable noise equivalent glucose concentration of 6.6 mg/dl was obtained under the given conditions.

Furthermore, an optimization with parameters such as laser pulse width, duty cycle and additional beam attenuation was carried out using cells of either 50 µm, 100 or 200 µm pathlength for recording an interval of 1800 to 950 cm-1 or of 1200 to 1000 cm-1 for glucose monitoring only. Dependent on the absorbance noise, detection limits for aqueous glucose have been estimated.
Raman spectroscopy is one of the most reviewed and studied technologies for non-invasive glucose sensing. Our research objective is to implement a complete Raman spectrometer on a single chip. This will significantly reduce the cost of continuous glucose monitoring and could finally enable an all-optical system.

The core component of the Raman system is the miniaturized spectral separation module. This module needs to be integrated with other components, such as lasers, filters and detectors. Based on these considerations, an arrayed waveguide grating (AWG) was chosen as an ideal solution for spectral separation.

In this paper, we investigate the feasibility of using an AWG as the optical dispersive component for in-vivo skin Raman spectroscopy. We simulated the structure optically and electrically, then fabricated and tested it. In the simulation, an AWG with operating range from 850nm to 880nm and resolution of 1nm was achieved. Based on the simulation results, we developed a fabrication process with oxide and oxynitride as the core and oxide as cladding materials. To select the best combination of waveguide width and depth, a testchip with multiple structures was designed. To integrate photodiodes on the chip as detectors, an unconventional waveguide formation method was adopted. In this approach, trenches were formed by etching the silicon wafer to define the waveguide channel, with oxide and oxynitride subsequently deposited. The manufacturing process, including trench filling, chemical-mechanical polishing and photodiode fabrication, is also discussed. After fabrication, we tested the various testchip structures to verify the simulations and correlate them to actual results.

9715-46, Session PMon
An optical fiber probe based on quantum-dots integrated cavity fabricated by femtosecond laser micromachining for single cell level temperature measurement
Qi Zhang, Lei Yuan, Clemson Univ. (United States); Jie Huang, Missouri Univ. of Science and Technology (United States); Hai Xiao, Clemson Univ. (United States)

Localized and real-time intracellular temperature sensing remains an issue for the optical sensing research area. Small size (less than 10 μm), high sensitivity and stability, invasive or cell-immunity, and minimized cross-talk with other parameters (e.g., pH, toxicity, concentration of surrounding medium, etc.) are highly needed for the intracellular temperature sensors. However, current available sensors are difficult to meet the requirements especially when applying to a single cell where most of the temperature sensors may be degraded making it almost impossible to be used as a long-term temperature sensor for cells undergoing different treatment conditions. In recent years, optical fiber based temperature sensors have attracted more and more interests attributed to their small size, light weight, remote control, non-toxicity, and immunity to cells and electromagnetic interference.

In this paper, we report a miniaturized optical fiber probe based on quantum dots integrated micro-cavity for single cell level temperature measurement. The micro-cavity is fabricated by femtosecond laser micromachining and subsequently tapering to the size of around 10 μm, in which quantum dots liquid is then filled via a syringe pump. The micro-cavity is then sealed with UV curable glue. By analyzing the back-reflected fluorescence signals generated from the quantum dots, the localized temperature of the micro-tip structure could be correlated. Experimental results of the proposed device will be presented and analyzed. Its novel characterizations of high spatial resolution, fast response, good repeatability and stability makes this temperature probe a very cost-effective tool for chemical/biological sensing, especially in the single cell level area.

9715-47, Session PMon
Single chip GMR-filter based self emission glucose monitoring system
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In this research, we designed an array of bandpass filters as a spectral separator for mid-infrared self-emission noninvasive glucose monitoring. The filters were based on the guided-mode resonance (GMR) effect. The human body is a good black body radiator that provides a stable temperature and continuous radiation energy in the mid-infrared range. Therefore, we use self-emission from human body to measure certain fingerprint peaks of glucose spectrum between 8 μm to 10 μm, which allows estimation of glucose concentration. The guided-mode resonance filter set includes at least four filters on one chip fabricated at the same time. With fixed thickness and material of the thin-film of the chip, a structure period adjustment can achieve multiple bandpass filters between the glucose fingerprint ranges and achieve these coplanar filters on a single chip. By using all CMOS-compatible materials, a COMSOL simulation result shows that a series peaks with 50% - 70% transmittances and 200nm bandwidths can be achieved. This filter set can be fabricated with just a few thin layers that can simplify the typical thin-film deposition process. The CMOS compatible single-chip fabrication process can reduce costs up to 300 times compared to commercially available discrete bandpass filters. We then use the filters to demonstrate noninvasive glucose monitoring, both in a model and in vivo. In this approach, we combine multiple bandpass filters with a thermopile to measure human body self-emission. The multiple individual bandpass filters can be replaced by our single-chip filter set and potentially make an all-optical glucose detection system feasible.

9715-48, Session PMon
Raman-spectroscopy based multiplexed detection of alternate glycemic marker panel
Rishikesh Pandey, Nicolas Spegazzini, Niyom Lue, Luis Galindo, Massachusetts Institute of Technology (United States); Ishan Barman, Johns Hopkins Univ. (United States); Gary L. Horowitz, Beth Israel Deaconess Medical Ctr. (United States) and Harvard Medical School (United States); Ramachandra R. Dasari, Peter T. C. So, Massachusetts Institute of Technology (United States)

Long-term glycemic markers notably glycated hemoglobin and glycated albumin reflect retrospective indices of integrated glucose value in the blood stream over the time span being unique to the biomarker. The secondary diabetic complications correlate more strongly with the elevated concentration of these biomarkers than the commonly used measurement of fasting glucose. Additionally, these biomarkers have gained traction in recent years for the prescreening of diabetic. While HbA1c measurement is affected by the conditions that alter erythrocyte survival, glycated albumin can be influenced by factors that affect albumin turnover. The combined determination of HbA1c and glycated albumin will provide a uniquely powerful metric in estimating the “true” glycemic history of a patient - a feature that is currently lacking in almost all clinical laboratories globally.

In this talk, we present the first demonstration of a Raman spectroscopy based novel approach to simultaneously detect and quantify glycemic marker panel in whole blood. We believe that this sensing approach can provide a powerful tool for simultaneous determination of this panel of biomarkers in routine clinical diagnostics in the future. We envision that the substantive advantages of Raman spectroscopy in terms of molecular specificity and its ability to couple to a fiber-probe for in vivo label-free acquisition will enable its ready translation to a point-of-care setting.
9715-49, Session PMon

**Comparison of production methods of a spiral inertial microfluidic cell separation device**

Mitchell Robinson, Haley L. Marks, Gerard L. Cote, Texas A&M Univ. (United States)

While centrifugation remains the gold standard for the removal of cellular components from blood plasma, a number of promising new lab-on-a-chip type devices have emerged in the last decade. In order to choose the most cost effective method with the fewest trade-offs in quality, we have tested and compared the effects of multiple production methodologies on the separation efficiency of an inertial microfluidic blood filter for optically probing biomarkers. Channel master molds were produced by machine milling, using soft photolithography techniques and an SU-8 negative photosist on silicon wafers, and printing by a stereolithographic 3D printer. The microfluidic devices were cast out of polydimethylsiloxane (PDMS), chosen for its transparency. Diluted whole blood was pumped through a spiral microchannel consisting of five concentric loops with a cross section measuring 500 μm x 1000 μm. The absorbance of the outlet solutions were collected and compared for each design as measure of red blood cell separation efficiency. The soft lithography method produced the best separation results with ~80% of cells removed, followed by 3D printing and then machine milling. Precision of microchannel dimensions proved to be the most crucial factor to successful cell separation, and while 3D printed molds are currently limited by the resolution of their generating printer and machine milled designs by the size of machining bits, we believe as 3D printers improve and machining bits become smaller, the application of these methods in the production of complex microfluidic devices will increase to the level of traditional lithographic techniques.

9715-50, Session PMon

**Probing focal cortical dysplasia in formalin fixed samples using tissue optical spectroscopy**

Suresh Anand, European Lab. for Non-linear Spectroscopy (Italy); Riccardo Cicchi, Istituto Nazionale di Ottica (Italy) and European Lab. for Non-linear Spectroscopy (Italy); Flavio Giordano, Anna Maria Buccoliero, Valerio Conti, Renzo Guerrini, Azienda Ospedaliera Univ. Anna Meyer (Italy); Francesco S. Pavone, European Lab. for Non-linear Spectroscopy (Italy) and Istituto Nazionale di Ottica (Italy)

Focal cortical dysplasia (FCD) is one of most common causes of intractable epilepsy in pediatric population and these are often insensitive to anti-epileptic drugs. FCD is characterized by a disarray in localized regions of the cerebral cortex and abnormal neurons which results them to misfire with incorrect signals. Resective neurosurgery to remove or disconnect the affected parts from the rest of the brain seems to be a viable option to treat FCD. Before neurosurgery the subject could undergo imaging studies including magnetic resonance imaging (MRI) or computed tomography (CT) scans. On the downside CT could be elusive in MRI images and may be practically invisible in CT scans. Furthermore, unnecessary removal of normal tissues is to be taken into consideration as this could lead to neurological defects. In this context, optical spectroscopy have been widely investigated as an alternative technique for the detection of abnormal tissues in different organ sites. Disease progression is accompanied by a number of architectural, biochemical and morphological changes. These variations are reflected in the spectral intensity and line shape. Here, in this proof of concept study we propose to investigate the application of tissue optical spectroscopy based on fluorescence excitation at two wavelength 378 and 445 nm coupled along with Raman spectroscopy for the detection of FCD on surgically excised formalin fixed tissue specimens from pediatric subjects. For fluorescence at both the excitation wavelengths FCD showed a decreased intensity at longer wavelength when compared to normal tissues. Also, differences exist in the Raman spectral profiles of normal and FCD. Further results coupled along with multivariate algorithms like principal component analysis (PCA) will be presented further.

9715-51, Session PMon

**Application of spectroscopic techniques for the analysis of kidney stones: a pilot study**

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Identification and characterization of kidney stone remains one of the important analytical tasks in the medical field. Kidney stone is a common health complication throughout the world, which may cause severe pain, obstruction and infection of urinary tract, and can lead to complete renal damage. It commonly occurs in both sexes regardless of age. Kidney stones have different composition, although each stones have a major single characteristic component. A complete understanding of a sample properties and their function can only be feasible by utilizing elemental and molecular information simultaneously. Two laser based analytical techniques; Laser Induced Breakdown spectroscopy (LIBS) and Raman spectroscopy have been used to study different types of kidney stones from different patients. LIBS and Raman spectroscopy are highly complementary spectroscopic techniques, which provide elemental and molecular information of a sample. Q-switched Nd:YAG laser at 355 nm laser having energy 17mJ per pulse at 10 Hz repetition rate was used for getting LIBS spectra. Raman measurements were carried out using a home assembled micro-Raman spectrometer. Using the recorded Raman spectra of kidney stones, we were able to differentiate different kinds of kidney stones. LIBS spectra of the same stones are showing the evidence of C, Ca, H, and O and also suggest the presence of certain pigments.

9715-52, Session PMon

**Microvascular contrast enhancement in optical coherence tomography using microbubbles**

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Exogenous contrast agents in optical coherence tomography (OCT) have been shown to improve detection and quantification of microcirculation. However, they are limited by their potential biological side effects. Micrometer-sized microbubbles, currently utilized as a contrast agent in diagnostic ultrasound, are investigated in this study as intravascular OCT contrast agents. Agar+Intralipid tissue phantoms with two embedded microtubes were fabricated to model blood flow via a double syringe pump. One microtube was filled with human blood, the other with a mixture of human blood and microbubbles. Swept-source OCT structural and speckle variance images were evaluated for flowing and stationary modes in both microtubes. OCT speckle decorrelation times, averaged over the microtube’s lumen, were estimated for different concentrations of microbubbles and blood flow velocities. 3D microcirculation maps were acquired to quantify the difference in speckle variance contrast in the absence and presence of microbubbles in blood.OCT signal intensity in the microtube’s lumen increased in the presence of microbubbles with the enhancement dependent on blood flow velocity. Faster decorrelation times were detected for the mixture of blood and microbubbles compared to only blood-only scenarios. Consequently, speckle variance contrast significantly increased compared to structural
contrast in the presence of microbubbles at the same blood flow rates. The potential for using microbubbles for tissue hemodynamic investigations and for microvasculature contrast enhancement in OCT angiography is thus demonstrated.

9715-53, Session PMon
Inter-and intraindividual differences in skin hydration and surface lipids measured with mid-infrared spectroscopy
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The optimal balance between skin hydration and surface lipids are important factors in the appearance and function of the skin [1]. A disruption of the balance between these two leads to a dermatologic condition known as “dry skin” that is observed in patients with Atopic Dermatitis [2-3]. The epidermal hydration in connection with skin surface lipids (hydro-lipid film) measured with conventional biophysical methods such as Corneometer and Sebumeter show significant differences between the dry skin of patients with Atopic Dermatitis and with the skin of healthy subjects [2]. From a cosmetic perspective, sufficient amount of skin hydration and sebum makes the skin appear smooth, soft and supple whereas lack of moisture can cause the skin to look dull and cracked, appearing older. Quantitative and simultaneous determination of these two biophysical parameters enable the clinicians to classify the skin types into Normal skin (N), Dry skin (D), Oily Skin (O), Oily-Hydrated skin (OH) and oily-dry skin (OD) and can provide personalized skin treatment solutions.

In spite of many technological developments throughout the years, until now no non-contact devices and methods have been reported for the quantitative and simultaneous measurement of skin hydration and sebum levels. To facilitate quantitative and simultaneous measurements of these two biophysical parameters, we have built an Infrared optical spectroscopic set-up in a spectral band around 1720 nm corresponding to the lipid vibrational bands that lie “in between” the prominent water absorption bands. Using this set-up, we have measured the inter-and intraindividual variations in skin hydration and sebum levels and its variations to external stimuli. Finally we map them onto a two-dimensional hydration-Sebum map to classify them into different skin types. Good correlation between experimental results and reference measurements using Corneometer and Sebumeter were found.

9715-54, Session PMon
A disposable, flexible skin patch for clinical optical perfusion monitoring at multiple depths
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Stable, relative localization of source and detection fibers is necessary for clinical implementation of quantitative optical perfusion monitoring methods such as diffuse correlation spectroscopy (DCS) and diffuse reflectance spectroscopy (DRS). A flexible and compact device design is presented as a platform for simultaneous monitoring of perfusion at a range of depths, enabled by precise location of optical fibers in a robust and secure adhesive patch. We will discuss preliminary data collected on human subjects in a lower body negative pressure model for hypovolemic shock. These data indicate that this method provides a lower noise floor for the collection of DCS data than previous, less flexible methods of fiber co-location, and facilitate simultaneous monitoring of perfusion at multiple depths and within multiple physiological compartments.

9715-19, Session 5
Assessment of phantom replicated cochlear blood flow changes with laser speckle system
Sungkon Yu, Jihoon Park, Myungjin Ha, Sangyeob Lee, Seulgi Jang, Edalat Radfar, Byungjo Jung, Yonsei Univ. (Korea, Republic of)

The purpose of this study is measurement of blood perfusion behind a phantom which was replicated bone tissue using laser speckle system. Laser speckle imaging has been developed as a non-invasive technique and widely used to study of blood flow. Recently researches suggested that the change of cochlear blood flow was known auditory ability problem. Cochlear blood flow occurred in a cochlear apex which is the diameter of 2mm and the part of the internal structure of the temporal bone. To determine the possibility of the measurement of cochlear blood flow existing the opposite side of bone tissue, using laser speckle flowmetry. Laser speckle imaging was calculated by 5x5 window to obtain laser speckle contrast index. Through variation in the total mean of index value in real time, perfusion was observed. The phantom for modeling the cochlear apex was made of silicon. Silicon was suitable for applying spin-coating technique developed in our laboratory for making the diameter of 2mm. In order to replicate blood perfusion, milk was poured into tube which was the diameter of 330um. Concern has been raised regarding the obstruction to get correct optical signal by optical properties of hard tissue. Our result demonstrated that the laser speckle imaging can detect the flow that take place behind phantom mimicking bone tissue.

9715-20, Session 5
A compact instrument to measure perfusion of vasculature in transplanted maxillofacial free flaps
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Complex facial reconstructive procedures that involve microvascular free transfer of composite tissue (bone, periosteum, skin, and muscle) involve anastomosis of recipient site blood vessels to the grafted tissue. Vascularization and resulting perfusion of transferred tissues are critical to the success of the free graft. Failure to detect perfusion of the graft after anastomosis can rapidly progress to total flap failure in the absence of intervention. To enable rapid clinical assessment of adequate tissue perfusion, we are developing an implantable instrument for the continuous monitoring of perfusion using diffuse correlation spectroscopy (DCS), and augmented with diffuse reflectance spectroscopy (DRS). This work
discusses instrument design and construction, including the implementation of a microprocessor-based correlator. We will also discuss verification experiments on tissue phantoms and porcine-implant flow occlusion studies. To assess DRS performance, we built optical phantoms incorporating polystyrene microspheres and hemoglobin to mimic the optical scattering and absorption properties of biological tissue. The DRS system acquired diffuse reflectance from phantoms with high SNR (-50) in less than 0.5 seconds.

9715-21, Session 5
Assessment of sacroccygeal pressure ulcers using diffuse correlation spectroscopy
David Diaz, Drexel Univ. (United States); Michael T. Neidrauer, Drexel Univ. (United States); Michael S. Weingarten, Drexel Univ. College of Medicine (United States); Alec Lafontant, Drexel Univ. (United States); Rose Ann DiMaria Ghaliili, Drexel Univ. (United States); Guy W. Fried, Magee Rehabilitation Hospital (United States); Peter A. Lewin, Leonid A. Zubkov, Drexel Univ. (United States)

Microcirculation is essential for proper supply of oxygen and nutritive substances to the biological tissue and the removal of waste products of metabolism. The determination of microcirculatory blood flow (mBF) is therefore of great interest to clinicians for assessing tissue health; particularly in pressure ulceration and suspected deep tissue injury. A clinical study is currently ongoing to assess deep-tissue pressure ulceration by non-invasively measuring mBF at depths of up to 1 cm using Diffuse Correlation Spectroscopy (DCS). DCS provides information about the rate of red cells in the capillary network by measuring the temporal correlation function of scattering light intensity (TCFI). A novel optical probe was developed in order to obtain measurements under the load of the subject’s body as pressure is applied (ischemia) and then released (reperfusion) on sacroccygeal tissue in a hospital bed. Prior to loading measurements, baseline readings of the sacral region are obtained by measuring the subjects in a sidely position. DCS measurements from the sacral region of twenty healthy volunteers have been compared to those of two medical patients who initially had similar non-blanchable redness. The TCFI of the patient whose redness later disappeared is similar to that of the average healthy subject. The second patient, whose redness developed into an advanced pressure ulcer two weeks later, has a drastic decrease in blood flow while under the loading position compared to healthy subjects. Preliminary results suggest the developed system may potentially predict whether non-blanchable redness will manifest as advanced ulceration or dissipate over time.

9715-22, Session 5
In vivo quantification with OCT of vascular changes in humans in response to heating
Rodney W. Kirk, Peijun Gong, Shaghayegh Es’haghian, Howard H. Carter, Ceri L. Atkinson, David D. Sampson, Daniel J. Green, Robert A. McLaughlin, The Univ. of Western Australia (Australia)

Optical coherence tomography (OCT) is an important tool for imaging the skin microvasculature. Cutaneous microvessels play a key thermoregulatory role in humans, helping to maintain core body temperature, blood pressure and cardiovascular homeostasis. During heating, vasodilation of the skin arterioles allows an increase in blood flow through the capillaries, thereby enabling more heat to be transferred via conduction, convection and evaporation. However, there is great individual variation in responses. Conditions such as obesity and diabetes can impair thermoregulation, whilst exercise training enhances function and cardiovascular integrity. We used OCT to investigate the changes that occur in the microvasculature during heating, comparing the response from exercise-trained athletes with untrained participants. The core temperature of participants was elevated via leg heating and forearm scans were acquired, both before and during heating. Blood vessels were segmented from OCT images using a speckle decorrelation algorithm. In tissue without bulk movement, areas of high decorrelation indicate blood flow and hence the location of blood vessels. The microvasculature was then quantified by calculating the average vessel diameter and vasculature area density. Through carefully co-located scans, we were able to identify changes in the microvasculature in response to heating, showing increases in both size and density of blood vessels, and relating these to other physiological measures. We believe this is the first time that OCT has been used to assess and quantify the thermoregulation system in humans.

9715-23, Session 5
Assessment of multi-wavelength pulse photometry for non-invasive dose estimation of circulating drugs and nanoparticles
Pratik Adhikari, Louisiana Tech Univ. (United States); Wakako M. Eklund, Louisiana Tech Univ. (United States) and Pediatrisk Medical Group of Tennessee, P.C. (United States); Eric Sherer, D. Patrick O’Neal, Louisiana Tech Univ. (United States)

The feasibility of multi-wavelength photoplethysmography for the real-time sensing of absorptive and scattering agents in pulsatile blood is discussed. The use of pulsatile signals extracted from trans-illumination of an accessible section of tissue allows us to calculate the concentration of the optically extinctive species in the pulsatile blood. This technology, initially used for pulse oximetry and dye densitometry, can be applied to monitor in vivo concentration and clearance of various absorptive species. Recently, our prototype has been used to monitor the concentration of therapeutic gold nanoparticles, antimarial quinine, antifungal amphotericin b, and indocyanine green (ICG). The assessment of the optical properties, device specifications, and signal quality for each compound are presented. We observe that this technology can be used to monitor numerous extinctive drug and nano-materials that present features in the 350-1100 nm range. The rationale for using this technology in a clinical setting would be to improve outcomes by real-time pharmaceutical feedback and/or control at point of care.

9715-24, Session 6
A wearable continuous-wave optical device for continuous monitoring during neoadjuvant chemotherapy infusions
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Early tumor response monitoring in patients receiving chemotherapy would allow for rapid changes in treatment regimens, potentially improving outcomes. We present here a wearable diffuse optical device aimed at investigating hemodynamic response of locally advanced breast cancer patients during a patient’s first neoadjuvant chemotherapy infusion. The imaging system design, calibration, and validation are described as well as the results from a proof-of-concept normal volunteer study. The optical infusion monitor is designed based on continuous-wave diffuse optical imaging technology. It consists of a flexible substrate that supports an array of surface-mount LED and photodiode pairs (i.e. optodes). Numerical simulations of diffusive light-tissue interactions using
Finite Element Modeling were performed to determine the geometry of optodes, the measurement sensitivity, the depth of measurement and the imaging resolution for expected changes in chromophore (oxy- and deoxyhemoglobin) concentrations for stage II and III breast tumors during cytotoxic and targeted chemotherapy. Prototype testing using silicone based tissue-simulation phantoms demonstrated high stability (±1% std. dev.), reconstructed image resolution (<8mm), and signal to noise ratio (>75dB). The accuracy of the wearable imaging system will be evaluated against a gold-standard frequency domain diffuse optical spectroscopy system. Results from a normal volunteer study of five female healthy subjects will be presented. For each volunteer, continuous measurement taking approximately five-hours will be conducted to evaluate normal hemodynamic fluctuations in breast tissue. Additionally, a cuff occlusion study will be performed to validate the functionality of the infusion monitor to measure fast dynamic changes in tissue oxygen saturation.

9715-25, Session 6
Multispectral imaging system for in vivo detection and therapeutic assessment of vulvar lichen sclerosis
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Vulvar lichen sclerosis (VLS) is a chronic, inflammatory and mucocutaneous disease of extragenital skin, which often goes undetected for years. The underlying cause is associated with the decrease of VEGF that reduces the blood oxygenation of vulva. Focused ultrasound has shown the encouraging clinical outcome in the treatment of VLS. However, few method is available for quantitative detection and therapeutic assessment of VLS. Clinician’s examination is subjective and may lead to misdiagnosis. Spectroscopy is a potentially effective method for noninvasive detection of VLS. In this paper, we studied the optical properties of vulvar tissue and developed a multispectral imaging system for quantitative assessment. First, a five-layered optical model was proposed for simulating diffuse reflectance characteristics of vulvar tissue. The multispectral wavelengths were optimized based on a second derivative method. Then a multispectral imaging system was developed for noninvasive detection of vulvar blood oxygenation. The system utilized a hyperspectral camera to collect the reflectance images of the entire vulva under Xenon lamp illumination. A wide gap second derivative reflectometric algorithm was used to reconstruct blood oxygenation distribution. Further image segmentation algorithm was developed to classify tissue into different types based on the different blood oxygenation of vulva. An IRB approved clinical trial was carried out to evaluate the clinical utility for VLS detection and for therapeutic assessment after the focused ultrasound treatment. Our pilot study has demonstrated the technical potential of using this multispectral imaging system for in vivo VLS detection and for outcome assessment of the focused ultrasound treatment.

9715-26, Session 6
A wearable, conformal bandage for noninvasive, two-dimensional imaging of skin oxygenation
Zongxi Li, Emmanuel Roussakis, Massachusetts General Hospital (United States); Emily Keeley, Massachusetts Institute of Technology (United States); Gabriela Apiou-Sbirlea, Massachusetts General Hospital (United States); Reginald Birngruber, Univ. zu Lübeck (Germany); Christene Huang, Conor L. Evans, Massachusetts General Hospital (United States)

The complex surface topology and soft mechanics of the skin poses a considerable challenge to the development of wearable, conformal sensors. As a results, current clinical assessments of healing-related skin parameters often rely on bulky and expensive optical systems that are difficult to deploy at the point of care. Here, using a rapid-drying, liquid bandage containing oxygen-sensing molecules, we created a wearable sensor bandage that conforms the surface geometry of skin and wounds, and provides two-dimensional maps of cutaneous oxygenation in a non-disruptive fashion. Custom oxygen sensing phosphors have been developed in house that are at least five times brighter than the commercial sensing molecules, enabling the visualization of oxygen concentration using a simple color camera or even by eye under ambient lighting conditions. The oxygen-sensing bandage has been applied to monitor tissue ischemia, graft integration, as well as the progression of burn in animal models. Recent studies have demonstrated its ability to track and quantify skin inflammation induced by complete Freund’s adjuvant in an in vivo porcine model.

9715-28, Session 7
Characterization of a multi-module tunable EC-QCL system for mid-infrared biofluid spectroscopy for hospital use and personalized diabetes technology
Herbert M. Heise, Thorsten Vahlssing, Fachhochschule Südwestfalen (Germany); Markus Grafen, Konstantinos Nalpantidis, Andreas Ostendorf, Ruhr-Univ. Bochum (Germany); Dieter Ihrig, Fachhochschule Südwestfalen (Germany)

Nowadays, sensing systems, especially for blood glucose monitoring, are important point-of-care devices for the hospital and personalized diabetes.
technology. FTIR-spectrometers have been successfully employed for the development of continuous bed-side monitoring systems designed in combination with micro-dialysis. In vivo applications for critically ill patients can be envisaged, especially as further analytes and parameters are accessible, which are of interest for intensive care such as lactate, urea, pCO2 and pH. For implementation in miniaturized portable systems, quantum cascade lasers (QCL) appear to be most suited. External cavity (EC) - QCLs promise broad tuning ranges, especially if the infrared fingerprint region has to be covered for quantitative multi-parameter analysis. An ultra-broadly tunable pulsed EC-QCL system covering a spectral range from 1920 to 780 cm⁻¹ (obtained with four “tuners”) has been characterised with regard to the spectral emission profile under different pulse durations and duty cycles. Wavenumber scale accuracy and stability have been tested using a high-resolution VERTEX 80v FTIR spectrometer. There are different options for QCL-operation, i.e. continuous tuning over a broad spectral interval or with programmed discrete wavenumbers. For the tuning ranges of the individual or combined tuners, different sparse multivariate calibration models were developed for, e.g. glucose and lactate based on dialysates of subcutaneous interstitial fluid and other parameters such as protein, cholesterol etc. derived from blood plasma spectra. Eventually, arrays of DBR-QCLs can be envisaged based on the optimum calibration models with discrete wavenumbers, intended for the clinical chemistry applications described.

9715-29, Session 7
A closed-loop dual modulation two wavelength polarimeter for glucose monitoring
Zhenfang Yu, Univ. of Electronic Science and Technology of China (China) and Texas A&M Univ. (United States); Casey W. Pirnstill, Gerard L. Cote, Texas A&M Univ. (United States)

Diabetes affects millions of people throughout the world. Current commercially available glucose monitoring devices require frequent patient blood draws leading to poor compliance. Polariometry for probing aqueous humor glucose concentrations has been shown as a potential method for ascertaining blood glucose concentrations noninvasively. One limiting factor for the realization of polarimetry is time-variant corneal birefringence due to motion. The presence of this birefringence confounds the optical activity due to glucose, which limits the approach from accurately predicting glucose levels in the aqueous humor. Previous polarimetric systems designed to overcome time-varying birefringence have used lasers that are powered with a constant DC voltage and polarization modulating devices such as Faraday rotators. This configuration requires two detectors for the two wavelength approach and the minimum achievable response speed of these prior systems was limited by the Faraday modulator frequency. In this report, we simultaneously utilize laser modulation and Faraday modulation for each wavelength to achieve near real-time close-loop polarimetry for glucose sensing in vitro. This approach allows for an increased response speed of the polarimeter. The new polarimetric design, setup, and in vitro glucose measurements will be presented demonstrating sensitivity and accuracy of the system at these higher frequencies. This approach can reduce noise and electromagnetic interference (EMI), improve the response speed, and hence improve the signal-to-noise ratio of the system in the presence of motion.

9715-30, Session 7
Glucose sensing through Fano resonances in mesoscale silica core-gold shell particles arrays
Francesca Pincella, Zhiwei Huang, National Univ. of Singapore (Singapore)

We report the development of a versatile, cheap and reusable plasmonic sensor able to detect glucose in the physiological concentration range by means of a simple label-free optical detection scheme. In order to achieve the aforementioned goal we applied a self-assembly deposition technique for the large-scale arraying of mesoscale gold nanoshell particles. Different from metallic nanospheres arrays, the localized surface plasmon resonances of gold nanoshells arrays extend in both the visible and near-infrared range, making them compatible with optical sensing in biological media. Furthermore, the optical response of mesoscale gold nanoshells arrays showed another remarkable characteristic, which is the presence of various Fano resonances, which have the advantage of enhancing the sensitivity of the plasmonic substrate to the external media thanks to their sharp features and increased spectral contrast. The plasmonic sensor was shown to have at isolated working range with a good linear response for large refractive index shifts, where a bulk refractive index sensitivity of 0.3%/RIU (RIU, refractive index units) was achieved experimentally. In addition, the plasmonic sensor could detect aqueous glucose solutions in the blood concentration range (0-25 mM), with a sensitivity of 0.24/M.

9715-31, Session 7
Single strand, fiber optic glucose and pH sensor
Krister Hammarling, Mid Sweden Univ. (Sweden); Dag R. Hjelme, Hogskolen i Sor-Trondelag (Norway) and Norwegian Univ. of Science and Technology (Norway)

Fiber optic blood glucose sensors based on phenyl boronic acid as glucose receptors are promising candidates for future continuous glucose monitoring, and several sensor platforms based on this technology is currently under development. However, one common challenge for all glucose sensor platforms under development is the inherent cross-sensitivity towards pH due to the pH-dependent binding coefficient of the glucose-boronic acid reaction. One possible solution to this issue is to integrate a pH-sensor and compensate for the pH cross-sensitivity. In this paper we evaluate the integration of two different pH-sensor technologies with an interferometric fiber optic glucose sensor based on stimuli-sensitive hydrogel. The two different pH sensors technologies evaluated are a) an inline interferometric sensor with a pH stimuli-sensitive hydrogel and b) a double fluorescent compound incorporated in the same polymer as the phenyl boronic acid. Both pH sensor systems a) and b) can be tailored so they work at a wavelength not disturbing the glucose sensor. The inline interferometer pH sensor is a Mach-Zehnder interferometer capable of sensing surrounding effective refractive index. Together with a pH stimuli-sensitive hydrogel made of 1.4-Butanediol Diacrylate and Piperazine one gets a very accurate pH sensor. The double fluorescent material has two distinct emission bands allowing ratiometric measurement resulting in accurate pH sensing insensitive to the excitation power.

9715-32, Session 7
Fresh calibration-free framework for continuous spectroscopic sensing of blood analytes: single prick glucose detection
Nicolas Spegazzini, Jeon Woong Kang, Rishikes Pandey, Massachusetts Institute of Technology (United States); Ishan Barman, Johns Hopkins Univ. (United States); Ramachandra R. Dasari, Peter T. C. So, Massachusetts Institute of Technology (United States)

An outstanding challenge in biophotonics research is to devise a method for continuous, non-invasive monitoring of blood analytes, which constitutes a significant component of critical care and point-of-care diagnostics. Vibrational spectroscopy potentially provides a powerful tool for simultaneous, quantitative and label-free measurement of
multiple analytes, due to its intrinsic biochemical specificity. Here, we propose a novel calibration framework that enables spectroscopy-based estimation of analyte information without necessitating extensive a priori concentration information. In a nutshell, a kinetic model of the investigated process provides a guide to the “missing” concentration portion of the ill-posed problem of concentration estimation. Using non-invasive blood glucose monitoring by Raman spectroscopy as an illustrative example. We demonstrate the efficacy of this novel approach in predicting glucose concentrations that match closely with the measured values in relation to those generated with conventional calibration methods. In this paradigm, the solution of the model of glucose variation in the human body also enables deeper insight into the physiological lag behavior in response to the initial glucose loading (stimulus) and may offer an alternate route at screening diabetic population. Using our newly developed algorithm, the glucose levels are compared with reference values, and we found that the prediction error is around 2-8% with a single finger stick maintaining clinically acceptable measurement accuracy for a period over one week.

9715-33, Session 8
Enhancing contrast and quantitation by spatial frequency domain fluorescence molecular imaging

Jessica Sun, Deep Hathi, Washington Univ. School of Medicine in St. Louis (United States); Haiying Zhou, Washington Univ School of Medicine in St Louis (United States); Monica Shokeen, Walter J. Akers, Washington Univ. School of Medicine in St. Louis (United States)

Diagnostic imaging modalities including CT, MRI and PET/SPECT enable non-invasive detection of primary and metastatic tumors throughout the body for staging of disease and pre-surgical planning. Unfortunately, these modalities require bulky instrumentation, use ionizing radiation or strong magnetic fields and have limited spatial and/or temporal resolution, limiting use in high-motion settings such as endoscopy or during oncologic surgery. These limitations have favored the development of real-time optical imaging systems for minimally invasive, non-contact imaging. Optical imaging using nonionizing light and targeted fluorescent molecular probes is a powerful technology for noninvasive detection of molecular signatures of cancer. Fluorescence imaging is scalable from nanometer resolution microscopy to deep tissue spectroscopy for biomedical diagnostics. Planar fluorescence reflectance imaging using digital camera technology and harmless excitation light can rapidly measure fluorescence signals over a large field of view. Moreover, tissue absorption and autofluorescence near infrared (NIR) region (700-900 nm) are minimal, increasing depth penetration and reducing background signal, respectively. Unfortunately, patient skin and peri-tumoral tissues are not uniform, varying by color and thickness and complicating subsurface fluorescence measurements. We present a novel strategy for rapid measurement of subsurface fluorescence measurement, spatial frequency domain fluorescence molecular imaging, to improve quantification of deep-seated fluorescence molecular probe accumulation. Utilizing structured illumination for fluorescence reflectance imaging rapidly provides correction of acquired fluorescence signals for both background autofluorescence and depth-dependent attenuation from overlying skin and subdermal tissues.

9715-34, Session 8
In vivo measurement of non-keratinized squamous epithelium using a spectroscopic microendoscope with multiple source-detector separations

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In the non-keratinized epithelia, dysplasia typically arises near the basement membrane and proliferates into the upper epithelial layers over time. We present a non-invasive, multimodal technique combining high-resolution fluorescence imaging and broadband sub-diffuse reflectance spectroscopy (sDRS) to monitor health at various tissue layers. This manuscript focuses on characterization of the sDRS modality, which contains two source-detector separations (SDSs) of 374 µm and 730 µm, so that it can be used to extract in vivo optical parameters from human oral mucosa at two tissue thicknesses. First, we present empirical lookup tables (LUTs) describing the relationship between reduced scattering (µs') and absorption coefficients (µa) and absolute reflectance. LUTs were shown to extract µs' and µa with accuracies of approximately 4% and 8%, respectively. We then present LUTs describing the relationship between µs', µa and sampling depth. Sampling depths range between 210-480 and 260-620 µm for the 374 and 730 µm SDSs, respectively. We then demonstrate the ability to extract in vivo µs', µa, hemoglobin concentration, bulk tissue oxygen saturation, scattering exponent, and sampling depth from the inner lip of thirteen healthy volunteers to elucidate the differences in the extracted optical parameters from each SDS (374 and 730 µm) within human oral mucosa.

9715-36, Session 8
Choice of spectroscopy method for tumor margin detection

Viacheslav Artyushenko, Andrey Bogomolov, art photonics GmbH (Germany); Hans-Peter Berlien, Evangelische Elisabeth Klinik (Germany); Franziska Schulte, Iskander Usenov, Ursula Zabarylo, art photonics GmbH (Germany)

Recent reports in the literature show that up to 30% of surgical procedures result in irradical (incomplete) tumor removal. The purpose of our work was to define tumor margins in-vivo using the optimal selection of the best spectroscopy method (or their combination), including Raman scattering, Mid IR-absorption, diffuse Vis/NIR-reflection and fluorescence. Comparison of all fiber spectroscopy methods was made with the normal and tumor tissue of the same organ (kidney, prostate, etc.) - using the same samples for all methods in short time (few hours). - to define the best one in sensitivity, specificity and accuracy. Looking for the future clinical applications all spectral systems or sensors must be used with flexible and tiny fiber probes – to be disposable or sterilizable. The most promising fiber solutions for cancer screening and tumor margin diagnostics will be compared, starting from the research spectroscopy systems used in a broad 0.3-16µm-range up to the most advanced fiber systems already used in clinics. Possible advantages of a multispectral tissue analysis will be discussed for selection of more sensitive, more specific and precise in-vivo detection of tumor margins. The optimal choice will enable to define future design of fiber sensors to detect tumor margins – with price and dimensions to be reduced compared research spectroscopy systems.
We find that HFD affected cardiac function throughout the lifecycles of the HFD (CLOCK-RNAi-HFD), with sample sizes 20, 26, 15 and 15, respectively. 

mutant Drosophila (CLOCK-RNAi), and CLOCK mutant Drosophila fed with Drosophila (WT), wild type Drosophila fed with HFD (WT-HFD), and WT.HFD as well as CLOCK-RNAi mutation can both lead to significant cardiac alterations in Drosophila, and HFD affected cardiac function more severely in CLOCK-RNAi mutants. Alterations in duty cycle and heart chamber size were also correlated with HFD and CLOCK-RNAi mutation, which further contributes to understanding obesity and gene related human heart disease.

Over 500,000 women per year in the United States drink during pregnancy, and 1 in 5 of this population also binge drink. As high as 20-50% of live-born children with prenatal alcohol exposure (PAE) present with congenital heart defects including outflow and valvuloseptal anomalies that can be life-threatening. Previously we established a model of PAE (modeling a single binge drinking episode) in the avian embryo and used optical coherence tomography (OCT) imaging to assay early-stage cardiac function/structure and late-stage cardiac defects. At early ages, alcohol/ethanol-exposed embryos had smaller cardiac cushions and increased retrograde flow. At late stages, they presented with gross morphological defects in the head and chest wall, and also exhibited smaller or abnormal atrio-ventricular (AV) valves, thinner interventricular septa (IVS), and smaller vessel diameters for the aortic trunk branches. In other animal models, the methyl donor betaine (found naturally in many foods such as wheat bran, quinoa, beets and spinach) ameliorates neurobehavioral deficits associated with PAE and the effects on heart structure are unknown. In our model of PAE, betaine supplementation led to a reduction in gross structural defects and appeared to protect against certain types of cardiac defects such as ventricular septal defects and abnormal AV valvar morphology. Furthermore, vessel diameters, IVS thicknesses and mural AV leaflet volumes were normalized while the septal AV leaflet volume was increased. These findings highlight the importance of betaine and potentially methylation levels in the prevention of PAE-related birth defects which could have significant implications for public health.

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Betaine supplementation reduces congenital defects after prenatal alcohol exposure

Ganga Karunamuni, Shi Gu, Yong Qiu Doughman, Megan M. Sheehan, Pei Ma, Lindsay M. Peterson, Case Western Reserve Univ. (United States); Kersti K. Linask, Univ. of South Florida (United States); Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States); Michiko Watanabe, Case Western Reserve Univ (United States)

Effect of high fat diet on heart development in wild-type and clock-mutant drosophila

Jing Men, Chao Zhou, Lehigh Univ. (United States)

Drosophila has orthologs of over 75% human disease genes. Research on structural and functional cardiac dysfunction induced by High-fat-diet (HFD) in Drosophila contributes to the understanding of the relationship between HFD induced obesity and the proportion-increasing heart disease of humans. Optical coherence tomography has been proved to be a powerful technique in conducting longitudinal cardiac studies on Drosophila. Here, we monitored the effect of HFD on heart development of wild type Drosophila with a customized optical coherence microscopy system. The effect that the mammalian circadian gene, CLOCK, has on cardiac development was also studied. Four groups were involved in this study, wild type control Drosophila (WT), wild type Drosophila fed with HFD (WT-HFD), CLOCK mutant Drosophila (CLOCK-RNAi), and CLOCK mutant Drosophila fed with HFD (CLOCK-RNAi-HFD), with sample sizes 20, 26, 15 and 15, respectively. We find that HFD affected cardiac function throughout the lifecycles of the four groups (from larva day 2 to adult day 1). Heart rate [HR] decrease was observed in the larval and pupal stages with the most impaction in larva. The CLOCK-RNAi also had reduced HR although, the reduction was not so severe as WT-HFD in some stages (larva, early and late pupa). HFD induced further reduction in HR of CLOCK-RNAi in all developmental stages except pupa day 1 and 2. Therefore, HFD as well as CLOCK-RNAi mutation can both lead to significant cardiac alterations in Drosophila, and HFD affected cardiac function more severely in CLOCK-RNAi mutants. Alterations in duty cycle and heart chamber size were also correlated with HFD and CLOCK-RNAi mutation, which further contributes to understanding obesity and gene related human heart disease.

Live dynamic OCT imaging of mouse embryonic cardiovascular structure and function with direct volumetric data acquisition at 1.5 MHz A-line rate

Shang Wang, Baylor College of Medicine (United States); Manmohan Singh, Univ. of Houston (United States); Andrew L. Lopez III, Baylor College of Medicine (United States); Chen Wu, Raksha Raghunathan, Alexander W. Schill, Jiasong Li, Univ. of Houston (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States) and Samara State Aerospace Univ. (Russian Federation); Irina V. Larina, Baylor College of Medicine (United States)

Efficient phenotyping of cardiovascular dynamics in live mouse embryos has significant implications on understanding of early mammalian heart development and congenital cardiac defects. Recent studies have established optical coherence tomography (OCT) as a powerful tool for live embryonic cardiovascular imaging in various animal models. However, current 4D OCT imaging of the beating embryonic heart largely relies on gated data acquisition or post-acquisition synchronization, which brings errors when cardiac cycles lack perfect periodicity and is time consuming and computationally expensive. Here, we report direct 4D OCT structural and functional imaging of cardiovascular dynamics in live mouse embryos achieved by employing a Fourier domain mode-locking swept laser source that enables 1.5 MHz A-line rate. Through utilizing both forward and backward scans of a resonant mirror, we obtained 6.4 kHz frame rate, which allows for a direct volumetric data acquisition speed of 43 Hz, around 20 times of the mouse embryonic heart rate. Our experiments were performed on E9.5 mouse embryos. Time-resolved 3D cardiodynamics clearly shows the heart structure and its motion characteristics. With speckle variance and Doppler processing algorithms, we present 4D dynamic imaging of blood flow in the embryonic cardiovascular system. Spatially-resolved measurement of blood flow velocity over time indicates the feasibility of this method for 4D hemodynamic analysis. Our results suggest that the combination of ultrahigh-speed OCT imaging with live mouse embryo culture could potentially be a useful embryonic cardiovascular phenotyping approach for mouse mutants modeling human congenital heart diseases.

Imaging of murine embryonic cardiovascular development using optical coherence tomography

Yongyang Huang, Lehigh Univ. (United States); Karl R.
Degenhardt, The Children’s Hospital of Philadelphia (United States); Sophie Astrof, Thomas Jefferson Univ. (United States); Chao Zhou, Lehigh Univ. (United States)

We have demonstrated the capability of spectral domain optical coherence tomography (SDOCT) system to image full development of mouse embryonic cardiovascular system. Monitoring morphological changes of mouse embryonic heart occurred in different embryonic stages helps identify structural or functional cardiac anomalies and understand how these anomalies lead to congenital heart diseases (CHD) present at birth. In this study, mouse embryo hearts ranging from E9.5 to E15.5 were prepared and imaged in vitro. A customized spectral domain OCT system was used for imaging, with a central wavelength of 1310nm, spectral bandwidth of -100nm and imaging speed of 47kHz A-scans/s. Axial resolution of this system was 8.3μm in air, and transverse resolution was 6.2 μm with 5X objective. Key features of mouse embryonic cardiovascular development such as vasculature remodeling into circulatory system, separation of atria and ventricles and emergence of valves could be clearly seen in three-dimensional OCT images. Optical clearing was applied to overcome the penetration limit of OCT system. With high resolution, fast imaging speed, 3D imaging capability, OCT proves to be a promising biomedical imaging modality for developmental biology studies, rivaling histology and micro-CT.

Optical mapping of conduction in early embryonic quail hearts with light-sheet microscopy

Pei Ma, Shi Gu, Yves T. Wang, Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Optical mapping (OM) using fluorescent voltage-sensitive dyes (VSD) to measure membrane potential is currently the most effective method for electrophysiology studies in early embryonic hearts due to its noninvasiveness and large field-of-view. Conventional OM acquires bright-field images, collecting signals that are integrated in depth and projected onto a 2D plane, not capturing the 3D structure of the sample. Early embryonic hearts, especially at looping stages, have a complicated, tubular geometry. Therefore, conventional OM cannot provide a full picture of the electrical conduction circumferentially around the heart, and may result in incomplete and inaccurate measurements. Here, we demonstrate OM of Hamburger and Hamilton stage 14 embryonic quail hearts using a new commercially-available VSD, Fluovolt, and depth sectioning using a custom built light-sheet microscopy system. Axial and lateral resolution of the system is 14μm and 8μm respectively. For OM imaging, the field-of-view was set to 900μmx900μm to cover the entire heart. 2D over time OM image sets at multiple cross-sections through the looping-stage heart were recorded. The shapes of both atrial and ventricular action potentials acquired were consistent with previous reports using conventional VSD (di-4-ANNEPS). With Fluovolt, signal-to-noise ratio (SNR) is improved significantly by a factor of 2-10 (compared with di-4-ANNEPS) enabling light-sheet OM, which intrinsically has lower SNR due to smaller sampling volumes. Electrophysioligic parameters are rate dependent. Optical pacing was successfully integrated into the system to ensure heart rate consistency. This will also enable accurately gated reconstruction of full four dimensional conduction maps and 3D conduction velocity measurements.

Optogenetic control of drosophila heart rhythm at different developmental stages

Aneesh Alex, Jing Men, Chao Zhou, Lehigh Univ. (United States)

Currently, electrical pacing is the gold standard for cardiac control. However, optical pacing of heart, which has high spatial and temporal precision, can provide non-invasive and more specific control of heart and has become a promising alternative to electrical stimulation. Drosophila, as a powerful model for research with orthologs of over 75% of human disease genes, has been studied widely in last decades. However, little work has been done on the developmental heart rhythm control despite the great value to understand cardiac dynamics and development in different life stages. Here, we developed an optic-controlled pacemaker by transgenic expression of a light-gated cation channel, channelrhodopsin-2 (ChR2) in Drosophila melanogaster. A customized optical coherence microscopy system with ultrahigh resolution and imaging speed was used to image the pacing process in vivo, and corresponding programs were also developed for the data analysis. By illuminating patterned blue light on specific areas of Drosophila hearts, successful pacing was observed over the whole life cycle (larva, early pupa and adult). The resting heart rate (RHR) and working pacing frequency for the three stages were determined. Pacing efficiency and excitation power thresholds were also quantified and analyzed between different developmental stages. Similar transmission electron microscope (TEM) images of paced and control flies confirmed the safety of our pacing protocol. Our work offers great potential in developing a new class of study in developmental cardiology by defining mechanisms of arrhythmia and developing new therapeutic strategies for treating arrhythmias.

Altering hemodynamics leads to congenital heart defects

Stephanie M. Ford, Univ. Hospitals Rainbow Babies & Children’s Hospital (United States); Matthew T. McPheeters, Yves T. Wang, Shi Gu, Yong Qiu Doughman, Case Western Reserve Univ. (United States); James P. Strainic, Univ. Hospitals Rainbow Babies & Children’s Hospital (United States); Andrew M. Rollins, Michiko Watanabe, Michael W. Jenkins, Case Western Reserve Univ. (United States)

The role of hemodynamics in early heart development is poorly understood. In order to successfully assess the impact of hemodynamics on development, we need to monitor and perturb blood flow, and quantify the resultant effects on morphology. Here, we have utilized cardiac optical pacing to create regurgitant flow in embryonic hearts and OCT to quantify regurgitation percentage and resultant morphology. Embryonic quail in a shell-less culture were optically paced at 3 Hz (well above the intrinsic rate of 1.33-1.67 Hz) on day 2 of development (3-4 weeks human) for 5 minutes. The pacing fatigued the heart and led to a prolonged period (> 1 hour) of increased regurgitant flow. Embryos were kept alive until day 3 (cardiac looping ~ 4-5 weeks human) or day 8 (4 chambered heart ~ 8 weeks human) to quantify resultant morphologic changes with OCT. All paced embryos imaged at day 3 displayed cardiac defects. The extent of regurgitant flow immediately after pacing was correlated with cardiac cushion size 24-hours post pacing (p-value < 0.01) with higher regurgitation leading to smaller cushions. Almost all embryos (16/18) surviving to day 8 exhibited congenital heart defects (CHDs) including 11/18 with valve defects, 5/18 with ventricular septal defects and 5/18 with hypoplastic right ventricles. Our data suggests that regurgitant flow leads to smaller cushions, which develop into abnormal valves and septa. Our model produces similar phenotypes as found in our fetal alcohol syndrome and velo-cardio-facial/ DiGeorge syndrome models suggesting that hemodynamics plays a role in these syndromes as well. Utilizing OCT and optical pacing to understand hemodynamics in development is an important step towards determining CHD mechanisms and ultimately developing earlier treatments.
Comparison of rotational imaging optical coherence tomography and selective plane illumination microscopy for embryonic study

Chen Wu, Manmohan Singh, David Mayerich, Univ. of Houston (United States); Irina V. Larina, Mary E. Dickinson, Baylor College of Medicine (United States); Kirill V. Larin, Univ. of Houston (United States)

The mouse is a common model for studying developmental diseases. Different optical techniques have been developed to investigate the mouse embryos. In this study, we imaged the E8.5 mouse embryo both with rotational imaging Optical Coherence Tomography (ri-OCT) and Selective Plane Illumination Microscopy (SPIM). Both methods can provide images with micrometer resolution. The ri-OCT technique is developed to increase the imaging depth of the mouse embryo by imaging at multiple angles. In SPIM, optical sectioning is achieved by illuminating the sample with a sheet of laser light. In this study, the images acquired from both techniques are compared with each other to evaluate the benefits and drawbacks for embryonic imaging. Since three dimension stacks of the SPIM could be obtained from different angles by rotation the sample, it might be possible to build a hybrid setup of two imaging modalities to combine the advantages of each technique in the future.

Live 4D optical coherence tomography for early embryonic mouse cardiac phenotyping

Andrew L. Lopez III, Shang Wang, Baylor College of Medicine (United States); Kirill V Larin, Univ. of Houston (United States); Paul A. Overbeek, Irina V. Larina, Baylor College of Medicine (United States)

Studying embryonic mouse development is important for our understanding of normal human embryogenesis and underlying causes of congenital defects. Our research focuses on imaging early development in the mouse embryo to specifically understand cardiovascular development using optical coherence tomography (OCT). We have previously developed imaging approach that combines static embryo culture, OCT imaging and advanced image processing to visualize whole live mouse embryos and obtain 4D (3D+time) cardioimages with cellular resolution. Here, we present the study of using 4D (3D+time) OCT for dynamic imaging of early embryonic heart in the mouse embryos to reveal mutant cardiac phenotypes during early development including dramatic cardiac looping defect. Our results indicate that the live 4D imaging approach is an efficient phenotyping tool that can reveal structural and functional cardiac defects at very early stages. Further studies integrating live embryonic cardiodynamic imaging with molecular genetic approaches in mouse mutants will help to elucidate the underlying signaling defects.

Label-free imaging of developing vasculature in zebrafish with phase variance optical coherence microscopy

Yu Chen, Jeff Fingler, Le A. Trinh, Scott E. Fraser, The Univ. of Southern California (United States)

Formation of the vasculature is a complex process that is critical in development and disease. The ability to detect the vasculature and image without perturbing the sample would be advantageous for our understanding of both healthy and malformed vessels. However, most studies in model systems such as zebrafish, rely on exogenous labels to visualize the vasculature.

To expand the opportunities for vascular imaging, we have developed a label-free imaging technique based on spectral-domain optical coherence microscopy (pvOCT), called phase variance OCT (pvOCT). pvOCT uses motion contrast caused by phase variations over time in the OCT signals. This allows for detection of flow dynamics in the vasculature.

We have built a spectral-domain OCT imaging system, with axial and transverse resolutions of 2 um in the tissue, and a frame rate of up to 150 Hz. Imaging of 2-5 days post-fertilization (dpf) zebrafish embryos with this OCT system shows that the detailed structure of somites, spinal cord, gut and notochord can be clearly identified. Visualization of the blood flow in the aorta, veins and intersegmental vessels was achieved with pvOCT, which corresponded to the confocal microscopic images of GFP-labelled vasculature in transgenic zebrafish. While the confocal microscopy only shows the vascular structure, pvOCT also provides the functional information of the blood flow that is crucial in the study of vascular development. Deeper structure, as well as older and larger zebrafish will be imaged with an extended focus OCT system, which has improved high-resolution depth of focus for imaging.

Use of a highly transparent zebrafish mutant for investigations in the development of the vertebrate auditory system

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Zebrafish, an auditory specialist among fish, offer analogous auditory structures to vertebrates and is a model for hearing and deafness in vertebrates, including humans. Nevertheless, many questions remain on the basic mechanics of the auditory pathway. Phase-sensitive Optical Coherence Tomography has been proven as valuable technique for functional vibrometric measurements in the murine ear. Such measurements are key to building a complete understanding of auditory mechanics. The application of such techniques in the zebrafish is impeded by the high level of pigmentation, which develops superior to the transverse plane and envelopes the auditory system superficially. A zebrafish double mutant for nacre and roy (mitfa-/-;roya-/- [casper]), which exhibits defects for neural-crest derived melanocytes and iridophores, at all stages of development, is pursued to improve image quality and sensitivity for functional imaging. So far our investigations with the casper mutants have enabled the identification of the specialized hearing organs, fluid-filled canal connecting the ears, and sub-structures of the semicircular canals. In our previous work with wild-type zebrafish, we were only able to identify and observe stimulated vibration of the largest structures, specifically the anterior swim bladder and tripus ossicle, even among small, larval specimen, with fully developed inner ears. In conclusion, this genetic mutant will enable the study of the dynamics of the zebrafish ear from the early larval stages all the way into adulthood.

An OCT-based approach to quantifying shear force and power dissipation in xenopus embryo cilia-driven fluid flow fields

Brendan K. Huang, Mustafa K. Khokha, Michael
Loewenberg, Yale Univ. (United States); Michael A. Choma, Yale School of Medicine (United States)

Ciliated surfaces are fluid transport organs that undergo poorly understood developmental processes during embryonic and fetal development. Quantifying flow performance during development is important for understanding congenital defects in flow performance and understanding the interaction between biomechanical forces and ciliated surface development. To date, most performance metrics have focused on flow velocity and simplified estimates of shear force. However, as with the developing heart, fluid transport performance requires an understanding of force and power generation under various loading conditions. Here, we present our initial work in quantifying shear force and net power dissipation from OCT-based cilia-driven fluid flow velocimetry. This work includes quantitative analysis of shear force and power dissipation metrics of cilia-driven fluid flow in Xenopus embryos, an important animal model of ciliated epithelial surface development. We report two different approaches to non-contact, all-optical shear force and power dissipation physiology. First, we developed a lumped-parameter model of flow driven by a ciliated surface. The lumped-parameter model yields semi-quantitative, Ohm’s law-type relationships (i.e. $F=U*R$ and $P=U*F$) between flow velocity ($U$), shear force ($F$), viscous resistance ($R$), and power dissipation ($P$). This model allows a lumped approach to evaluate force and power performance under viscous loading. Second, we numerically estimate shear force and power dissipation using flow velocity fields acquired using OCT. Specifically, the velocity gradient tensor estimated from the flow velocity field contains the required information to estimate both shear force and net power dissipation.

9716-13, Session 3

Three-dimensional imaging of the developing mouse female reproductive organs with optical coherence tomography

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Infertility is known to be a major health concern and is estimated to impact ~15% of couples in the US. A majority of failed pregnancies occur before or during implantation of fertilized embryo into the uterus. The mouse has been widely used as a model for mammalian reproduction studies. Understanding the mechanisms regulating the development of the mouse reproductive organs could significantly contribute to an improved understanding of normal development of reproductive organs and developmental causes of infertility in humans. Toward this goal, we report a 3D imaging study of the developing mouse reproductive organs (ovary, oviduct and uterus) using optical coherence tomography (OCT). OCT is used for 3D imaging of reproductive organs without endogenous contrast and provides micro-scale spatial resolution. Experiments are conducted on the developing mouse reproductive organs ranging from the embryonic day 14.5 to the fully developed adult tissue ex vivo. Reproductive organs in adult females are also visualized in vivo using imaging approaches that we recently developed. Structural features of the ovary, oviduct and uterus are presented through developmental stages. Comparison with traditional histological analysis is presented. These results provide a basis for a wide range of infertility studies in mouse models. Through integration with traditional genetic and molecular biology approaches, this imaging method can improve our understanding of ovary, oviduct and uterus development and function, which can potentially contribute to our understanding of fertility and infertility.

9716-14, Session 3

OCT imaging of craniofacial anatomy in xenopus embryos

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The etiology of craniofacial defects is incompletely understood. The ability to obtain large amounts of gene sequence data from families affected by craniofacial defects is opening up new ways to understand molecular genetic etiological factors. One important link between gene sequence data and clinical relevance is biological research into candidate genes and molecular pathways. We present our recent research using OCT as a nondestructive phenotyping modality of craniofacial morphology in Xenopus embryos, an important animal model for biological research in gene and pathway discovery. We define 2D and 3D scanning protocols for a standardized approach to craniofacial imaging in Xenopus embryos. We define standard views and planar reconstructions for visualizing normal anatomy and landmarks. We compare these views and reconstructions to traditional histopathology using alcin blue staining. In addition to being 3D, nondestructive, and having much faster throughout, OCT can identify craniofacial features that are lost during traditional histopathological preparation. We also identify quantitative morphometric parameters to define normative craniofacial anatomy. We also note that craniofacial and cardiac defects are not infrequently present in the same patient (e.g. velocardiofacial syndrome). Given that OCT excels at certain aspects of cardiac imaging in Xenopus embryos, our work highlights the potential of using OCT and Xenopus to study molecular genetic factors that impact both cardiac and craniofacial development.
are necessary for yolk lipoprotein transportation and oogenesis in C. elegans. Moreover, the established experimental method can be further used for the screening and examination of undiscovered genes related to lipid transportation and developmental biology.

9716-16, Session 4

**Bessel beam fluorescence lifetime tomography of live embryos**

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Optical tomography allows isotropic 3D imaging of embryos. Scanning-laser optical tomography (SLOT) has superior light collecting efficiency than wide-field optical tomography, making it ideal for fluorescence imaging of live embryos.

We previously reported an imaging system that combines SLOT with a novel Fourier-multiplexed fluorescence lifetime imaging (FmFLIM) technique named FmFLIM-SLOT. FmFLIM-SLOT performs multiplexed FLIM-FRET readout of multiple FRET sensors in live embryos. Here we report a recent effort on improving the spatial resolution of the FmFLIM-SLOT system in order to image complex biochemical processes in live embryos at the cellular level.

Optical tomography has to compromise between resolution and the depth of view. In SLOT, the commonly-used focused Gaussian beam diverges quickly from the focal plane, making it impossible to achieve high resolution imaging in a large volume specimen. We thus introduce Bessel beam laser-scanning tomography, which illuminates the sample with a spatial-light-modulator-generated Bessel beam that has an extended focal depth. The Bessel beam is scanned across the whole specimen. Fluorescence projection images are acquired at equal angular intervals as the sample rotates. Reconstruction artifacts due to annular-rings of the Bessel beam are removed by a modified 3D filtered back projection algorithm.

Furthermore, in combination of Fourier-multiplexing fluorescence lifetime imaging (FmFLIM) method, the Bessel FmFLIM-SLOT system is capable of perform 3D lifetime imaging of live embryos at cellular resolution. The system is applied to in-vivo imaging of transgenic Zebrafish embryos. Results prove that Bessel FmFLIM-SLOT is a promising imaging method in development biology research.

9716-17, Session 4

**OCT-based three-dimensional, three vector component imaging of cilia-driven fluid flow for developmental biology**

Brendan K. Huang, Yale School of Medicine (United States); Kevin C. Zhou, Yale Univ. (United States); Ute A. Gamm, Yale School of Medicine (United States); Vineet Bhandari, Yale Univ. (United States); Mustafa K. Khokha, Michael A. Choma, Yale School of Medicine (United States)

One critical barrier to the robust study of cilia-driven fluid flow in developmental biology is a lack of methods for acquiring three-dimensional (3D) images of three vector component (3C) measurements of flow velocities. A 3DSC map of cilia-driven fluid flow quantifies the flow speed along three axes (e.g. three Cartesian vector components $v_x$, $v_y$, $v_z$) at each point in 3D space. 3DSC quantification is important because cilia-driven fluid flow is not amenable to simplifying assumptions (e.g. parabolic flow profile). Such quantification may enable systematically detailed characterization of performance using shear force and power dissipation metrics derived from 3DSC flow velocity fields.

We report our OCT-based results in developing methods for the 3DSC quantification of cilia-driven flow fields. First, we used custom scan protocols and reconstruction algorithms to synthesize 3DSC flow velocity fields from 2D2C fields generated using correlation-based methods (directional dynamic light scattering and digital particle image velocimetry). Xenopus results include flow driven by ciliated embryo skin and flow driven by ciliated ependymal cells in developing brain ventricles. Second, we developed a new approach to particle tracking velocimetry that generates 2D2.5C ($2.5C: v_x, |v_y|, v_z$) velocity fields from single-plane 2D image acquisitions. We demonstrated this particle streak velocimetry method in calibrated flow phantoms and in flow driven by ciliated Xenopus embryo skin. Additionally, we have preliminary results extending particle streak velocimetry to 3DSC in calibrated flow phantoms with ongoing work in Xenopus embryos.

9716-18, Session 4

**Time multiplexing super resolution using a 2D Barker-based array**

Asaf Ilovitsh, Tali Ilovitsh, Eyal Preter, Bar-Ilan Univ. (Israel); Nadav Levanon, Tel Aviv Univ. (Israel); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

We propose the use of a new encoding mask in order to improve the performance of the conventional time multiplexing super resolution method. The encoding is performed by a 2D Barker-based array, which is a 2D generalization of the standard 1D Barker code. The decoding process involves using a mismatched array. The 2D Barker-based array enables achieving two dimensional super resolution image using only one dimensional scan, by exploiting the unique cross correlation between the Barker-based array and the mismatched array. The cross correlation has a perfect peak to sidelobes ratio, making it ideal for the super resolution process. Also, instead of placing the 2D Barker-based array onto the object, we propose the projection of this array onto the object using a phase-only spatial light modulator. Projecting the array eliminates the need for printing it, mechanically shifting it, and having a direct contact with the object, which is not feasible in many imaging applications. 13 phase masks, which generate shifted Barker-based arrays, were designed using a revised Gerchberg-Saxton algorithm. A sequence of 13 low resolution images were captured using these phase masks, and were decoded using the mismatched arrays, resulting with a high resolution image. The proposed 2D Barker-based array, mismatched array, and the design process of the phase masks are presented, and the method is validated by a laboratory experiment.

9716-19, Session 4

**Watching embryonic development in a new light: elasticity specific imaging with dual Brillouin/Raman microspectroscopy**

Zhaokai Meng, Jessica Hanson, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Mechanical properties of tissues play an important role in biological development. However, the current elasticity-specific imaging techniques are either destructive / invasive, or have a limited spatial and/or temporal resolution. Recently, we introduced Brillouin microscopy imaging as a local non-invasive probe of microscopic viscoelasticity in cells and tissues. In this study, by taking advantage of Brillouin spectroscopy, we imaged the viscoelasticity properties of different compartments of living zebrafish embryos, including yolk-sac, skin, spine and heart. Brillouin and Raman spectra were collected simultaneously at each location using a recently developed Brillouin/Raman microscope [1]. We also performed those experiments for different stages of embryonic development.

Super resolved optical system using circular gratings for objects with finite sizes
Asaf Ilovitsh, Bar-Ilan Univ. (Israel); Vicente Mico, Univ. de València (Spain); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

We present a real time all optical super resolution (SR) method for exceeding the diffraction limit of an imaging system which has a circular aperture. In Field of view (FOV) SR the resolution improvement is achieved by exploiting unused parts of the FOV. Diffractive gratings are used in order to optically encode (and later on decode) the high resolution spatial data that is diffraction limited by the aperture. The high resolution information is also encoded into other areas in the FOV, thus there is a trade-off between the possible resolution improvement and the size of the inspected object. In our method, the SR is obtained using two fixed circular symmetric gratings which are placed in predetermined positions. The proposed method has several advantages over previous ones, where 2D Dammann Cartesian gratings were used for the SR process. Mathematically, the proposed circular gratings are 1D gratings as they are only radius dependent. As such, their design is simplified. In addition, since the gratings are rotating angle independent, the optical setup requires less calibration and alignment.

Furthermore, the circular gratings generate synthetic circular duplications of the aperture. Thus, they seem to be the best choice for an optical system which has a circular aperture. The method is applicable for both spatially coherent and incoherent illuminations, as well as for white light illumination. The proposed method is presented analytically, demonstrated via numerical simulations, and validated by laboratory experiments.

Blood flowing state analysis in outflow tract of chick embryonic heart based on spectral domain optical coherence tomography
Yuqian Zhao, Zhenhe Ma, Yi Wang, Shidan Dou, Northeastern Univ. (China)

The cardiac development is a complicated process affected by genetic and environmental factors. Wall shear stress (WSS) and periodic stress (WPS) are the components which have been proved to influence the morphogenesis during early stages of cardiac development. The vessel wall will be deformed by the blood flow pressure and produce natural elastic force acting on the blood. Because blood flowing in different flow state and show different characteristics of fluid, which influence the calculation of WSS and WPS directly. It is necessary to study the blood flow state. In this paper, we introduce a method to quantify the blood flowing state of early stage chick embryonic heart based on high speed spectral domain optical coherence tomography (SDOCT). 4D (x,y,z,t) scan was performed on the outflow tract (OFT) of HH18 (~3 days of incubation) chick embryonic heart. By processing the structural image, the geometric parameters, such as radius, were obtained. Import UG software into the geometric contour line to reconstruct the 3D model. After phase synchronization, OFT boundary segmentation, and OFT center line calculation, Doppler angle of the blood flow in the OFT can be achieved. Combining with the Doppler OCT results, we calculate absolute blood flow velocity distribution in the OFT. The 3D model is imported into the finite element analysis software, and the grid division and boundary conditions are set up. Numerical simulation analysis of blood flow in the early chick embryo heart blood outflow tract was carried out by computational fluid dynamics method. Hemodynamic parameters were obtained at different times during the cardiac cycle, such as Reynolds number and Womersley.
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9717-1, Session 1

Multi-actuator adaptive lens for wavefront correction in optical coherence tomography and two-photon excitation fluorescence microscopy

Stefano Bonora, IFN-CNR LUXOR Lab. (Italy); Sujin Lee, Yifan Jian, Michelle Cua, Simon Fraser Univ. (Canada); Edward N. Pugh, Robert J. Zawadzki, Univ. of California, Davis (United States); Marinko V. Sarunic, Simon Fraser Univ. (Canada)

We present a new type of adaptive lens with 18 actuators that can correct up to the 4th order of aberration. The Multi-actuator Adaptive Lens (M-AL) can guarantee a good level of aberration correction for many applications and, with respect to deformable mirror, it allows the realization of more compact and simple optical systems. The adaptive lens is based on the use of piezoelectric actuators and, without any obstruction or electrodes in the clear aperture, can guarantee a fast response time, in the order of about 10ms. The clear aperture of the M-AL allows its use in “classical” Adaptive Optics configuration together with a wavefront sensor. To introduce a further simplification to the optical system design we show that the M-AL lens can be also driven with a wavefront sensorsless control algorithm during in vivo optical coherence tomography of the human retina and for two-photon excitation fluorescence microscopy. In the experimental setup we used two aberration correcting devices: a commercial adaptive lens (AL) with a high dynamic range to correct for defocus and the Multi-actuator Adaptive Lens (M-AL) to correct for the Zernike aberrations up to the 4th order. Experimental results show that the ocular aberrations of human eyes can be successfully corrected with our M-AL for pupils of 5mm and that retinal cones can be readily imaged.

9717-2, Session 1

Stand-alone scattering optical device using photopolymer

Jongchan Park, KyeoReh Lee, YongKeun Park, KAIST (Korea, Republic of)

When a light propagates through a highly disordered medium, its optical parameters such as amplitude, phase and polarization states are completely scrambled because of multiple scattering events. Since the multiple scattering is a fundamental optical process that contains extremely high degrees of freedom, optical information of a transmitted light is totally mingled. Until recently, the presence of multiple scattering in an inhomogeneous medium is considered as a major obstacle when manipulating a light transmitting through the medium. However, a recent development of wavefront shaping techniques enable us to control the propagation of light through turbid media: a light transmitting through a turbid medium can be effectively controlled by modulating the spatial profile of the incident light using spatial light modulator.

In this work, stand-alone scattering optical device is proposed; a holographic photopolymer film, which is much economic compared to the other digital spatial light modulators, is used to record and reconstruct permanent wavefront to generate optical field behind a scattering medium. By employing our method, arbitrary optical field can be generated since the scattering medium completely mixes all the optical parameters which allow us to access all the optical information only by modulating spatial phase profile of the impinging wavefront. The method is experimentally demonstrated in both the far-field and near-field regime where it shows promising fidelity and stability. The proposed stand-alone scattering optical device will open up new avenues for exploiting the randomness inherent in disordered medium.

9717-3, Session 2

Adaptive aberration correction for single molecule switching nanoscopy

Martin J. Booth, Univ. of Oxford (United Kingdom); Debora Andrade, Jacopo Antonello, Daniel Burke, Brian Patton, Univ. of Oxford (United Kingdom); Fang Huang, Joerg Bewersdorff, Yale School of Medicine (United States)

Single molecule switching (SMS) nanoscopy is a super-resolution concept that encompasses STORM, PALM, GSDIM and other variants. The methods use stochastic switching of fluorescent molecules permit localization with precision far below the diffraction limit. This is achieved by ensuring that the image of any emitting molecule is well separated from others, permitting estimation of the emitter’s position. Acquisition and analysis of thousands of these images permits the formation of image representations with, in effect, vastly improved resolution on the nanometer scale. However, these SMS methods are susceptible to the effects of specimen-induced aberrations, as the model used for the estimation is based upon an aberration free system. This mismatch between model and data leads to inaccurate localization. We investigate the effects of different aberration modes on the performance of these microscopes. In particular, we consider versions of SMS nanoscopy providing three-dimensional resolution, including astigmatic, biplanar and helix methods. These methods are more widely applicable for biological investigations and also more sensitive to aberration errors. It is found that each of these SMS methods is affected differently by aberrations, but in all cases erroneous image representations can result. These observations have significant consequences for the design of adaptive optics strategies, as correction of certain aberration modes can be far more important than others.

9717-4, Session 2

Adaptive optics in digital micromirror based confocal microscopy

Paolo Pozzi, Dean Wilding, Oleg A. Soloviev, Gleb V. Vdovin, Michel Verhaegen, Technische Univ. Delft (Netherlands)

We present a wide field, high-speed, optical sectioning fluorescence microscope with adaptive optics capabilities.

Adaptive optics implementation in standard confocal microscopy is a challenging goal, as the low level of fluorescence intensity makes the use of a wavefront sensor difficult, and the low frame rate of the acquisition makes optimization algorithms based on image metrics extremely slow. The system we present is a modified epifluorescence microscope, with High power LED excitation, a deformable mirror for the optimization of both the excitation and fluorescence wavefronts, and a Digital Micromirror Device (DMD) in an image plane between the dichroic and the objective. The DMD is a pixelated mirror, in which each element can be individually tilted in order to deviate light in the optical path, acting like both a point source for excitation and a confocal pinhole for detection, thus granting optical sectioning. An image is acquired by separately switching on all the DMD elements once during a single camera exposure. The obtained images have a considerably
wider field of view and higher frame rate compared to a standard confocal microscope. Moreover, the fluorescence light rejected by the digital pinholes can be detected by a separate camera with short exposures, acquiring information about the point spread functions generated by single micromirrors. Such information can be used as a metric for real time wavefront optimization algorithms, working at frequencies close to the dynamic limit of the deformable mirror.

9717-5, Session 2

Wavelet-based denoising of the Fourier metric for real-time sensorless adaptive optics single molecule localization microscopy

Kayvan F. Tehrani, Peter Kner, The Univ. of Georgia (United States)

Sensorless Adaptive Optics (AO) approaches for the correction of aberrations induced by biological specimens require intensity independent properties of the image as measures of fitness. Image intensity cannot be used as metric for Single Molecule Localization (SML) microscopy because the intensity of blinking fluorophores follows exponential statistics. Therefore a robust intensity-independent metric is required. We previously reported a Fourier Metric (FM) that is relatively intensity independent. The Fourier metric was successfully tested on a Genetic Algorithm and a Particle Swarm Optimization approach for wavefront correction about 50 µm inside the Central Nervous System (CNS) of Drosophila melanogaster. Because raw SML images contain sparse collections of single molecules, the images can be dominated by noise, resulting in a noisy fitness metric. By adding denoising to the images before calculating the metric, the performance of the metric can be improved. Here we present wavelet-based approaches to reducing noise in the raw images and producing a more consistent metric. We use simulations to compare different approaches and demonstrate wavefront correction on images of blinking molecules.

9717-6, Session 2

Overcoming the resolution limit in retinal imaging using the scattering properties of the eye

Dino Carpentras, Timothé Laforest, Demetri Psaltis, Christophe Moser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

In-vivo imaging of the eye's fundus is widely used to study eye's health. State of the art Adaptive Optics devices can resolve features up to a lateral resolution of 1.5 um. This resolution is still above what is needed to observe sub-cellular structures such as cone cells (1-1.25 um diameter). This limit in resolution is due to the small numerical aperture of the eye when the pupil is fully dilated (max 0.24).

In our work, we overcome this limit using a non-standard illumination scheme. A laser beam is shined on the lateral choroid layer, whose scattered light is illuminating the eye's fundus. Thanks to a Spatial Light Modulator the scattered light from the choroid layer can be manipulated to produce a scanning focus spot on the fundus. The intensity of the reflected light from the fundus is collected from the pupil and used for reconstructing the image.

9717-7, Session 3

Strategies for aberration control in dual-objective lens nanoscopy (Invited Paper)

Xiang Hao, Edward S. Allgeyer, Mary Grace M. Velasco, Yale School of Medicine (United States); Martin J. Booth, Univ. of Oxford (United Kingdom); Joerg Bewersdorf, Yale School of Medicine (United States)

The development of fluorescence microscopy, which allows live-cell imaging with high labeling specificity, has made the visualization of cellular architecture routine. However, for centuries, the spatial resolution of optical microscopy was fundamentally limited by diffraction. The past two decades have seen a revolution in far-field optical nanoscopy (or “super-resolution” microscopy). The best 3D resolution is achieved by optical nanoscopy like the ISOLED or the iPALM/4Pi-SMS, which utilize two opposing objective lenses in a coherent manner. These system are, however, also more complex and the required interference conditions demand precise aberration control. Our research involves developing novel adaptive optics techniques that enable high spatial and temporal resolution imaging for biological applications. In this talk, we will discuss how adaptive optics can enhance dual-objective lens nanoscopy. We will demonstrate how adaptive optics devices provide unprecedented freedom to manipulate the light field in isoSTED nanoscopy, allow to realize automatic beam alignment, suppress the inherent side-lobes of the point-spread function, and dynamically compensate for sample-induced aberrations. We will present both the theoretical groundwork and the experimental confirmations.

9717-8, Session 3

Fast method of cross-talk effect reduction in biomedical imaging

Maciej Nowakowski, Sylwia M. Kolenderska, Dawid Borycki, Maciej Wojtkowski, Nicolaus Copernicus Univ. (Poland)

Optical imaging of biological samples or living tissue structures requires light delivery to a region of interest and then collection of scattered light or fluorescent light in order to reconstruct an image of the object. When the coherent illumination light enters bulky biological object, each of the scattering centers (single molecule, group of molecules or other sample feature) acts as a secondary light source. As a result, scattered spherical waves from these secondary sources interact with each other, generating cross-talk noise between optical channels (eigenmodes). The cross-talk effect have serious impact on the performance of the imaging systems. In particular it reduces an ability of optical system to transfer high spatial frequencies thereby decreasing its resolution. In this work we present a fast method to eliminate all unwanted waves combination, that overlap at image plane, suppressing recovery of high spatial frequencies by using the spatio-temporal optical coherence manipulation (STOC, [1]). In this method a number of phase mask is introduced to illuminating beam by spatial light modulator in a time of single image acquisition. We use a digital mirror device (DMD) in order to rapid cross-talk noise reduction (up to 22KHz modulation frequency) when imaging living biological cells in vivo by using full-field microscopy setup with double pass arrangement. This, to our best knowledge, has never been shown before.


9717-9, Session 3

Wavefront sensorless approaches to adaptive optics for in vivo fluorescence imaging of mouse retina

Daniel J. Wahl, Bengt K. Haunerland, Oscar Sánchez Mata, Simon Fraser Univ. (Canada); Stefano Bonora, CNR-Istituto
High resolution fluorescence images are vital to small animal vision research and an increase in resolution for non-invasive imaging is highly desirable for longitudinal studies. Adaptive optics (AO) is necessary to utilize the high numerical aperture available in the mouse eye. In order to obtain cellular resolution, we have implemented wavefront sensorless adaptive optics for in vivo fluorescence imaging of mouse retina. Our approach includes a lens-based system with a variable focus lens and MEMS deformable mirror for aberration correction. The system is compact and inexpensive which is important to increase the availability of AO systems.

Our AO system includes a simultaneous reflectance channel for structural images and fluorescence channel for functional images. The structural imaging is used in real-time for navigation on the retina using landmarks such as blood vessels. We have also implemented a tunable liquid lens to select the retinal layer of interest at which to perform the optimization. At the desired location on the mouse retina, the optimization algorithm uses the fluorescence image data to drive a modal hill-climbing algorithm using an intensity-based image quality metric. The optimization requires ~30 seconds to complete an exhaustive search up to the 20th Zernike mode. We have acquired in vivo images of ganglion and microglia cells that clearly demonstrate the AO performance. We have also implemented a sharpness image quality metric and explored optimization methods that are potentially suitable for in vivo imaging, including pupil segmentation. These sensorless approaches use fluorescence images as feedback information for aberration corrections.

9717-11, Session 3

Adaptive stimulated emission depletion (STED) microscopy for 3D super-resolution imaging of thick specimens (Invited Paper)

Brian R. Patton, Univ. of Oxford (United Kingdom); Debora Andrade, Daniel Burke, Univ. of Oxford (United Kingdom); Joerg Bewersdorf, Yale School of Medicine (United States); Martin J. Booth, Univ. of Oxford (United Kingdom)

The development of super-resolution microscopy, in which structures significantly smaller than the wavelength of light are imaged, has encompassed both scanning methods (STED, RESOLFT, etc.) and stochastic wide field methods (PALM, STORM, GSDIM, etc.). The demanding nature of the imaging formation process means that aberrations induced by the sample can significantly compromise resolution and signal beyond that seen in conventional, diffraction-limited methodologies. We have been investigating the incorporation of adaptive optics technologies into super-resolution microscopes in order to correct for these aberrations and allow effective imaging in otherwise unsuitable samples. In particular, we will demonstrate how, by combining a deformable mirror with a spatial light modulator in a STED microscope, we can image at depths where a conventional STED microscope would fail to return any images. We address the challenges in performing aberration correction in STED, with particular emphasis on allowing imaging in the 3D STED mode. We demonstrate 2D and 3D STED imaging at depths of over 50 microns in tissue, showing that adaptive optics will open a much wider range of sample types to imaging with STED.

9717-10, Session 3

Pulse front adaptive optics in multiphoton microscopy

Patrick S. Salter, Bangshan Sun, Martin J. Booth, Univ. of Oxford (United Kingdom)

The accurate focusing of ultrashort laser pulses is extremely important in multiphoton microscopy, in order to achieve high resolution imaging in biological specimens. Recent advances have shown the improvements to imaging through the inclusion of an adaptive optic element in the microscope. Using a device such as a liquid crystal spatial light modulator or a deformable mirror to modify the wavefront of the incident ultrafast laser beam, it is possible to nullify any aberrations induced by the specimen, leading to improved resolution and efficiency. In an alternative configuration, the adaptive optic element may be used shape the incident beam in the spectral domain to compensate for any dispersion in the optical system and minimise the pulse duration at the focus, leading to an improved fluorescence yield. In this talk we demonstrate the newly developed concept of pulse front adaptive optics as a further mechanism for control in a multiphoton microscope. A deformable mirror and a spatial light modulator are operated in concert to give control over the incident pulse fronts (contours of constant intensity in space and time within an ultrashort pulse). By this approach, pulse fronts can be generated which are spatially variant across the beam. A quadratic time delay in the pulse front from the centre to the edge of the beam is of particular interest in multiphoton microscopy since it is an equivalent deformation to the propagation time delay (PTD) introduced to ultrashort pulses by many lenses. By varying the amount of PTD in the incident beam using adaptive optics, we demonstrate an enhancement in the measured fluorescence from a two photon microscope with non-uniform pulse fronts.

9717-12, Session 4

What advances in microscopy are required for functional brain imaging? (Keynote Presentation)

David Kleinfeld, Univ. of California, San Diego (United States)

This overview talk will focus on forward-looking scientific needs and physical limits to images of neuronal processes. The challenge in nervous systems is that the basic unit for “switching” events in the nervous system occurs on the one micrometer scale of synaptic spines, while computations involve communication between individual neurons across the full expanse of cortex, which is ten millimeters for mouse cortex. I will address hoped-for advances in optical microscopy, within the context of existing and proposed contrast mechanisms of neuronal function, that span the four orders of magnitude of length scales for neuronal processing.

9717-13, Session 4

Large field-of-view wavefront control for high resolution in vivo neuroimaging (Invited Paper)

Jung-Hoon Park, Meng Cui, Howard Hughes Medical Institute (United States)

The biggest obstacle for deep tissue imaging is the scattering of light due to the heterogeneous distribution of biological tissue. In this respect, multiphoton microscopy has an inherent advantage as the scattering is significantly reduced by the use of longer excitation wavelengths. However, as we go deeper into the brain, effects of scattering still accumulate resulting in a loss of resolution and increased background noise. Adaptive optics is an ideal tool of choice to correct for such distortions of the excitation wavefront; the incident light can be tuned to cancel out the...
wavefront distortion experienced while propagating into greater depths resulting in a diffraction limited focus at the depth of interest. However, the biggest limitation of adaptive optics for in vivo brain imaging is its limited corrected field-of-view (FOV). For typical multiphoton laser scanning microscopes, the wavefront corrector for adaptive optics is placed at the pupil plane. This means that a single correction wavefront is applied to the entire scanned FOV which results in inefficient correction as the correction is averaged over the entire FOV. In this work, we demonstrate a novel approach to measure and display different correction wavefronts over different segments of the FOV. The application of the different correction wavefronts for each segment is realized in parallel resulting in fast aberration corrected imaging over a large FOV for high resolution in vivo brain imaging.

9717-14, Session 5

Adaptive optics without guide stars (Invited Paper)
Jerome Mertz, Jiang Li, Devin Beaulieu, Hari P. Paudel, Roman Barankov, Thomas G. Bifano, Boston Univ. (United States)

Adaptive optics is a strategy to compensate for sample-induced aberrations in microscopy applications. Generally, it requires the presence of “guide stars” in the sample to serve as localized reference targets. We describe an implementation of conjugate adaptive optics that is amenable to widefield (i.e. non-scanning) microscopy, and can provide aberration corrections over potentially large fields of view without the use of guide stars. A unique feature of our implementation is that it is based on wavefront sensing with a single-shot partitioned-aperture sensor that provides large dynamic range compatible with extended samples. Combined information provided by this sensor and the imaging camera enable robust image de-blurring based on a rapid estimation of sample and aberrations obtained by closed-loop feedback. We present the theoretical principle of our technique and experimental demonstrations using both trans-illumination and fluorescence microscopes. Finally, we apply our technique to mouse brain imaging.

9717-15, Session 5

Dynamic performance of MEMS deformable mirrors for use in an active/adaptive two-photon microscope
Christian C. Zhang, Warren B. Foster, David L. Dickensheets, Montana State Univ. (United States)

Active optics, including fast focus and aberration control, can be useful to enable in vivo two-photon microscopic imaging. We are investigating fast focus control mirrors used in concert with aberration correction mirrors to control the axial position of focus and system aberrations dynamically during scanning. With an adaptive training step, sample-induced aberrations may be compensated as well. If sufficiently fast and precise, active optics may be able to compensate under-corrected imaging optics, for example for miniaturized instruments, to maintain diffraction-limited performance throughout the field of view. Toward this end we have measured a Boston Micromachines Corporation (BMC) 140 element deformable mirror, and a Revibro Optics 4-zone focus control mirror to characterize dynamic performance. Tests included both step response and sinusoidal sweeps of specific Zernike modes. For the step response we measure 90% settling times for the target Zernike amplitude. AC sweeps identified the 3 dB bandwidth of the mirror when attempting to form a sinusoidal amplitude trajectory for a specific Zernike mode. We find settling times generally less than 300 us and 3 dB frequencies in excess of 3 kHz for all tested Zernike modes on the BMC mirror, with shorter settling times for modes like astigmatism that have zero mean surface deflection, compared to defocus or spherical aberration that have non-zero mean deflections. The Revibro mirror showed a 3 dB frequency in excess of 2 kHz, and settling times less than 200 us. These speeds are sufficient for intra-scan correction at scan rates typical of two-photon microscopy.

9717-16, Session 5

Depth-enhanced in vivo imaging using wavefront shaping optical coherence tomography
HyeonSeung Yu, Jaehyun P. Lee, KyeoReh Lee, Yong Jeong, YongKeun Park, KAIST (Korea, Republic of)

Optical coherence tomography provides 3-D tomographic images of biological tissues using low-coherence interferometry. Due to the utilization of single back scattered signal as imaging contrast, OCT inherently suffers from the limited penetration depth in the presence of multiple light scattering. In most biological tissues, multiple light scattering becomes dominant and thus penetration depth is limited up to 172 mm. Extending the penetration depth through control of multiple light scattering may open new possibilities of OCT applications.

In this work, we demonstrate in vivo imaging with the penetration depth enhancement using a spectral domain OCT (SD-OCT) system in combination with wavefront shaping techniques. Exploiting a digital micro-mirror device (DMD) for a high-speed modulation of incident light fields, the penetration depth is OCT images is significantly enhanced. We demonstrate the enhancement of the penetration depth and signal-to-noise ratio using various in vivo and ex vivo tissues: ex vivo chicken breast tissue, an ex vivo mouse ear tissue and an in vivo mouse tail. For the in vivo mouse tail imaging, the present approach clearly visualize multilayered structures, whereas a conventional OCT approach only provides a superficial layer.

9717-17, Session 5

An optical tomography PSF almost insensitive to aberrations: the benefit of a spatial incoherent illumination
Peng Xiao, Mathias Fink, Claude Boccara, Institut Langevin (France)

An aberrated imaging system PSF is broadened; this broadening is responsible of the blurring of the images. A lot of effort has been carried out to correct the effects of aberrations on OCT images for eye examination or biological samples. We have worked on quantifying the effect of geometrical aberrations on Full-Field OCT images and found that there is mostly no loss of resolution but a decrease of the signal level. This is obviously why we use these signals as metric to correct the wavefront distortion. Moreover we found that this absence of blurring, which is due to the fact that we record the dot product of a diffraction limited reference signal and the distorted sample signal, is specific to the use of an incoherent illumination and did not show up with OCT approaches that use spatially coherent sources. More precisely the loss in signal is roughly proportional to the square root of the Strehl ratio: for example, a Strehl ratio of 1/9, which is considered to give a low quality image, would only be 1/3 in Full-Field OCT while keeping the sharpness of the image. Using both an USAF resolution target and a transmissive SLM we have demonstrated this unique feature of sharpness conservation. It was also confirmed by using biological samples. We think that we can thus restrict the aberration corrections in eye examination to the main aberrations (e.g. focus and astigmatism) that will increase the speed of the correction.
Volumetric imaging of fast biological dynamics in deep tissue via wavefront engineering (Invited Paper)
Lingjie Kong, Meng Cui, Purdue Univ. (United States)

In vivo volumetric imaging of biological dynamics in deep tissue is highly desirable in biomedical research to study the signaling dynamics and the cellular interactions in large cell populations at high spatiotemporal resolutions. Laser scanning multiphoton microscopy is now the standard for in vivo deep tissue imaging. However, the aberration and random scattering, induced by the heterogeneous refractive index distribution of biological tissue, deteriorate the imaging resolution and limit the maximum imaging depth. On the other hand, the speed of current volumetric imaging methods is usually limited by the inertia of axial scanning hardware, given sufficient signal level. Here we exploit wavefront engineering as the solution of high-speed volumetric imaging of biological dynamics in deep tissue. We have developed the Iterative MultiPhoton Adaptive Compensation Technique (IMPACT) for wavefront distortion measurement and compensation, and achieved deep imaging of cerebral cortex down to ~ 700 μm. Recently, we developed the volumetric imaging technique by using an optical phase-locked ultrasound lens (OPLUL) for generating oscillating defocusing wavefronts. By integrating IMPACT with the volumetric imaging system based on OPLUL, we demonstrate volumetric imaging of fast biological processes in deep tissue, and show its applications in neuroscience and immunology.

Aberrations correction for stimulated emission depletion microscopes with coherent optical adaptive technique
Wei Yan, Shenzhen Univ. (China) and Clemson Univ. (United States); Tong Ye, Clemson Univ. (United States); Xiao Peng, Junle Qu, Shenzhen Univ. (China)

Stimulated emission depletion (STED) microscopy create images with resolution beyond the diffraction limit by employing a combination of optical and photophysical effects. But the aberration introduced by the refractive index structure of specimens is often deteriorate the spatial resolution, especially imaging from deep within biological tissue specimens. Here we show that by using coherent optical adaptive technique (COAT) to correct the aberration for STED microscopy, and improve the spatial resolution. In the system of aberration correction, we employ spatial light modulation (SLM) as the aberration correction device, which plays a dual role in providing the beam-shaping phase mask and aberration correction. The COAT STED will have widely promising for applications that require images from deep within biological tissue specimens.

Effects of absorption on light transmission channels in random media (Invited Paper)
Hui Cao, Yale Univ. (United States)

Recently it has been shown that shaping the wavefront of an incident laser beam can significantly enhance the total transmission of light through strong scattering media [1]. This is done by coupling light to high transmission channels. However, optical absorption would modify such transmission channels. In a disordered system with uniform absorption, the maximal transmission channel changes from diffusive to ballistic-like transport [2]. This ballistic-like transport may enable new modes of imaging in absorbing media. If the absorption is distributed non-uniformly in space, the high transmission channels redirect the energy flows to circumvent the absorbing regions to minimize loss. Thus the attenuation of high transmission channels by inhomogeneous absorption becomes lower than that by homogeneous absorption [3]. Since the maximum transmission channel is the most efficient in bypassing the absorbing region, the ratio of its transmittance to the average transmittance increases with absorption, eventually exceeds the ratio without absorption. The finding that inhomogeneous absorption may have a weaker impact on open channels than homogeneous absorption is promising for practical applications.


Optical wavefront shaping for the enhancement of Raman signal in scattering media
Vladislav V. Yakovlev, Texas A&M Univ. (United States); Graham Throckmorton, Baylor Univ. (United States); Jonathan Thompson, Brett H. Hokr, Texas A&M Univ. (United States)

The ability to non-invasively focus light through scattering media has significant applications in many fields ranging from nanotechnology to deep tissue sensing. Until recently, the multiple light scattering events that occur in complex media such as biological tissue have inhibited the focusing ability and penetration depth of optical tools. Through the use of optical wavefront shaping, the spatial distortions due to these scattering events can be corrected, and the incident light can be focused through the scattering medium. Here, we demonstrate that wavefront shaping can be used to non-invasively enhance the Raman signal of a material through a scattering medium. Raman signal enhancement was achieved using backscattered light and a continuous sequential algorithm. Our results have the potential to make wavefront shaping an important addition to non-invasive medical diagnostics.

Enhanced second-harmonic-generation imaging of collagen by means of optical wavefront shaping
Vladislav V. Yakovlev, Texas A&M Univ. (United States); Graham Throckmorton, Baylor Univ. (United States); Jonathan Thompson, Texas A&M Univ. (United States)

Second-harmonic generation (SHG) has proven to be an effective method to both image and detect structural variations in fibrillar collagen. The ability to detect these differences is especially useful in studying diseases like cancer and fibrosis. SHG techniques have historically been limited by their ability to penetrate and image through strongly scattering tissues. Recently, optical wavefront shaping has enabled light to be focused through highly scattering media such as biological tissue. Here, we demonstrate that wavefront shaping can be used to focus light within skin and enhance the SHG field from the collagen fibrils. In an experimental set up, the SHG field from collagen fibrils in chicken skin were enhanced by a factor of 5 using a continuous sequential algorithm.
Second-harmonic generation imaging enhancement through scattering media via wavefront shaping

Sophie Brasselet, Hilton B. Barbosa de Aguiar, Institut Fresnel (France); Sylvain Gigan, Lab. Kastler Brossel (France)

Nonlinear microscopy is a powerful technique for label-free biological imaging. Despite its tremendous impact, all of nonlinear imaging modalities can only image at shallow depths, mostly because of sample scattering. Wavefront shaping, which consists in manipulating high spatial frequencies of the incident wavefront using spatial light modulators, has shown to be able to re-establish a focus through a highly scattering media. In this work we explore the possibility to use such a method to recover a second harmonic generation signal from a sample placed behind a scattering medium. While the refocus is optimized for the linear incident beam at the fundamental frequency, nonlinear emission is probed by scanning this focus in the sample. We show remarkably high nonlinear signal enhancements through scattering media mimicking biological samples conditions, at thicknesses that can reach a transport mean free path. The linear optimization scheme used here exhibits several advantages comparing to techniques based on the direct optimization of a nonlinear signal, in particular for low signal to noise ratios. Highly contrasted nonlinear images could be formed in model nonlinear nanocrystals as well as collagen. Interestingly, the polarization state of the focus is seen to be highly correlated with the incident polarization, even at a few transport mean free paths depth. This allows polarization resolved imaging and analysis of a sample without the need of multiple runs of the refocusing algorithm. These results show that the combination of wavefront shaping and nonlinear microscopy can potentially reach label-free imaging at large depths in biological media.

Energy leakage in highly scattering media due to the limited numerical aperture and its effects on wavefront shaping techniques

HyeonSeung Yu, KyeoReh Lee, YongKeun Park, KAIST (Korea, Republic of)

The efficient delivery of energy through scattering media is a topic of fundamental physical studies with a particularly important implications for biomedical imaging and light therapeutic applications. It has been theoretically predicted from the random-matrix theory that a perfect transmission through scattering media is attainable in the diffusive regime. Recent studies show that a partial measurement of a scattering matrix is inevitable in optical systems due to limited numerical aperture (NA) and this imperfect measurement prohibits the access and realization of the perfect transmission channel. Although these observations were confirmed by both experimentally and theoretically, the energy loss induced from imperfect measurements has not been fully investigated.

In this work, we systematically study leaky modes originating from partial measurements of the scattering matrix of disordered media by conducting numerical simulations. In a measurement of a transmission matrix (TM), a leaky mode, defined as uncollected transmission out of the NA, produces additional energy transmission. In a measurement of a reflection matrix (RM), a zero reflection channel is always observed even with a highly limited NA, from which one can expect the perfect transmission. However, the actual realization of the zero reflectivity channel produces high energy loss through a leaky mode, defined as uncollected reflection out of the NA, and the transmission of the energy is highly suppressed. The behaviors of leaky modes are also studied for samples with various thickness.

Accelerated wavefront determination technique for optical imaging through scattering medium

Hexiang He, Kam Sing Wong, Hong Kong Univ. of Science and Technology (Hong Kong, China)

With the help of the Spatial Light Modulator (SLM) and the memory effect of a thin scattering medium, wavefront shaping technique has been developed to be a promising imaging method in biological systems. Usually, the most commonly way is to use an optimization algorithm to control and calculate a modulation mask to compensate the scattering effect of a biological tissue. Due to the control loop of SLM and feedback detector, this kind of method is of very low efficiency. Especially for the low refresh rate most widely used Liquid-Crystal SLM, it usually needs tens of minutes to get a satisfied imaging result. We introduce an improved optimized wavefront modulation value determined method to speed up the whole process. The core of the proposed method is “detection and compensation.” Specifically, the disturbed wavefront is detected with a phase retrieval method, then, the modulation mask is calculated directly from its phase conjugated wavefront. With the help of the fast implementation method, the LC-SLM based wavefront correction system can complete within several seconds. The proposed method develops a novel solution to speed up the wavefront shaping technique to overcome the slow refresh rate of LC-SLM. In addition to the application on imaging, with the help of the massive controllable unit of the SLM, the object wavefront can be manipulated according to different requirements, such as optical illusion. Here we discuss the ability to modulate the object beam and generate the equivalent visual illusion effect using the wavefront shaping technique. Furthermore, we introduce the detection-compensation system to discuss the possibility of dynamic illusion function.

Biophotonic applications of eigenchannels in a scattering medium (Invited Paper)

Moonseok Kim, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Wonjun Choi, Univ. of Exeter (United Kingdom); Youngwoon Choi, Changhyeong Yoon, Wonshik Choi, Korea Univ. (Korea, Republic of)

When waves travel through disordered media such as ground glass and skin tissues, they are scattered multiple times. Most of the incoming energy bounces back at the superficial layers and only a small fraction can penetrate deep inside. This has been a limiting factor for the working depth of various optical techniques. We present a systematic method to enhance wave penetration to the scattering media. Specifically, we measured the reflection matrix of a disordered medium with wide angular coverage for each orthogonal polarization states. From the reflection matrix, we identified reflection eigenchannels of the medium, and shaped the incident wave into the reflection eigenchannel with smallest eigenvalue, which we call anti-reflection mode. This makes reflectance reduced and wave penetration increased as a result of the energy conservation. We demonstrated transmission enhancement by more than a factor of 3 by the coupling of the incident waves to the anti-reflection modes. Based on the uneven distribution of eigenvalues of reflection eigenchannels, we further developed an iterative feedback control method for finding and coupling light to anti-reflection modes. Since this adaptive control method can keep up with sample perturbation, it promotes the applicability of exploiting reflection eigenchannels. Our approach of delivering light deep into the scattering media will contribute to enhancing the sensitivity of detecting objects hidden under scattering layers, which is universal problem ranging from geology to life science.
Towards deep focusing in scattering tissues using the angular memory effect of scattered light and ultrafast wavefront shaping (Invited Paper)

Laurent Bourdieu, Ecole Normale Supérieure (France); Sam Schott, Ecole Normale Supérieure (France) and Univ. of Cambridge (United Kingdom); Jacopo Bertolotti, Ecole Normale Supérieure (France) and Univ. of Exeter (United Kingdom); Baptiste Blochet, Walther Akemann, Jean-François Léger, Cathie Ventalon, Stéphane Dieudonnè, Benjamin Mathieu, Ecole Normale Supérieure (France); Sylvain Gigan, Ecole Normale Supérieure (France)

The propagation of light in biological tissues is dominated by scattering. For coherent light, the recombination of the different paths creates a complex interference known as a speckle pattern. Recently, different techniques of wavefront shaping have been developed to control the intensity of a single speckle grain. This technical breakthrough opens the possibility to focus light through complex media and eventually to image in them by scanning the focused spot in the tissue. However the generalization of these methods to obtain images with large field of view faces two difficulties. On the one hand, the use of scattered photons for imaging exploits angular correlations in transmitted light, but those correlations ('angular memory effect') are of very short range. From measurements on neural tissues and complementary simulations, we found that strong forward scattering in biological tissues can enhance the memory effect range (and thus the possible field-of-view) by more than an order of magnitude compared to isotropic scattering for 1mm thick tissue layers. On the other hand, the intrinsic temporal dynamics of biological tissues creates a fast decorrelation of the speckle pattern. Therefore, focusing and imaging through biological tissues requires ultrafast wavefront shaping devices. We have developed two approaches to address this issue. The first one is based on micro-mirrors and allows optimization of a speckle pattern in tissues at a refresh rate of 500Hz. The second uses acousto-optic deflectors, which we managed to use as ultrafast wavefront shapers, whose phase mask can be changed at a rate of 100 kHz.

Biological elements carry out optical tasks in coherent imaging systems (Invited Paper)

Pietro Ferraro, Vittorio Bianco, Melania Paturzo, Lisa Miccio, Pasquale Memmolo, Francesco Merola, Valentina Marchesano, Istituto di Scienze applicata e Sistemi Intelligenti (Italy)

Digital Holography (DH) is a well assessed technique providing label-free, quantitative 3D microscopy of biological samples. Imaging through turbid media, hologram denoising and resolution enhancement are among the most investigated issues in DH. Many efforts have been spent so far to recover the scattered information of samples hidden behind turbid media, and moving diffusers are often employed to get rid of speckle artifacts (multi-look DH). Here we show how biological elements, like live bacteria species and Red Blood Cells (RBCs) can accomplish optical functionalities in DH systems. Indeed, turbid media made of biological elements allows coherent microscopy despite the strong light scattering these provoke, acting on light just as moving diffusers. Scattering events are highly uncorrelated in time, so that a multi-look strategy can be successfully adopted to achieve quantitative microscopy through turbidity. Experiments aimed at observing test targets and biological specimens through high-density bacteria volumes and turbid blood flows are reported, demonstrating that a turbid medium can have a positive effect on a coherent imaging system, mimicking the action of a coherent noise decorrelation device. It is remarkable that restored holographic reconstructions through turbidity yield an image quality significantly higher, in terms of Signal-to-Noise Ratio (SNR), than the quality achievable through a transparent medium in similar recording conditions. Moreover, suspended RBCs are demonstrated here to behave as tunable liquid micro-lenses, whose focus is controllable changing the buffer features. This capability is demonstrated through dynamic wavefront characterization and direct imaging, opening new scenarios in biophotonics for endoscopic vision and diagnostics.

Imaging of biological objects using spatio-temporal optical coherence (STOC) modulation

Sylwia M. Kolenderska, Maciej Nowakowski, Nicolaus Copernicus Univ. (Poland); Grzegorz Wilczynski, Nencki Institute of Experimental Biology (Poland); Maciej Wojtkowski, Dawid Borycki, Nicolaus Copernicus Univ. (Poland)

The coherent light reflected from the optically rough surface or propagating in the non-uniform medium generates the random intensity distribution which is known as the speckle pattern. This e?ect results from the incidental interference of many wavelets emitted from the secondary sources located at di?erent positions of the surface or within the medium. The speckle pattern detrimentally influences the capabilities of any optical method which exploits the coherent light sources. For this reason it is strongly desirable to control or at least reduce this e?ect without a?ecting the ability of the light source to generate highly directional fields, i.e. the optical beams. To achieve this task we intend to use the spatio-temporal optical coherence manipulation (STOC, [1]) technique, which performs diagonalization of the spectral coherence matrix by modulating the light with phase masks that compose an orthonormal basis. We demonstrate that such approach can be employed for universal imaging through diffusive layers without the need of feedback control systems, determination of the transmission matrix nor numerical reconstruction algorithms.

In the presentation, we will show theoretical fundamentals of the method as well as the imaging of biological samples.


Hybrid iterative wavefront shaping for high-speed focusing through scattering media

Ashton S. Hemphill, Washington Univ. in St. Louis (United States); Lihong V. Wang, Washington Univ. in St. Louis (United States)

One of the prime limiting factors of optical imaging in biological applications is the diffusion of light by tissue, which prevents focusing at depths greater than ~1 mm in the body. To overcome this issue, phase-based wavefront shaping alters the phase of sections of the incident wavefront to counteract aberrations in phase caused by scattering. This enables focusing through scattering media beyond the optical diffusional limit and increases signal compared to amplitude-based compensation. However, in previous studies, speed of optimization has typically been limited by the use of a liquid crystal spatial light modulator (SLM) for measurement and display. SLMs usually have refresh rates of less than 100 Hz and require much longer than the speckle decorrelation time of tissue in vivo, usually on the order of milliseconds, to determine the optimal wavefront. Here, we present a phase-based iterative wavefront shaping method based on an on-axis digital micromirror device (DMD) in conjunction with an electro-
overcoming multiple scattering for detection and imaging in strongly scattering media (Invited Paper)
Amaury Badon, Dayan Li, Geoffroy Lerosey, Claude Boccara, Mathias Fink, Alexandre Aubry, Institut Langevin (France)

Our approach first consists in measuring a time-gated reflection matrix associated to a scattering medium using a spatial light modulator at the input and a CCD camera at the output. An interferometric arm allows to discriminate the scattered photons as a function of their time of flight. Inspired by previous works in acoustics, a random matrix approach then allows to get rid of multiple scattering. This improves by far the detection and imaging of targets embedded in or hidden behind a highly scattering medium. As proof of concept, we tackle with the issue of imaging ZnO micrometric beads across a highly scattering paper sheet whose optical thickness is of 12.5 λ, with λ is the scattering mean free path. This experimental situation is particularly extreme, even almost desperate for imaging. The ballistic wave has to go through 25 λ back and forth, thus undergoing an attenuation of 10^-31 in intensity. For an incident plane wave, 1 scattered photon over 1000 billions is associated to the target beads. In optical coherence tomography, the single-to-multiple scattering ratio is of 5x10^-4 which prevents from any target detection and imaging. On the contrary, our approach allows to get rid of most of the multiple scattering contribution in this extreme situation. By means of the time-reversal operator, the ballistic echoes associated to each bead are extracted and allow to reconstruct a satisfying image of the targets. The perspective of this work is to apply this promising approach to in-depth imaging of biological tissues.

high-speed channel demixing by scanning interferometric focusing with binary transmission matrix (Invited Paper)
Xiaodong Tao, Univ. of California, Santa Cruz (United States); Dare Bodington, Univ. of Rochester (United States); Marc R. Reining, Joel Kubby, Univ. of California, Santa Cruz (United States)

In this paper, we demonstrate a fast binary intensity modulation based on the measurement of the binary TM. At each correction, the binary TM was calculated based on measurements of the intensity change at the target with a series of input masks. After loading the measurement masks, the DMD can run at full speed during measurement. Compared with the optimization method, no feedback information is needed during the measurement. The proposed method only requires one measurement for each input mode. The total time for a single correction is only 75 ms for 1024 input modes. To avoid the low SNR during the measurement caused by the low intensity of the reference field, the reference optimization method by scanning the speckles on the target is demonstrated. It provides more stable focusing, especially for focusing through a fast changing media or two dimensional scanning through a slowly changing scattering media. The system allows dynamic focusing at 12.5 Hz with 1024 input modes, and more than 60 times intensity enhancement. The proposed method was tested on both stationary and moving samples with different decorrelation times. Finally we demonstrate focusing light through a highly dynamic scattering sample, a live drosophila embryo.

optogenetic control of cell signaling pathway through scattering skull using wavefront shaping (Invited Paper)
YongKeun Park, KAIST (Korea, Republic of)

We present the first demonstration of optogenetic control through a highly scattering skull layer in vitro using a wavefront shaping technique. Despite significant potentials on optogenetics, attempts toward non-invasive in vivo optogenetics have been stymied by a fundamental limitation—light scattering; multiple light scattering significantly limits light delivery through turbid media such as brain or skull layers. To address this issue, we propose and experimentally demonstrate that the controlling the wavefront of an excitation beam impinging through the skull layer using a spatial light modulator, which enables the spatiotemporal regulation of light-sensitive protein activities through the intact skull.

The present work presents the important proof-of-concept for non-invasive optogenetics using in vitro models and it provides the first demonstration of the key importance of wavefront shaping techniques towards in vivo applications of optogenetics.

adaptive wave-front shaping for flow-field measurements
Nektarios Koukourakis, Bob Fregin, Lars Büttner, Jürgen W. Czarske, TU Dresden (Germany)

Adaptive optical imaging has paved the way to a new era of microscopy and general imaging. Supported by the technological progress of spatial light modulators and wave front sensing techniques, flexible control of light-fields enabled to overcome the limited penetration depth light has, when it propagates inside turbid media. This resulted in unprecedented measurements deep into and through highly scattering samples e.g. tissue. These and other experiments underlined the strong potential of this vivid research field.

In this contribution we analyze the effect of scattering or fluctuating media on the measurement accuracy of image correlation based flow field measurements. We show that time-reversal and wave front shaping techniques used for both turbidity suppression and aberration correction have the potential to increase the measurement accuracy and thus to strongly improve the quality of the measurements. Experimental and simulated results for process engineering and biomedical applications underline the capability of our approaches.

universal structures of transmission eigenchannels inside random media (Invited Paper)
Zhou Shi, Azriel Z. Genack, Queens College (United States)

Since scattering is a deterministic process, the fields on the input and output of a scattering medium are ultimately linked via the transmission matrix (TM). There has been considerable progress in focusing and controlling transmission through opaque samples by manipulating the eigenchannels
of the TM. However, the intensity distribution for individual eigenchannel inside the sample has remained largely an unexplored subject. Based upon recursive Green's functions simulations, we find universal expressions for average energy profile of eigenchannel, $W(x)$, within the sample, where $x$ is the value of transmission. We show that $W(x)$ can be expressed as a product of the perfectly transmitting eigenchannel, $W=1(x)$, and a source term in a generalized diffusion equation, $S(x)$. The source term depends only on $x$ and is independent of details of the sample for diffusive waves. Furthermore, we find that $W=S(x)=1+Fl(x)$, where $Fl(x)$ is a symmetrical function peaked in the middle of the sample equal to the probability of return of the wave to a cross section of the sample at $x$. An expression for $W=S(x)$ is given in terms of an auxiliary localization length introduced by Dorokhov to consider the scaling of transmission for individual eigenchannel. Though $W=S(x)$ cannot be measured directly, the integrated energy can be determined from the derivative with angular frequency of the average phase shift of the eigenchannel accumulated in transmission through the sample. These results reveal the rich structure of transmission eigenchannels and enable the control of the energy distribution inside random media.

9717-37, Session 10
Selective coupling of optical energy into the fundamental diffusion mode of a scattering medium using of optical wavefront shaping

Oluwafemi S. Ojambati, Hasan Yilmaz, Univ. Twente (Netherlands); Ad Lagendijk, Allard P. Mosk, Willem L. Vos, Univ. Twente (Netherlands)

Diffusion equation describes the energy density inside a scattering medium such as biological tissues and paint [1]. The solution of the diffusion equation is a sum over a complete set of eigensolutions that shows a characteristic linear decrease with depth in the medium. It is of particular interest if one could launch energy in the fundamental eigensolution, as this opens the opportunity to achieve a much greater internal energy density. For applications in optics, an enhanced energy density is vital for solid-state lighting, light harvesting in solar cells, low-threshold random lasers, and biomedical optics. Here we demonstrate the first ever selective coupling of optical energy into a diffusion eigensolution of a scattering medium of zinc oxide (ZnO) paint. To this end, we exploit wavefront shaping to selectively couple energy into the fundamental diffusion mode, employing fluorescence of nanoparticles randomly positioned inside the medium as a probe of the energy density. We observe an enhanced fluorescence in case of optimized incident wavefronts, and the enhancement increases with sample thickness, a typical mesoscopic control parameter. We interpret successfully our result of the fundamental eigensolution of the diffusion equation, and we obtain excellent agreement with our observations, even in absence of adjustable parameters [2].

References

9717-38, Session 11
Deep-tissue, high-resolution imaging with collective accumulation of single-scattered waves (Invited Paper)

Sungsam Kang, Seungwon Jeong, Wonshik Choi, Korea Univ. (Korea, Republic of)

Optical microscopes have served as one of the most important tools for biomedical science for its high spatial resolution, molecular contrast, and non-invasiveness. However, when dealing with scattering medium like as biological tissues, optical microscopes lose their resolving power due to the multiple light scattering. Here we introduce a new microscopic technique called collective accumulation of single scattering (CASS) microscope, which can obtain high resolution image for targets embedded deep within a thick scattering medium. By exploiting the spatio-temporal correlation of single-scattered waves, it can collectively extract the image information in the strong background of multiple scattering. We also present various interesting features of CASS microscope such as the quantitative behaviors of multiple scattering, 3D imaging, and dealing with specimen induced aberrations.

9717-39, Session 11
Retrieving time-dependent Green’s functions in optics with low-coherence interferometry: application to diffuse optical imaging

Amaury Badon, Dayan Li, Geoffroy Lerosey, Claude Boccara, Mathias Fink, Alexandre Aubry, Institut Langevin (France)

We recently showed how the correlations of a broadband and incoherent wave-field can directly yield the time-dependent Green’s functions between scatterers of a complex medium [Badon et al., Phys. Rev. Lett., 2015]. In this study, we apply this approach to the imaging of optical transport properties in complex media. A parallel measurement of millions of Green’s functions at the surface of several strongly scattering samples (ZnO, TiO2, Teflon tape) is performed. A statistical analysis of this Green’s matrix allows to investigate locally the spatio-temporal evolution of the diffusive halo within the scattering sample. An image of diffusion tensor is then obtained. It allows to map quantitatively the local concentration of scatterers and their anisotropy within the scattering medium. The next step of this work is to test this approach on biological tissues and illustrate how it can provide an elegant and powerful alternative to diffuse optical imaging techniques.

9717-40, Session 11
Dense sampled transmission matrix for high resolution angular spectrum imaging through turbid media via compressed sensing

Hwanchol Jang, Gwangju Institute of Science and Technology (Korea, Republic of); Changhyeong Yoon, Wonshik Choi, Korea Univ. (Korea, Republic of); Tae Joong Eom, Heung-No Lee, Gwangju Institute of Science and Technology (Korea, Republic of)

We provide an approach to improve the quality of image reconstruction in wide-field imaging through turbid media (WITM). In WITM, a calibration stage which measures the transmission matrix (TM), the set of responses of turbid medium to a set of plane waves with different incident angles, is preceded to the image recovery. Then, the TM is used for estimation of object image in image recovery stage. In this work, we aim to estimate highly resolved angular spectrum and use it for high quality image reconstruction. To this end, we propose to perform a dense sampling for TM measurement in calibration stage with finer incident angle spacing. In conventional approaches, incident angle spacing is made to be large enough so that the columns in TM are out of memory effect of turbid media. Otherwise, the columns in TM are correlated and the inversion becomes difficult. We employ compressed sensing (CS) for a successful high resolution angular spectrum recovery with dense sampled TM. CS
is a relatively new information acquisition and reconstruction framework and has shown to provide superb performance in ill-conditioned inverse problems. We observe that the image quality metrics such as contrast-to-noise ratio and mean squared error are improved and the perceptual image quality is improved with reduced speckle noise in the reconstructed image. This results shows that the WITM performance can be improved only by executing dense sampling in the calibration stage and with an efficient signal reconstruction framework without elaborating the overall optical imaging systems.

9717-41, Session 11

Effects of aberrations in vortex-beams generated with amplitude diffraction gratings
Carlos Cuartas-Vélez, René Restrepo, Santiago Echeverri Chacón, Univ. EAFIT (Colombia)

We present a mathematical model for the generation of vortex-beams by using a square amplitude diffraction with arbitrary topological charge. The framework of aberrations in the forked-shape diffraction grating is analyzed, and the resulting diffracted pattern is simulated. Experimental optical vortices are generated by using a transmission spatial light modulator, which is used as a dynamic diffraction grating, allowing us to aberate the diffraction grating. We show the effect of aberrations in the experimental diffracted vortex-beams and compare it with the numerical simulation. Finally, we make a comparison between experimental abrated on-axis Laguerre-Gauss modes with off-axis vortex-beams.

9717-42, Session 11

Computational adaptive optics for broadband interferometric tomography of tissues and cells (Invited Paper)
Steven G. Adie, Jeffrey A. Mulligan, Cornell Univ. (United States)

Adaptive optics (AO) can shape aberrated optical wavefronts to physically restore the constructive interference required for high-resolution imaging. With access to the complex optical field, however, many functions of optical hardware can be achieved computationally, including focusing and the compensation of optical aberrations to restore the constructive interference required for image formation. Holography, which employs interferometric detection of the complex optical field, was developed based on this connection between hardware and computational image formation, although this link has only recently been exploited for 3D tomographic imaging in scattering biological tissues. This talk will present the underlying imaging science behind computational image formation with optical coherence tomography (OCT) – a beam-scanned version of broadband digital holography. Analogous to hardware-based AO (HAO), we demonstrate computational adaptive optics (CAO) and optimization of the computed pupil correction in ‘sensorless mode’ (Zernike polynomial corrections with feedback from image metrics) or with the use of ‘guide-stars’ in the sample. Results using CAO will be shown in scattering tissues ex vivo, 3D cell culture in vitro, and through in vivo imaging of human retinal photoreceptors. We present the concept of an ‘isotomic volume’ as the volumetric extension of the ‘isoplanatic patch’ introduced in astronomical AO. We also discuss the advantages and disadvantages of HAO vs. CAO for the effective shaping of optical wavefronts. Finally, we discuss the future prospects of CAO, including ongoing work towards hybrid approaches that synergistically combine the unique advantages of hardware and computational methods for rapid volumetric tomography with cellular resolution.

9717-43, Session 12

Dynamic focusing in the beating zebrafish heart (Invited Paper)
Jorge Ripoll, Univ. Carlos III de Madrid (Spain) and Instituto de Investigación Sanitaria del Hospital Gregorio Marañón (Spain); Laura Andrés, Nadia Mercader, Ctr. Nacional de Investigaciones Cardiovasculares (Spain)

One of the current challenges on in-vivo cardiac imaging is being able to follow cells dynamics within the beating heart. Of the large amount of animal models available for cardiac research, the zebrafish is extremely valuable due to its transparency during early stages of development. For this reason the zebrafish is one of the in-vivo models most used in laser sheet microscopy, a technique which is opening new ways to study development with cellular resolution. In this presentation a dual illumination laser sheet microscope with simultaneous dual camera imaging is used to image the beating heart at 200fps, dynamically and selectively focusing inside the beating heart through the use of a Focus Tunable Lens. This dual color dynamic focusing enables 3D imaging of the full beating heart at high framerates, allowing dynamic imaging with cellular resolution. In this talk the approaches used to implement this dynamic focusing in a user-friendly fashion will be presented, together with in-vivo time-lapse results of the developing zebrafish heart.

9717-44, Session 12

Light-sheet optimization for microscopy
Dean Wilding, Paolo Pozzi, Technische Univ. Delft (Netherlands); Oleg A. Soloviev, Delft Univ. of Technology (Netherlands) and Flexible Optical B.V. (Netherlands); Gleb V. Vdovin, Technische Univ. Delft (Netherlands) and Flexible Optical B.V. (Netherlands); Michel Verhaegen, Technische Univ. Delft (Netherlands)

Light-sheet fluorescence microscopy is developing into a routine imaging technique for clinical and biological research. The advantages of this technique are the low photo-bleaching and toxicity associated with its use. Whilst the advantages of this technique are well-documented it still suffers from a number of disadvantages, such as artefacts due to absorption, aberration and scattering in the specimens that ensure diffraction-limited performance is seldom ever achieved in normal use. To overcome these limitations we present an approach to a general purpose light-sheet microscope system that uses a spatial light modulator for generation of the light-sheet. Using this phase modulator we are able overcome the limitations of static correction and allow for the engineering of the illumination light. The addition of a third objective in the standard LSFM configuration, which is used for the detection of transmitted laser light. By the use of a specifically designed and tuneable image metrics the transmitted light is then used for the optimization of the light-sheet profile. Using this optimization procedure it is possible to both correct for the aberrations introduced by the sample and to also increase the depth-of-field of the illumination such that the axial resolution is more uniform over the field-of-view.

9717-45, Session 12

Structured adaptive focusing through reconfigurable scattering media
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Wavefront shaping for single fiber aberrations and stage drifts, therefore excellent for imaging nanostructures. Our method is robust against optical and a field of view of 10 μm x 10 μm. 

a collection of nanospheres with a deconvolved Abbe resolution of 116 nm. We demonstrate two-dimensional fluorescence images of our algorithm which discards ambiguities therefore ensures convergence to Fourier components of standard-resolution images as a new constraint in Fourier correlation effect known as the optical memory effect. A series of standard-pattern is raster-scanned over the fluorescent nanospheres using a speckle pattern with a very fine structure that illuminates the fluorescent imaging method that uses a scattering layer with a high-index substrate as an alternative solution for the same problem: by using wavefront shaping, modal scrambling in multimode fibers can be compensated. Then, the fiber can be inserted in tissue and used as an imaging device. Multimode fibers are extremely thin (down to 100 μm), and therefore this can be seen as a minimally invasive way to obtain images from deep inside tissue. Here, we demonstrate confocal reflection imaging through fibers, as a way to increase contrast when imaging unstained biological specimens. With a transmission matrix approach, we create focused spots at the distal end of a fiber, and collect the backscattered signal back through the fiber. Then, from the returning field measured in the proximal end, we can reconstruct the backscattered field as it came back from the sample in the distal end. This reconstructed field can be filtered with a digital pinhole, achieving the desired confocal effect. We also propose an alternative correlation-based filtering technique, which also achieves confocal filtering but with reduced computational loads. Results are shown for the imaging of human epithelial cells and polystyrene beads. We also show that the proposed technique can resolve the interfaces of a cover glass. These results show that the confocal approach can significantly enhance the contrast in images obtained through multimode fibers.

Ultrathin endoscopes: nonlinear imaging at the tip of a multi-mode fiber

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Current state-of-the-art micro-endoscopes are not thin enough for applications such as neuroimaging and optogenetics. These applications require shrinking the diameter up to a few tens of microns, making multimode fibers an attractive alternative. Unfortunately, the different velocities of propagating modes, scattering and spatial and temperature perturbations scramble the transmitted image. A theoretical solution was proposed long ago, in 1976, by Yariv based on phase conjugation. However, not until recently advances in wavefront control, spatial light modulators, and computational power, have made the use of multimode fibers a realistic option. Nevertheless, significant developments are still needed to translate this principle into practical biomedical applications.

In this talk, we discuss the different existing approaches to use a multimode fiber as endoscope. We present our high-speed focusing and imaging technique based on a transmission matrix approach and the use of a digital micromirror device to create a fiber scanning endoscope. Furthermore, we will discuss our latest results comparing the performance of a variety of multimode fibers with different properties (diameter, index profile, etc). The performance is quantified in terms of robustness to spatial perturbations and the quality of the focus created.
Recent advances in wavefront shaping techniques have enabled so-called lensless endoscopes using fiber probes. Unlocking the full potential of such endoscopes call for the capability of optically sectioned and/or label free imaging. Or in other words, imaging through fibers must retain the functionality of a nonlinear microscope. This is a key challenge due to the temporal broadening of ultrashort pulses in fibers owing to modal dispersion.

Here, we detail the first ever demonstration of two photon fluorescence imaging at the distal tip of a conventional graded index (GRIN) multimode fiber. GRIN fibers possess a high mode density, excellent throughput and limited temporal broadening. These features, in addition to its ready availability, make them attractive candidates for ultrathin endoscopes. In our approach, we apply the transmission matrix formalism and treat these fibers akin to highly scattering media. This lets us retrieve combinations of input modes that would generate intense focal spots throughout the field of view. Furthermore, we identify a regime where the modal dispersion in the fiber is minimal and two-photon excitation with femtosecond light pulses is possible. This allows us to perform two-photon imaging with ultrashort pulses in an epi-detection configuration analogous to conventional nonlinear microscopes. Finally, these concepts are validated by acquiring optically sectioned two photon fluorescence images of 3D samples with cellular resolution. We believe this first report of an ultrathin rigid endoscope of only 125 μm thickness would further accelerate the development of novel tools for demanding applications in biological imaging and opto-genetics.

9717-50, Session 13

**Two-photon excitation endoscopy through a multimode optical fiber**

Edgar E. Morales Delgado, Demetri Psaltis, Christophe Moser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

The large number of modes that can propagate through multimode optical fibers provides a larger information capacity than that allowed in fiber bundles. Therefore, in the field of imaging, multimode fibers potentially allow the transmission of images with higher resolution. However, image transmission through multimode fibers is not direct as in the fiber bundle case, in which each of the fiber cores can relay a portion of the distal image. In multimode fibers, a distribution of intensity is scrambled by the propagating modes, leading on the other side to a speckle-like pattern that doesn’t resemble the initial distribution.

Here we demonstrate for the first time two-photon excitation imaging of fluorescent beads through a multimode optical fiber. We show that our method maintains the advantages of two-photon excitation microscopy compared to single-photon excitation such as reduced photon-bleaching, deeper penetration depth and sectioning capability. Our method is based on time-gated digital phase conjugation, which allows the generation of focused pulses on the other side of a multimode fiber. To acquire an image, the focused femtosecond pulse is scanned in a three-dimensional mesh, producing two-photon excitation on each spatial location of the sample. By collecting the fluorescence through the fiber, a 3D two-photon image is reconstructed.

9717-51, Session 13

**Two-photon fluorescence imaging through multicore fiber with digital phase conjugation**

Nicolino Stasio, Donald Conkey, Christophe Moser, Demetri Psaltis, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

We present near diffraction limited two photon fluorescence (TPF) imaging through a lensless, multi-core fiber (MCF) endoscope utilizing digital phase conjugation. The ultra-small size of MCFs make them desirable tools for imaging deep into the body. TPF imaging enables optical sectioning and is widely used in brain and biological imaging and is a desired modality for fiber endoscopes. Previous implementations of TPF imaging through MCFs focus and scan the light from individual cores for image formation. In such systems the resolution is limited by the MCF core spacing, although a lens may be used to improve the resolution at the expense of the field of view. A recent work has improved the resolution limitation using custom built MCFs for focusing and scanning of ultrafast pulses using wavefront shaping. Here we present digital phase conjugation for ultrafast pulse focusing through a MCF for an imaging resolution independent of core spacing. Furthermore, the phase conjugation technique does not require the use of a lens at the fiber end for focus formation and is compatible with commercially available MCFs with a large number of cores. Here, we present a 3000 core MCF endoscope and demonstrate ultrafast pulse focusing with sufficient focus spot contrast and power for TPF endoscope imaging. We construct TPF images by digitally scanning the phase conjugated focus on the target object and collecting the emitted fluorescence through the MCF. This work demonstrates the viability of digital conjugation combined with commercially available MCFs for higher resolution lensless, two photon endoscopy.

9717-52, Session 13

**Label free imaging system for measuring blood flow speeds using a single multi-mode optical fiber**

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Wavefront shaping for focusing and controlling light fields through multi-mode optical fibers (MMFs) is an attractive platform for developing next-generation micro-endoscopes for deep tissue imaging and targeted light delivery for biomedical applications. While recent progress in the field was geared at imaging fluorescent markers at the distal end of the MMF, there are a number of label-free (non-fluorescent) imaging modalities that are also beneficial for deep-tissue imaging. In this work we demonstrate a single MMF-based label-free optical imaging system for measuring blood flow speeds at the distal end of the fiber. We use the transmission-matrix (TM) approach to raster-scan a focal spot across the distal fiber facet, and image the cross-polarized back-reflected light at the proximal facet using a camera. Light back-scattered light by moving scatterers, collected by the same multimode fiber, experiences reduction in its spatial coherence, which can be quantified as spatial speckle contrast. By computing the mean back-reflected speckle contrast value across the proximal fiber facet for every focal spot position we obtain spatially-resolved flow speed maps across a 100 μm wide field of view. Furthermore, physiologically-relevant flow speeds (1-10 mm/s for typical capillary flow speeds) are measured by selecting an appropriate camera integration time. We demonstrate this approach in-vitro using microfluidic capillaries carrying blood-mimicking intralipid phantom. The proposed system can find application in minimally-invasive studies of neurovascular coupling in deep brain structures, such as the hippocampus, and can be particularly suited for the study of animal models of stroke and Alzheimer’s disease.

9717-53, Session 13

**Fluorescence and optical-resolution photoacoustic imaging through capillary waveguides**

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Endoscopy can be used to obtain high-resolution images at large depths in biological tissues. Usually endoscopic devices have a diameter ranging from 1 to few millimeters. Using Digital Phase Conjugation (DPC), it is possible to adapt ultrathin multimode fibers (MMFs) to endoscopic purposes [1-5]. Fluorescence imaging with MMFs was demonstrated in [4] and photoacoustic imaging in [5]. Recently, we demonstrated that a 330 μm diameter, water-filled silica capillary waveguide (CWG) can guide high frequency ultrasound waves through a 3 cm thick fat layer, allowing optical resolution photoacoustic imaging. In this case, the photoacoustic signal was created by focusing light through a microscope objective placed on the sample side [6]. Here we demonstrate that using DPC, the same water-filled CWG (3 cm long) can be used as an endoscopic probe to obtain both fluorescence and optical resolution photoacoustic imaging, with no optical or acoustic elements at the tip of the waveguide.

References

9717-54, Session 14
Time-reversed ultrasonically encoded (TRUE) optical focusing inside scattering media with high power gain
Cheng Ma, Washington Univ. in St. Louis (United States); Xiao Xu, Washington Univ. in St. Louis (United States); Lihong V. Wang, Washington Univ. in St. Louis (United States)

Focusing light deep inside scattering media plays a key role in such biomedical applications as high resolution optical imaging, control, and therapy. In recent years, wavefront shaping technologies have come a long way in controlling light propagation in complex media. A prominent example is time-reversed ultrasonically encoded (TRUE) focusing, which allows noninvasive introduction of “guide stars” inside biological tissue to guide light focusing. By measuring the optical wavefront emanating from an ultrasound focus created at the target location, TRUE determines the desired wavefront non-iteratively, and achieves focusing at the target position via a subsequent optical time reversal. Compared to digital counterparts that employ slow electronic spatial light modulators and cameras, analog TRUE focusing relies on nonlinear photorefractive crystals that inherently accommodate more spatial modes and eliminate the troublesome alignment and data transfer required by digital approaches. However, analog TRUE focusing suffers from its small gain, defined as the energy or power ratio between the focusing and probing beams in the focal volume. Here, by implementing a modified analog TRUE focusing scheme that squeezes the duration of the time-reversed photon packet below the carrier-recombination-limited hologram decay time of the crystal, we demonstrated a photon flux amplification much greater than unity at a preset focal voxel in between two scattering layers. Although the energy gain was still below unity, the unprecedented power gain will nevertheless benefit new biomedical applications.

9717-55, Session 14
High-speed wavefront measurement using a lock-in camera for time-reversal based optical focusing inside scattering media
Yan Liu, Cheng Ma, Yuecheng Shen, Washington Univ. in St. Louis (United States); Lihong V. Wang, Washington Univ. in St. Louis (United States)

Optical focusing plays a central role in biomedical optical imaging, manipulation, and therapy. However, in scattering media, direct optical focusing becomes infeasible beyond ~10 mean free paths. To break this limit, time-reversed ultrasonically encoded (TRUE) optical focusing phase-conjugates ultrasonically tagged diffuse light back to the ultrasonic focus, thus forming a focus deep inside scattering media. In previous works, the speed of wavefront measurement was limited by the low frame rate of the camera used to record the four images required for phase-shifting holography. Moreover, most of the bits of a pixel value were used to represent an informationless background caused by the large amount of untagged light, increasing the amount of data to transfer and necessitating the use of costly high-resolution analog-to-digital converters (ADCs). Here, we developed a digital TRUE focusing system based on a lock-in camera (300?300 pixels), in which each pixel performs analog lock-in detection on chip. Since only the information of the signal, not that of the background, is digitized, the lock-in camera reduces the amount of data to transfer, and enables the use of cheap low-resolution ADCs. Using this lock-in camera, we were able to measure the wavefront of ultrasonically tagged light in less than 0.3 ms, and to achieve TRUE focusing in between two ground glass diffusers. Even when the signal-to-background ratio dropped to 6.32?10^-4, a phase sensitivity as low as 0.51 rad could still be realized, which is more than enough for digital optical phase conjugation.

9717-56, Session 14
Focusing light in deep tissue with time-reversed ultrasound microbubble encoded light
Haowen Ruan, Mooseok Jang, Changhui Yang, California Institute of Technology (United States)

Optical scattering of biological tissue limits the penetration depth of conventional optical techniques, which rely on the detection of ballistic photons. Recent developed optical phase conjugation (OPC) technique breaks through this depth limit by shaping an optical wavefront that can “undo” the optical scattering. Assisted with an ultrasound focus, this technique enables optical focusing inside biological tissue in a freely addressable fashion. However, ultrasound modulation efficiency is low and the focusing resolution is limited by the ultrasound. Here we present a new technique, time-reversed ultrasound microbubble encoded (TRUME) optical focusing, which is able to provide high focusing efficiency and sub-ultrasound resolution. This technique achieves the wavefront solution by taking the difference of the optical fields captured outside the sample before and after ultrasound-driven microbubble destruction. We experimentally demonstrate that a focus with ~2 mm size was formed through a 2-mm thick biological tissue using this method. While the size the microbubble sets the resolution of an individual focus, the scale of the ultrasound focus limits the focusing addressability of this technique. Importantly, by utilizing the nonlinear destruction of microbubbles, the TRUME technique breaks the addressable focus resolution barrier imposed by the ultrasound focus. We experimentally demonstrate a 2-fold improvement in addressability using this effect. Since microbubbles are widely used as ultrasound contrast agents in human, this technique provides a promising solution for focusing light deep inside biological tissue.
Controlling the light distribution through turbid media with wavefront shaping based on volumetric optoacoustic feedback

Xosé L. Deán-Ben, Héctor Estrada, Ali Özbek, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

Wavefront shaping based on optoacoustic (photoacoustic) feedback has recently emerged as a promising tool to control the light distribution in optically-scattering media. In this approach, the phase of a short-pulsed light beam is spatially-modulated to create constructive light interference (focusing) at specific locations in the speckle pattern of the scattered wavefield. The optoacoustic signals generated by light absorption provide a convenient feedback mechanism to optimize the phase mask of the spatial light modulator in order to achieve the desired light intensity distribution. The optimization procedure can be done by directly considering the acquired signals or the reconstructed images of the light absorption distribution. Recently, our group has introduced a volumetric (three-dimensional) optoacoustic wavefront shaping platform that enables monitoring the distribution of light absorption in an entire volume with frame rates of tens of Hz. With this approach, it is possible to simultaneously control the volumetric light distribution through turbid media. Experiments performed with absorbing microparticles distributed in a three-dimensional region showcase the feasibility of enhancing the light intensity at specific points, where the size of particles also determines the maximum attainable signal enhancement. The advantages provided by optoacoustic imaging in terms of spatial and temporal resolution anticipate new capabilities of wavefront shaping techniques in biomedical optics.

Iterative calibration of a digital optical phase conjugation system

Mehdi Azimpour, Farid Atry, Ramin Pashaie, Univ. of Wisconsin-Milwaukee (United States)

Digital optical phase conjugating (DOPC) is an emerging technique for suppressing scattering in biological tissues. This system has potential applications in deep tissue fluorescence tomography, laser therapy, or targeted optogenetic stimulation. Optical phase conjugation process extracts the complex field information of the scattered light by employing a scientific camera and holography techniques, and then, it uses a spatial light modulator (SLM) to modulate a collimated reference beam and produce a time-reversed version of the sample beam. DOPC system requires precise pixel-to-pixel registration between the wave front sensor and the SLM and misalignments and imperfections in the optical components can reduce the efficiency of the system significantly. In this paper, we propose an iterative calibration process based on the Zernike polynomials to compensate optical imperfections in a DOPC system such as substrate curvature of the spatial light modulator and also aberrations in the reference beam without adding extra complexity or optical components to the original system. The proposed calibration process was applied after initial alignment of a DOPC system and an improvement by approximately one order of magnitude in the peak to background ratio of the system was observed for a highly scattering sample.

Transmissive liquid-crystal device correcting primary coma aberration and astigmatism in laser scanning microscopy

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Laser scanning microscopy allows 3D cross-sectional imaging in a biospecimen. However, some aberrations degrade the quality of images. We previously developed transmissive liquid-crystal devices compensating for spherical aberrations that were predominant during observation especially at deep regions in samples. The device, inserted between the objective lens and the revolver of the microscope, improved the quality of fluorescent images in fixed mouse-brain slices, which was originally degraded by spherical aberration, in a two-photon excitation laser scanning microscope. In this study, we newly developed a transmissive device that corrects primary coma aberration and astigmatism because these asymmetric aberrations can also often deteriorate image quality considerably even during observation at surface of samples. The device’s performance was evaluated by observing fluorescent beads using single-photon excitation laser scanning microscopy. The fluorescent intensity in a bead image, artificially degraded mainly by coma aberration in the x-direction, was increased by 1.6 times after correction with the device. Furthermore, the x- and z-widths of the bead image were reduced to 60%. On the other hand, the fluorescence intensity of the image degraded mainly by astigmatism was increased by 1.4 times after correction. The difference between the x- and y-widths of the bead image affected by astigmatism reduced to 20%. In addition, the focal-plane lateral width and the z-width were reduced to 80% and 70%, respectively. Our device successfully corrected several asymmetric aberrations to improve the fluorescent signal and spatial resolution, and it is expected to be useful for observing various biospecimens.

Use of a GPU for fast deconvolution in wavefront coding light sheet imaging

Jacob Licea-Rodriguez, David Castillo-Andreo, Omar E. Olarte, Pablo Loza-Alvarez, ICFO - Institut de Ciències Fotòniques (Spain)

The observation of fast dynamic processes with high spatiotemporal resolution is one of the most important requirements of biological sciences. Some advanced optical imaging approaches, such as light-sheet fluorescence microscopy (LSFM), have been used to acquire images up to a few volumes-per-second (vps). However, this is not enough for some biological experiments, such as the imaging of the propagation of calcium waves in a living organism, which require imaging at volumetric videorate of about 30 vps. An interesting passive alternative for fast 3D imaging is the use of wavefront coding to extend the depth-of-field (DOF) of the collection arm of a light-sheet microscope. By doing this, the light-sheet can be rapidly swept within the extended DOF capturing the 3D features of the sample at volumetric videorates [1]. As in this approach the DOF is extended by coding the pupil function of the imaging lens by using a custom-designed phase mask, a deconvolution is required to decode the information of the captured images. Therefore, to preserve the overall speed of the imaging chain this deconvolution strategy should be optimized. In this work we present the use of a custom-developed deconvolution software, based on a Graphical Processor Unit (GPU), to perform fast 3D deconvolutions. We present results on biological samples using two designed phase masks for both medium and high numerical aperture configurations.

Analysis of design for Hartmann-Shack measurements under usage of Fourier-iteration and Zernike approximation wavefront reconstruction methods

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The measurement of a wavefront is a powerful tool for characterization of optical systems. The most commonly used wavefront measurement technique is the method of local-light aberrometry. The conventional version of this kind of measurement principle is the Hartmann-Shack wavefront sensor. This method returns the result of the matrix of spatially resolved gradients of the wavefront. However, the last and crucial step of the wavefront analysis is the reconstruction of the wavefront from the measured data packets. The issues of the measurement preparation and design are interesting in the same volume. The work presented here describes the comparison between a Fourier-Iteration algorithm and the Zernike approximation method for the wavefront reconstruction in relation to the measurement design. In the context of this work, the term “design” of the measurement refers to the issue of the number and relative position of the measurement points. In this work, the behavior of the reconstruction method under usage of the Monte-Carlo simulation was analyzed. The optimum number of points and point distribution were found and validate the parameter to describe the impact of measurement errors on the analysis results. Based on this parameter, the Monte-Carlo based simulation to make the design of the experiment with the highest accuracy was realized. The presented comparison technique was applied to determine the optimum measurement point distribution over the beam’s surface.

Spatially resolving the optical energy density inside a scattering medium

Oluwafemi S. Ojambati, Ad Lagendijk, Allard P. Mosk, Willem L. Vos, Univ. Twente (Netherlands)

In nature, there are numerous complex materials that strongly affect wave propagation due to scattering. Due to strong scattering of waves, the materials become opaque and it is quite challenging to probe inside the materials, such as biological tissue and paint. The diffusion equation is known to accurately describe the energy density distribution inside a scattering medium when a plane wave is incident on the medium [1]. With an optimized incident wavefront, however, the spatial distribution of the energy density inside the scattering medium remains to be observed experimentally.

Recently, we have measured the spatially-integrated energy density inside a scattering medium when the incident wavefront is optimized [2]. Nevertheless the energy density was not spatially resolved, which will be addressed here. We retrieved the spatial energy density inside the scattering medium by detecting the fluorescent intensity from single and isolated fluorescent probes located inside the medium. We managed to determine the depth of the fluorescent particles from the size of the diffuse fluorescent intensity spot at the back surface of the sample. Our experimental data reveal a characteristic distribution of the energy density, which is comparable to the distribution of the fundamental diffusion mode, which is expected.

References

9718-1, Session 1

The evolution of interferometry from metrology to biomedical applications (Keynote Presentation)

James C. Wyant, The Univ. of Arizona (United States)

Interferometry is a powerful tool often used for the metrology of surfaces with many applications in industries such as optical fabrication, data storage, machine tool, and semiconductor. For many years interferometers have been built into microscopes so surface microstructure can be measured. Phase-shifting interferometric techniques have provided an extremely accurate rapid way of getting the interferogram data into the computer and the inherent noise in the data taking process is so low that in a good environment angstrom or sub-angstrom surface height or thickness measurements can be performed. The recent development of single-shot phase-shifting techniques has made it possible to perform accurate phase measurement techniques in less than ideal environments and to make movies showing how surface shape or optical thickness is varying with time. These same interferometric techniques can be applied to biomedical applications. This paper will trace the history of the development of these interferometric techniques and the application of these techniques to looking at cells and tissues.

9718-2, Session 1

Non-iterative adaptive optical microscopy using wavefront sensing (Invited Paper)

Joel Kubby, Xiaodong Tao, Oscar A. Azucena Jr., Marc R. Reinig, Qinggele Li, Univ. of California, Santa Cruz (United States); Dare Bodington, Univ. of Rochester (United States)

This talk will review the development of wide-field, confocal and two-photon microscopes with wavefront sensing and adaptive optics for correcting refractive aberrations and compensating scattering when imaging through thick tissues (Drosophila embryos and mouse brain tissue). To make wavefront measurements in biological specimens we have modified the laser guide-star techniques used in astronomy for measuring wavefront aberrations that occur as star light passes through Earth’s turbulent atmosphere. Here sodium atoms in Earth’s mesosphere, at an altitude of 95 km, are excited to fluoresce at resonance by a high-power sodium laser. The fluorescent light creates a guide-star reference beacon at the top of the atmosphere that can be used for measuring wavefront aberrations that occur as the light passes through the atmosphere. We have developed a related approach for making wavefront measurements in biological specimens using cellular structures labeled with fluorescent proteins as laser guide-stars. An example is a fluorescently labeled centrosome in a fruit fly embryo or neurons and dendrites in mouse brains. Using adaptive optical microscopy we show that the Strehl ratio, the ratio of the peak intensity of an aberrated point source relative to the diffraction limited image, can be improved by an order of magnitude when imaging deeply into live dynamic specimens, enabling near diffraction limited deep tissue imaging.

9718-3, Session 1

High resolution quantitative phase imaging of live cells with constrained optimization approach

Vimal Prabhu Pandiyian, Indian Institute of Technology Hyderabad (India); Kedar B. Khare, Indian Institute of Technology Delhi (India); Renu John, Indian Institute of Technology Hyderabad (India) and Indian Institute of Technology Delhi (India)

Quantitative phase imaging (QPI) aims at studying weakly scattering and absorbing biological specimens with sub-wavelength accuracy without any external staining mechanisms. Use of a reference beam at an angle is one of the necessary criteria for recording of high resolution holograms in most of the interferometric methods used for quantitative phase imaging. The spatial separation of the dc and twin images is decided by the reference beam angle and Fourier-filtered reconstructed image will have a very poor resolution if hologram is recorded below a minimum reference angle condition. However, this is always inconvenient to have a large reference beam angle while performing high resolution microscopy of live cells and biological specimens with nanometric features. In this paper, we treat reconstruction of digital holographic microscopic images as a constrained optimization problem with smoothness constraint in order to recover only complex object field in hologram plane even with overlapping dc and twin image terms. We solve this optimization problem by gradient descent approach iteratively and the smoothness constraint is implemented by spatial averaging with appropriate size. This approach will give excellent high resolution image recovery compared to Fourier filtering while keeping a very small reference angle. We demonstrate this approach on digital holographic microscopy of live cells by recovering the quantitative phase of live cells from a hologram recorded with nearly zero reference angle.

9718-4, Session 1

Label-free three-dimensional refractive-index acquisition by micro-manipulations of cells in suspension

Natan T. Shaked, Tel Aviv Univ. (Israel)

Our latest methods for non-invasive label-free acquisition of the three-dimensional (3-D) refractive-index maps of live cells in suspension are reviewed. These methods are based on the acquisition of off-axis interferograms of single or multiple cells in suspension from different angles using an external interferometric module, while fully rotating each cell using micro-manipulations. The interferometric projections are processed via computed tomographic phase microscopy reconstruction technique, which considers optical diffraction effects, into the 3-D refractive-index structure of the suspended cell. Till now, tomographic phase microscopy was obtained by acquiring a series of interferograms of the light transmitted through the sample in different angles by either using an entire sample rotation, or patch clamping a single cell, which is invasive to the cells, or alternatively, using various angles of illumination, which causes a limited acceptance angle, and an incomplete 3-D Fourier spectrum. In contrast, our methods allow fast acquisition with full angular range, and thus obtain an accurate 3-D refractive-index map of the imaged cell. By inspection of the 3-D refractive-index distribution of cells in suspension, the proposed methods can be useful for high-throughput, label-free characterization of biological processes and cellular transformations from healthy to pathological conditions.

9718-5, Session 1

Holographic microscopy in low coherence

Radim Chmelik, Jiri Petracek, Vera Kollarova, Michala Slaba, CEITEC Brno Univ. of Technology (Czech Republic)

Low coherence of the illumination substantially improves the quality of holographic and quantitative phase imaging (QPI) by elimination of
the coherence noise and various artefacts and by improving the lateral resolution of the holographic microscope. Coherence-controlled holographic microscope (CCHM) that is an off-axis holographic system allowing QPI within the range from complete coherent to incoherent refocusing verified these advantages.

Next important positive effect of low coherence illumination is coherence gating which constraints imaging of some spatial frequencies of an object axially thus forming an optical section in the wide sense. In this way the depth discrimination capability of the microscope is introduced at the price of restricting the axial interval of possible numerical refocusing. We describe theoretically these effects for the whole range of illumination spatial and temporal coherence. We also show that the axial refocusing constraints can be overcome using advanced mode of imaging based on mutual lateral shift of reference and object image fields in CCHM.

Lowering the spatial coherence of illumination means increasing its numerical aperture. We study how this change of the illumination geometry influences 3D objects QPI and especially the interpretation of live cells QPI in terms of the dry mass density measurement.

In this way a strong dependence of the imaging process on the light coherence is demonstrated. The theoretical calculations and numerical simulations are supported by experimental data including time-lapse observations of live cells.

9718-9, Session 1

Three-axes digital holographic microscopy for studying bacteria hydrodynamics
Silvio Bianchi, Filippo Saglimbeni, Roberto Di Leonardo, Sapienza Univ. di Roma (Italy)

Digital Holographic Microscopy allows to numerically retrieve three dimensional information encoded in a single 2D snapshot of the coherent superposition of a reference and a scattered beam. Since no mechanical scans are involved, holographic techniques have a superior performance in terms of achievable frame rates. Unfortunately, numerical reconstructions of scattered field by back-propagation leads to a poor axial resolution. Here we show that overlapping the three numerical reconstructions obtained by tilted red, green and blue beams results in a great improvement over the axial resolution and sectioning capabilities of holographic microscopy.

We use our technique to study the swimming features of E. coli. This rod-shaped bacterium propels itself rotating its flagella which form a single helical bundle. Most experiments on E. coli swimming have been performed using two-dimensional microscopy techniques. However there is a number of problems, in particular hydrodynamic interactions between cells and solid interfaces, that are intrinsically three dimensional and thus require a volumetric imaging. Our technique furnishes 3D tracking of size, position, and orientation of cells swimming towards a solid wall. Understanding the hydrodynamics of the near-wall swimming of bacteria is of high interest for shedding light on the first stage of biofilm formation.

9718-10, Session 2

Phase microscope imaging in phase space (Invited Paper)
Colin J. Sheppard, Istituto Italiano di Tecnologia (Italy); Shalin B. Mehta, Marine Biological Lab. (United States)

Imaging in a bright field or phase contrast microscope is partially coherent. We have found that the image can be conveniently considered and modeled in terms of the Wigner distribution function (WDF) of the object transmission. The WDF of the object has a simple physical interpretation for the case of a slowly varying object. Basically, the image intensity is the spatial marginal of the spatial convolution of the object WDF with the phase space imager kernel (PSI-kernel), a rotated version of the transmission cross-coefficient. The PSI-kernel can be regarded as a partially-coherent generalization of the point spread function.

This approach can be extended to consider the partial coherence of the image itself. In particular, we can consider the mutual intensity, WDF or ambiguity function of the image. It is important to note that the spatial convolution of the object WDF with the PSI-kernel is not a WDF, and not the WDF of the image. The phase space representations of the image have relevance to phase reconstruction methods such as phase space tomography, or the transport of intensity equation approach, and to the three-dimensional image properties.
Study the effect of resolution on focusing process via optical phase conjugation

Te-Jen Kung, Chia-Ta Tseng, Snow H. Tseng, National Taiwan Univ. (Taiwan)

Optical phase conjugation (OPC) is a method on focusing light through a macroscopic scattering medium. In order to demonstrate this method, we simulate the propagation of monochromatic light through the scattering medium using the two-dimensional pseudospectral time-domain (PSTD) simulation technique. We analyze the effect of resolution on focusing process: resolution of the phase conjugate mirror (PCM), the discretization of the recorded phase. Simulation results show that, focused light intensity increases with higher resolution of the phase profile.

Propagating light through a scattering medium with specific amplitude and phase

Snow H. Tseng, Te-Jen Kung, Min-Lun Yu, National Taiwan Univ. (Taiwan)

By means of numerical solutions of Maxwell’s equations, we model the complex light scattering phenomenon. Light propagation through scattering medium is a deterministic process; with specific amplitude and phase, light can propagate to the target position via multiple scattering. By means of numerical solutions of Maxwell’s equations, the complex light scattering phenomenon can be accurately analyzed. The reported simulation enables qualitative and quantitative analyses of the effectiveness of directing light through turbid media to a targeted position.

Single shot recovery of hologram from speckle field

Vini R. V., Indian Institute of Space Science and Technology (India); Kyoohyun Kim, KAIST (Korea, Republic of); Atul S. Somkuwar, Indian Institute of Space Science and Technology (India); YongKeun Park, KAIST (Korea, Republic of); Rakesh K. Singh, Indian Institute of Space Science and Technology (India)

Non invasive imaging of objects through and inside random media is a demanding area in optics with important applications in the field of biomedical imaging. The random media such as the biological tissues induces light scattering and the information is scrambled into a complex interference patterns called laser speckles. Numerous amounts of innovative techniques for imaging through and inside random media are introduced till date such as inverse scattering, time gating, phase conjugation and wave front shaping, holography and digital holography, speckle correlation, ghost imaging, etc.

In this paper we describe and demonstrate an alternative approach for the recovery of hologram from the laser speckle which will be useful for imaging of the object lying behind the opaque scattering medium. We demonstrate that a single image (single shot imaging) of the scattered field from the random media is sufficient to recover the hologram generating behind the medium. The technique utilizes concepts of speckle holography together with speckle correlation (two point intensity correlation) at the far field for the recovery of hologram at the scattering plane. The object information behind the random media is encoded as an interference pattern and is made to illuminate the random phase screen. The speckle field with scrambled hologram information is Fourier transformed using a lens, which gives the opportunity to replace ensemble averaging with spatial averaging by the assumption of spatial stationarity and ergodicity of the speckle field. The speckle correlation at the Fourier plane results in the intensity correlation function which is analogous to squared modulus of the complex coherence function. The measured intensity correlation function is able to give only the modulus of the coherence function where the phase information is lost. In order to retrieve the complex coherence function an independent reference speckle field generated from an off axis point source is coherently added with the speckle field from the scattering plane. Thus a digital hologram at the scattering plane can be recovered from the speckle field using intensity correlation function measured at the Fourier plane and by using van Cittert-Zernike theorem. Our proposed technique is able to recover the hologram with high accuracy and thereby give opportunity to retrieve the object information using digital holography principles.

Reversibility of scattered fields

Renjie Zhou, Massachusetts Institute of Technology (United States), Taewoo Kim, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

In recent years, tremendous efforts have been spent on deep tissue imaging using phase conjugation, a technique used to undo the effects of light scattering in a thick tissue. Despite the early debates between Yariv and Wolf, it is still not well understood physically how deep can a field propagate into biological tissue and still be phase conjugated. In order to answer this question, we developed a light scattering theory to describe the evolution of the phase associated with a field scattered by a thick tissue block. The multiple scattering through the sample is simplified to a series of single scattering through consecutive thin tissue slices. With this theory, we identify the limits of the phase conjugation operation and recover the previous results by Yariv and Wolf, which asserts that phase conjugation is rooted in small angle approximation. Importantly, we discover the fundamental principle that rules phase conjugation: the mean axial wavenumber of a field progressively decreases to zero as it scatters multiple times. At this point, phase becomes a spatially random variable and phase conjugation becomes impossible. This result describes a fundamental phenomenon: the interaction between a deterministic object and a deterministic field can result in a random scattered field. We show that this phenomenon is rooted into Heisenberg’s uncertainty principle.

Quantitative phase imaging with a hybrid diffractive-refractive optical lens doublet (Invited Paper)

Alexander Jesacher, Walter H. Harm, Stefan Bernet, Monika Ritsch-Marte, Medizinische Univ. Innsbruck (Austria)

We use a hybrid diffractive-refractive optical lens doublet for quantitative phase microscopy. The combination of a varifocal Moiré Fresnel lens and a polymer lens of tunable refractive power allow one to separately change the Abbe number and the refractive power. Thus the hybrid lens may be tuned to an optimal operating regime for quantitative phase microscopy based on a two-color transport of intensity (TIE) approach which utilizes chromatic aberrations (rather than intensity recordings) in adjacent planes to reconstruct the optical path length of a phase object. The method is applied to the quality assessment of laser-written waveguides in fused silica.

Conference 9718: Quantitative Phase Imaging II

9718-11, Session 2

9718-12, Session 2

9718-13, Session 2

9718-14, Session 2

9718-15, Session 2
9718-16, Session 2

Spectral interferometric techniques for high-sensitivity measurement of sperm morphology

Yizheng Zhu, Chengshuai Li, Virginia Polytechnic Institute and State Univ. (United States)

Morphological assessment of spermatozoa is of critical importance for in vitro fertilization (IVF), especially intracytoplasmic sperm injection (ICSI)-based IVF. In ICSI, a single sperm cell is selected and injected into an egg to achieve fertilization. The quality of the sperm cell is found to be highly correlated to IVF success. Current observation relies on conventional techniques such as differential interference contrast microscopy and polarized light microscopy. These features suit well in the imaging of live sperm cells, which are small, dynamic objects with only low to moderate levels of phase and birefringence contrast. Therefore they are capable of highly sensitive phase and birefringence imaging. We will introduce the operation of both techniques and demonstrate their application to measuring the phase and birefringence morphology of sperm cells.

9718-17, Session 2

GPU-based rapid reconstruction of cellular 3D refractive index maps from tomosgraphic phase microscopy

Gili Dardikman, Natan T. Shaked, Tel Aviv Univ. (Israel)

We present highly parallel and efficient algorithms for real-time reconstruction of the quantitative three-dimensional (3-D) refractive-index maps of biological cells without labeling, as obtained from the interferometric projections acquired by tomosgraphic phase microscopy (TPM). The new algorithms are implemented on the graphics processing unit (GPU) of the computer using CUDA programming environment. The reconstruction process includes two main parts. First, we used parallel complex wave-front reconstruction of the TPM-based interferometric projections acquired at various angles. The complex wave-front reconstructions are done on the GPU in parallel, while minimizing the calculation time of the Fourier transforms and phase unwrapping needed. Next, we implemented on the GPU in parallel the 3-D refractive index map retrieval using the TPM filtered-back projection algorithm. The incorporation of algorithms that are inherently parallel with a programming environment such as Nvidia's CUDA makes it possible to obtain real-time processing rate, and enables high-throughput platform for label-free, 3-D cell visualization and diagnosis.

9718-18, Session 2

Enhanced resolution phase retrieval transport of intensity equation techniques based on inclined illumination

Juan Martínez-Carranza, Konstantinos Falaggis, Tomasz Kozacki, Warsaw Univ. of Technology (Poland)

Quantitative Phase Imaging Techniques (QPITs) have recently emerged as practical tools for measuring the phase information of non-absorbing specimens [1–3] and are routinely employed over a wide range of wavelengths that go from the optical to the x-ray regime [4–6]. These techniques allow an accurate phase retrieval of the phase properties of the sample under study with an experimental system of low complexity. For these reasons, QPITs have gained increased interest in recent years and are used in several fields of science and technology [5,7,8]. An important example of these techniques is the Transport of Intensity Equation (TIE) that relates the phase of an object to the intensity distribution by linear operators [1]. Based TIE QPITs have shown their capabilities to obtain accurate phase information [9,10]. However, the lateral resolution of TIE based techniques are limited by the numerical aperture of the optical system. In order to break this limit, the employment of structured illumination have been proposed [11]. With this approach, the directional derivatives of the phase are obtained and the final phase can be found when integrating the gradient maps [11,12] with an enhanced lateral resolution [11]. Therefore, this approach is sensitive to phase discontinuities and noise [13]. In this work, we show that the lateral resolution of the phase mapping can be improved when employing tilted illumination and the classical TIE solver. This configuration allows recovering a different portion of the frequency space according to the angle of the tilted wavefront. Thus, the retrieved phase will have an enhanced lateral resolution. Additionally, to suppress the Low Frequency Artifacts (LFAs) that affects the TIE based techniques we employ the Multi-Filter TIE approach [14], which uses a few planes to suppress effectively LFAs. The conclusions of this work are supported with numerical and experimental results.

References
9718-19, Session 2

**Tomographic wavefront retrieval using the geometric sensor**

José Manuel Rodríguez Ramos, Juan Manuel Trujillo-Sevilla, Juan José Fernández-Valdivia, Univ. de La Laguna (Spain)

The geometric sensor, a new variety of the classic curvature wavefront sensor, restores at full optical resolution the wavefront phase at the pupil of the imaging system. It is based on the Van Damm-Lane idea of using the Radon Transform to locate the same beam, the same photon, in the two blurred images. But these authors expand the phase map on Zernike polynomials, which causes a smoothing on the restored phase even when the first few hundred Zernike polynomials are used in the development.

In our case, we propose an expansion on complex exponential functions, based on a new Cartesian estimator for the phase gradients. This gets sharper phase maps (higher spatial frequencies), faster power calculation (complex exponentials contain the FFT kernel) and square pupils restoration is achieved if necessary.

We have already demonstrated lightfield generation from the focal stacks (Rodríguez Ramos et al, 2015). And lightfield acquisition allows to extract tomographical wavefront phases, a 3D map of the refractive index changes from plenoptic sensor and from combined use of the plenoptic and the geometric sensor (J.M. Trujillo-Sevilla et al, 2014). In this paper, we will show tomographical wavefront restoration from the geometric sensor, making acquisition of several defocused images varying a liquid-lens to generate the focal stack.

- Rodríguez-Ramos et al., Spanish Patent, 2015

9718-20, Session 3

**Quantitative phase-digital holographic microscopy: a new imaging modality to identify original cellular biomarkers of diseases (Invited Paper)**

Pierre Marquet, Institut Univ. en Santé Mentale de Québec, Univ. Laval (Canada) and Ctr. Hospitalier Univ. Vaudois (Switzerland) and Brain and Mind Institute, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Pascal Jourdain, Ctr. Hospitalier Univ. Vaudois (Switzerland) and Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Christian Depeursinge, Pierre J. Magistretti, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (Switzerland) and King Abdullah Univ. of Science and Technology (Saudi Arabia)

During the last decades numerous of quantitative phase (QP) microscopy techniques based on interferometer or non-interferometer approaches have been developed. Considerably simpler to implement than classical interference microscopy, while providing a reliable and QP mapping of the observed specimen, they have started to be efficiently used in the field of cell biology. In this presentation, the main advantages of QP measurement based on a digital holographic microscopy approach, allowing in particular to propagate the whole object wave (amplitude and phase) diffracted by the observed specimen during the numerical reconstruction of the digitally recorded holograms, will be explained in particular as far as living cells are concerned.

On the other hand, QP mapping, depending on both the size of the observed specimen as well as the difference between its refractive index and that of the surrounding medium, contains a wealth of information. However, its interpretation in terms of specific biological processes remains an issue. Consequently, some strategies and technical developments including QP tomography to calculate from the QP mapping relevant cell parameters will be discussed. It will be also presented how such relevant cell parameters can be used to study specific cell processes. Finally, it will be outlined how QP signal in multimodality approaches combining, e.g., fluorescence, second-harmonic generation, should allow simultaneous measurements of a large number of relevant and specific parameters yielding an extended cell profiling highly suitable for the identification of new original biomarkers of diseases, in particular psychiatric disorders.

9718-21, Session 3

**Quantification of neurotoxic effects on individual neuron cells using optical diffraction tomography**

Jonghee Yoon, Su-a Yang, Kyoohyun Kim, YongKeun Park, KAIST (Korea, Republic of)

Parkinson’s disease (PD) is a common neurodegenerative disease that causes symptoms of postural instability and slowness of movement. Neurodegeneration in dopaminergic neurons at the substantia nigra has been reported as pathologic features, however, detailed mechanisms underlying neurodegeneration are still remain unclear. To investigate a neurodegenerative process, various imaging tools including phase contrast microscopy, electron microscopy, and fluorescence microscopy are utilized. However, these imaging methods provide qualitative information and require invasive approaches such as the use of fluorescence agents or chemical fixation procedures that disturb normal physiological conditions of neuron cells.

In order to quantify the neurodegenerative process in a non-invasive manner, we exploited optical diffraction tomography (ODT). ODT is a 3D quantitative phase imaging method that measures 3D refractive index (RI) distributions of a sample which provide quantitative structural (volume, surface area, sphericity) and biochemical (protein concentration, total cellular dry mass) information. We investigated neurotoxic effects of MPP+ on SH-SY5Y cells by using quantitative information obtained from 3D RI distributions. We also performed temporal measurements of 3D RI distributions of an individual SH-SY5Y cell to analyze neurotoxic effects on intracellular vesicle dynamics.

9718-22, Session 3

**Monitoring in-vitro bovine embryo development during the first days after fertilization**

Mikhail E. Kandel, Marcello Rubessa, Daniel Fernandes, Tan H. Nguyen, Matthew B. Wheeler, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Conventional label-based contrast enhancement techniques (e.g., fluorescence) frequently modify the genetic makeup of tagged cells, making them poor candidates for use in in-vitro fertilization applications. Instead, we choose a label-free form of contrast, based on interferometric imaging, sensitive to optical path length differences. Compared to, single HeLa cells, typical mammalian ova and embryos are more than an order of magnitude thicker. As a result, regions of large phase variation lead to phase wrapping and an overall reduction in signal intensity occurs due to multiple scattering. These effects manifest themselves in low-spatial frequencies (blurs), with the desired details buried in the background. We present a
Prospects and challenges of quantitative phase imaging in tumor cell biology

Björn Kemper, Westfälische Wilhelms-Univ. Münster (Germany)

During the past decade various quantitative phase imaging (QPI) techniques were developed and continuously further improved for high resolution label-free quantitative live cell imaging. Quantitative phase imaging is based on the detection of the specimen induced optical path length changes against the surrounding environment. QPI can be integrated modular into common research microscopes for multimodal imaging and requires only low light intensities which minimizes the interaction with the sample. Thus, the technology is in particular suitable for minimally invasive long term live cell imaging. In various proof of concept studies different application areas, e.g., research on blood cells, bacteria or neuro science as well as the usage in digital pathology were explored. Here, in an overview, for the example of digital holographic microscopy (DHM), the prospects and challenges of quantitative phase imaging in tumor cell imaging are presented. This includes results from the usage of DHM in migration and motility assays to quantify cellular motions changes that are caused by different surface coatings or genetic modifications. Furthermore, label-free imaging of growth and morphology properties of different cell types and the response of tumor cells to drugs during wound healing in-vitro is illustrated. Finally, application prospects of QPI for quantitative tumor cell imaging during chemotaxis in a two or three-dimensional environment is demonstrated. In conclusion, quantitative phase microscopy has a high potential to become a powerful tool in various different areas of tumor cell biology and cancer cell research.

Quantitative label-free sperm imaging by means of transport of intensity

Praveen Kumar Poola, Vimal P. Pandiyan, Renu John, Indian Institute of Technology Hyderabad (India); Varshini Jayaraman, Univ. of Hyderabad (India)

Sperm cells are almost transparent and imaging these cells using bright-field microscopy (BFM) is very difficult without addition of external contrast agents. Zernike’s phase contrast and Nomarski’s differential interference contrast (DIC) are often-used techniques to improve the contrast. However these techniques only provide a qualitative estimation of the phase. Most common quantitative phase imaging techniques are based on interferometry and would require additional resources like coherent lasers and complex experimental set-ups. In this paper, we report non interferometric quantitative phase imaging of live sperm cells from a regular bright field microscope using the principle of transport of intensity equations (TIE). In this work we record three bright field images; one in focus, and two out of focus (+z, -z) images and implement the TIE algorithm to retrieve the 3-D phase information. Experimental results would demonstrate the capability of this technique in 3-D volume estimation of sperm cells with nanometric sensitivity. We also quantify the sperm parameters like acrosome area, head length, width and tail length. This real-time imaging technique would be highly promising for imaging phase samples without any preprocessing. This technique shows high potential as a promising candidate for real time characterization of sperm cells without any sample preparation steps and identifying the anomalies associated with the sperm cells as per WHO standards.
analysis tool is based directly on the optical path delay profile of the sample and does not necessitate decoupling refractive index and thickness in the cell quantitative phase profile; thus, it can be calculated using a single-frame acquisition. Nowadays, it is not possible to detect small spaces inside living cells, such as vacuoles and lipid droplets, which has different refraction index using bright-field microscopy without labeling. Our experimental system includes low-coherence wide-field interferometer, combined with simultaneous fluorescence microscopy system for validation. We used this system and analysis tool for studying vacuole formation in sperm cells and lipid droplets formation in adipocytes. The latter demonstration is relevant for various cellular functions as lipid metabolism, protein storage and degradation to viral replication. These processes are functionally linked to several physiological and pathological conditions, including obesity and metabolic diseases. Quantification of these biological phenomena based on the texture changes in the cell phase map has a potential as a new cellular diagnosis tool.

9718-28, Session 3

Detecting neuronal activity using two new QPI systems
Olivier Thouvenin, Mathias Fink, Claude Boccara, Institut Langevin (France)

Active neurons tend to have a different dynamical behavior compared to resting ones. Non-exhaustively, vesicular transport towards the synapses is increased, since axonal growth becomes slower. Previous studies also reported small phase variations occurring simultaneously with the action potential. Such changes exhibit times scales ranging from milliseconds to several seconds on spatial scales smaller than the optical diffraction limit. Therefore, QPI systems are of particular interest to measure neuronal activity without labels. Here, we report the development of two new QPI systems that should enable the detection of such activity. Both systems can acquire full field phase images with a sub nanometer sensitivity at a few hundreds of frames per second. The first setup is a synchronous combination of Full Field Optical Coherence Tomography (FF-OCT) and Fluorescence wide field imaging. The latter modality enables the measurement of neurons electrical activity using calcium indicators. In cultures, FF-OCT exhibits similar features to Digital Holographic Microscopy (DHM), except from complex computational reconstruction. However, FF-OCT is of particular interest in order to measure phase variations in tissues. The second setup is based on a Quantitative Differential Interference Contrast setup mounted in an epi-illumination configuration with a spectrally incoherent illumination. Such a common path interferometer exhibits a very good mechanical stability, and thus enables the measurement of phase images during hours. Additionally, such setup can not only measure a height change, but also an optical index change for both polarization. Hence, one can measure simultaneously a phase change and a birefringence change.

9718-29, Session 3

Highly sensitive kinesin-microtubule motility assays using quantitative phase imaging
Mikhail E. Kandel, Univ. of Illinois at Urbana-Champaign (United States); Kai Wen Teng, Paul R. Selvin, Univ. of Illinois (United States); Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

We provide an experimental demonstration of Spatial Light Interference Microscopy (SLIM), as a tool for measuring the motion of 25nm Tubulin structures without the use of fluorescence labels. Compared to intensity imaging methods such as phase contrast or DIC, our imaging technique relies on the ratios of images associated with optically introduced phase shifts, thus implicitly removing background illumination. The result is a precise topological map showing objects below 0.02 rad path length difference, without computational background stabilization or other post-processing steps. In contrast to other QPI techniques, we show that SLIM’s unobstructed common-path configuration leads to exceptional phase stability by avoiding the vibration, sampling error, and low-light conditions typically associated with indirect measurements from fringes or lens arrays. To demonstrate our new found capabilities, we characterize kinesin-based motility continuously over long periods- a configuration where fluorescence would typically photobleach. As our method requires an order of magnitude less exposure time compared to fluorescent techniques, relevant to the function of adherent cells, we exploit this new method to compare the motility of microtubules at low ATP concentrations, with and without the fluorescent tagging proteins formerly required to perform these studies.

9718-30, Session 4

Online quantitative phase imaging of vascular endothelia cells under continuous flow utilizing digital holographic microscopy
Maria Odenthal-Schnittler, Westfälische Wilhelms-Univ. Münster (Germany) and MOS Technologies (Germany); Angelika Vollmer, Hans Joachim Schnittler, Björn Kemper, Westfälische Wilhelms-Univ. Münster (Germany)

Fluid shear stress (FSS) is an established in-vitro tool to simulate the physiological conditions in a vascular environment which induces endothelial cell conversion into an arterial phenotype. Protein remodeling and expression can be analyzed with high specificity by proteomics and fluorescence microscopy. However, the continuous online quantification of FSS induced morphology changes (morphodynamics) of the cells is challenging. Quantitative phase imaging with digital holographic microscopy (DHM) has been demonstrated to be a versatile tool for high resolution non-destructive analysis of surfaces and multi-modal minimally-invasive monitoring of living cell cultures in-vitro. As quantitative phase imaging is based on the detection of optical path length changes the method is label-free and only requires low light intensities for object illumination which minimizes the interaction with the sample. We have explored the utilization of quantitative phase imaging with digital holographic microscopy (DHM) as a novel tool for quantifying morphological alterations of confluent cell layers during application of FSS. Primary human umbilical vein endothelial cells (HUVECs) were exposed to continuous fluidic shear stress in a transparent cone/plate flow system (BTF-system) and observed with a modular setup for quantitative DHM phase imaging for up to 75 h. The resulting series of quantitative phase images were analyzed for the temporal dependence of the average cell layer surface roughness. In addition, cell motility was quantified by automated digital holographic cell tracking. Our experimental results demonstrate that quantitative phase imaging is a reliable powerful tool to quantify the morphological response of endothelial cells to continuously applied FSS.

9718-31, Session 4

Label-free classification of white blood cell population using optical diffraction tomography
Jonghee Yoon, Kyoohyun Kim, Min-hyeok Kim, Suk-Jo Kang, YongKeun Park, KAIST (Korea, Republic of)

White blood cells (WBC) have crucial roles in immune systems which defend the host against from disease conditions and harmful invaders. Various WBC subsets have been characterized and reported to be involved in many
pathophysiologic conditions. It is crucial to isolate a specific WBC subset to study its pathophysiological roles in diseases. Identification methods for a specific WBC population are rely on invasive approaches, including Wright-Gimza staining for observing cellular morphologies and fluorescence staining for specific protein markers. While these methods enable precise classification of WBC populations, they could disturb cellular viability or functions.

In order to classify WBC populations in a non-invasive manner, we exploited optical diffraction tomography (ODT). ODT is a three-dimensional (3-D) quantitative phase imaging technique that measures 3-D refractive index (RI) distributions of individual WBCs. To test feasibility of label-free classification of WBC populations using ODT, we measured four subtypes of WBCs, including B cell, CD4 T cell, CD8 T cell, and natural killer (NK) cell. From measured 3-D RI tomograms of WBCs, we obtain quantitative structural and biochemical information and classify each WBC population using a machine learning algorithm.

9718-32, Session 4

Using quantitative interference phase microscopy for sperm acrosome evaluation

Michal Balberg, Ksawery Kalinowski, Mattan Levi, Natan T. Shaked, Tel Aviv Univ. (Israel)

We demonstrate quantitative assessment of sperm cell morphology, primarily acrosomal volume, using quantitative interference phase microscopy (IPM). The acrosome contains digestive enzymes that break down the outer membrane of the ovum, and its form and volume are important for selecting a normal spermatozoon for in-vitro fertilization (IVF). Normally, the area of the acrosome is assessed using dyes that stain the acrosomal part of the cell. Without staining, it is difficult to assess the volume of the acrosome. We have imaged fixed individual sperm cells using IPM. Following, the sample was stained and the same cells were imaged using bright field microscopy (BFM). As the same cells were imaged using both techniques, we identified the acrosome using the stained BFM image, and used it to define a quantitative corresponding area in the IPM image and determine a quantitative threshold for evaluating the volume of the acrosome. These initial findings may assist in selecting unstained sperm cells for in-vitro-cytoplasmic injection (ICSI) in the future.

9718-33, Session 4

Characterizations of individual human red blood cells from patients with diabetes mellitus

SangYun Lee, KAIST (Korea, Republic of); Seongsoo Jang, Univ. of Ulsan (Korea, Republic of) and Asan Medical Ctr. (Korea, Republic of); HyunJoo Park, YongKeun Park, KAIST (Korea, Republic of)

We systematically measured the morphological, biochemical, and biomechanical properties of individual human red blood cells (RBCs) from patients with diabetes mellitus using quantitative phase imaging technique to characterize the diabetic red cells with respect to those of the healthy. The 3-D refractive index tomograms and 2-D dynamic membrane fluctuation maps of individual RBCs are reconstructed from a set of the retrieved complex optical fields at various laser incidence angles using the Common-path diffraction optical tomography, from which volume, surface area, sphericity, hemoglobin (Hb) concentration, Hb content, and membrane fluctuation are obtained simultaneously. The correlation relations among the retrieved red cell indices of diabetic and healthy RBCs are also investigated with capabilities of individual cell measurement.

As expected, there are no significant alterations in morphologies (cellular volumes, surface area, and sphericity) between diabetic and healthy RBCs. However, despite the minute mean corpuscular Hb differences in cell blood count datasheet, the measured Hb concentrations and Hb contents of diabetic RBCs are statistically higher than those of healthy RBCs, which might be related to the glycation of Hb molecules by hyperglycemia. Meanwhile, the membrane fluctuations of diabetic RBCs are clearly diminished compared to healthy red cells, implying the significantly decreased RBC deformability. In particular, it seems that the membrane fluctuations have mild negative relationships with the reported HbA1c levels.

9718-34, Session 4

Automatic diagnosis system for prostate cancer using quantitative phase images and machine learning

Tan H. Nguyen, Shamira Sridharan, Univ. of Illinois at Urbana-Champaign (United States); Virgilia Marcias, Andre Balla, Univ. of Illinois at Chicago (United States); Minh N. Do, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

In a previous paper, we showed that refractive index measurements of transparent samples can be used to numerically stain different regions of the tissue based on their textural changes. The morphological features, i.e., shape, convexity, solidity of this stain map can be used in a simple diagnosis problem of classifying Gleason Grade 3 and Grade 4 using a Random Forest algorithm. However, this feature is not good enough in resolving the difficult cases. For example, morphological features of the high grade prostatic intraepithelial neoplasia (HGPIN) and the high grade cancer are very similar. However, one is normal, the other is malignant. The only feature that can be used to discriminate them is an existence of a thin layer of basal cells surrounding the gland. The morphological feature is not good enough to describe this layer since it contains significant contributions from non-basal pixels. Here, we push the capability of our method further by combining the morphological feature with the local textural features. They are complementary to each other and, therefore, allow us to handle more complex variation in the tissue. Here, we introduce new cases to our test bench including 131 normal cases, 60 HGPIN cases and 29 benign prostatic hyperplasia cases, all imaged using Spatial Light Interferometry Microscopy (SLIM). The accuracy of our automatic diagnosis scheme is evaluated based on comparison with ground truth verified by certified pathologists.

9718-107, Session 4

Investigation of CD4 (helpers) and CD8 (killers) T-lymphocytes with coherent phase microscopy

Anastasiya A. Bolotova, Tatiana V. Vyshenskaya, Vladislav D. Zverzhkovskiy, Moscow State Univ. of information Technologies, Radioengineering and Electronics (Russian Federation); I. V. Vasilenko, Russian Medical Academy of Post-Graduate Education (Russian Federation)

It was shown that a significant number of phase images of donor’s CD8 T-lymphocytes (killers) have an increased phase volume of cytoplasm in compare with CD4 T-lymphocytes (helpers). This phenomenon can be explained by the presence of perforin granules in the cytoplasm which cause the cytotoxic effects. Using phase microscopy and a software for calculating the integral functions of the phase portrait in frames of a previously developed 4-layer spherical cell model a cytotoxic potential of CD8 T-lymphocytes can be estimated.
Modern microscopy and "crossing the chasm" with new technologies (Keynote Presentation)

Jim Sharp, Carl Zeiss Microscopy, LLC (United States)

This presentation will explore the ZEISS 170 Year company history of optical innovation, discuss today's modern multi-dimensional/modal microscopy and examine the challenges of moving disruptive microscope technologies to mainstream.

*Geoffrey A. Moore

Phi optics: from image to knowledge (Invited Paper)

Catalin Chiritescu, Phi Optics, Inc. (United States)

Phi Optics, Inc. is an optical microscopy company that empowers life science researchers to explore deeper, discover more, and ultimately cure better. We target the live cell imaging research market, with applications in: fundamental cell science, disease identification, drug discovery, embryonic and stem cells manipulation. Phi Optics patented technology – Spatial Light Interference Microscopy (SLIM) - provides faster and more sensitive imaging of live cells and tissues than currently possible with the state-of-the-art technology. Phi Optics focus is supported by VC funding, the NSF SBIR program, and industry partners. The company is a graduate of the NSF Innovation Corps program (March 2012 cohort) and currently resides in the University of Illinois Enterprise Works Research Park hub, in Champaign, Illinois. Phi Optics was founded to commercialize technology developed at University of Illinois at Urbana-Champaign. We launched our first commercial product in February 2014 and had first sales the same year. A second generation instrument was launched in February 2015 to enable fully automatic functionality and integration with existing microscopy platforms.

We will introduce the company's history and outline the trajectory of the company from idea to invention and product development. We will share the lessons learned during Phi Optics participation in the NSF Innovation Corps and how they applied to the product development and customer discovery process.

Taking laser research results to quantitative phase imaging and beyond (Invited Paper)

Benoit F. Wattellier, Marie-Begoña Lebrun, PHASICS S.A. (France)

We describe several examples of technology transfer from academic laboratories to PHASICS. PHASICS was created in 2003 as a spin-off of LULI an academic laboratory working on plasma physics and developing high power lasers to create such objects which temperature and pressure conditions are close to those at the center of stars. In order to optimize the intensity at laser focus, several thesis treated the subject of adaptive optics for lasers. LULI decided to collaborate with ONERA who just invented a technique for wave front sensing called multiwave lateral shearing interferometry. Though developed at first for infrared metrology applications, this technique proved to be very efficient with lasers because it was able to analyze wave front of modulated beams with sharp edges. Before being industrialized the technique was further improved to a compact version called quadriwave lateral shearing interferometry.

As soon as PHASICS was created, we felt the potential of making wave front images from transparent objects because of its wide high spatial resolution. PHASICS and Institut Fresnel started a collaboration to study applications in microscopy imaging. Research subjects include biological imaging, CARS microscopy, anisotropy imaging, or laser damage testing. The results of research were then included in PHASICS products but sometimes only a tool developed during the project became a product.

We will present research works that led to transfers as well as the method we used to ensure fruitful collaboration and transfer.

Holomonitor M4: holographic imaging cytometer for real-time kinetic label-free live-cell analysis of adherent cells (Invited Paper)

Mikael Sebesta, Peter J. Egelberg, Anders Langberg, Jens-Henrik Lindskov, Kersti Alm, Birgit Janicke, Phase Holographic Imaging AB (Sweden)

Live-cell imaging enables studying dynamic cellular processes that cannot be visualized in fixed-cell assays. An increasing number of scientists in academia and the pharmaceutical industry are choosing live-cell analysis over or in addition to traditional fixed-cell assays. We have developed a time-lapse label-free imaging cytometer HoloMonitor M4. HoloMonitor M4 assists researchers to overcome inherent disadvantages of fluorescent analysis, specifically effects of chemical labels or genetic modifications which can alter cellular behavior. Additionally, label-free analysis is simple and eliminates the costs associated with staining procedures.

The underlying technology principle is based on digital off-axis holography. While multiple alternatives exist for this type of analysis, we prioritized our developments to achieve the following: a) All-inclusive system – hardware and sophisticated cytometric analysis software; b) Ease of use enabling utilization of instrumentation by expert- and entry-level researchers alike; c) Validated quantitative assay end-points tracked over time such as optical path length shift, optical volume and multiple derived imaging parameters; d) Reliable digital autofocus; e) Robust long-term operation in the incubator environment; f) High throughput and walk-away capability; and finally g) Data management suitable for single- and multi-user networks.

We provide examples of validated HoloMonitor M4 applications from routine cell culture QC to cell proliferation, viability, mitosis and multiple modes of cell death.

Optical diffraction tomography using a digital micromirror device (Invited Paper)

Seungwoo Shin, Kyohyun Kim, Sangchon Na, Taehong Kim, Kihyun Hong, Tomocube (Korea, Republic of)

Refractive index (RI) is an intrinsic optical property which provides structural and chemical information of a sample. Optical diffraction tomography (ODT) techniques reconstruct quantitative three-dimensional (3-D) RI distribution of samples noninvasively. From measured optical fields scattered by a sample from various illumination angles, 3-D RI distribution is reconstructed via the Fourier diffraction theorem. Typically, a dual-axis galvanometer has been used to control illumination angles of plane waves impinging on a sample. However, nonlinear response of the galvanometer at high voltage values and electric noise can cause positioning and jittering errors. In addition, the rotating plane of the galvanometer is difficult to be conjugated with the sample plane due to the geometry of the galvanometer. Recently, in order to control the illumination angles stably without mechanically moving parts, a spatial light modulator (SLM) has been used, but the widespread usage is restricted by the slow response and the expensive cost of the SLM.
In this work, we present an ODT technique using a digital micromirror device (DMD) for fast and stable measurement of 3-D RI distribution. Displaying binary amplitude holograms on a DMD with suitable spatial filtering, the illumination angles can be controlled with high speed and stability. Diffracted optical fields by the sample for various incident angles are measured via Mach-Zehnder interferometry. To demonstrate the presented ODT technique using a DMD, we measured 3-D RI distribution of biological samples and 3-D Brownian motion of colloidal particles with the tomogram acquisition rate of 100 Hz.

9718-40, Session 5
From university research to commercial product (Invited Paper)
Philip Mathuis, Ovizio Imaging Systems (Belgium)

Ovizio Imaging Systems, a quantitative microscopic imaging spin-off of the Université Libre de Bruxelles, Belgium, was founded in the beginning of 2010 by Philip Mathuis, Serge Jooris, Prof. Frank Dubois and Dr. Catherine Yourassowsky. The company has launched a range of specialized microscopy instruments for quantitative imaging mainly focused on the bioprocessing and diagnostics fields within the life sciences market.

During my talk I will present the story of how an idea, emerged from the research labs of the University made it to a manufactured and sold product. The talk will look at many aspects of entrepreneurship and setting up a company, finding the funding for the project, attracting people, industrialization and product design and commercialization. It will also be focused on choices one has to make during the start-up phase and methodologies that can be applied in many different settings.

9718-106, Session 5
Coherence-controlled holographic microscopy principle embodiment into Q-PHASE microscope: story of a successful technology transfer (Invited Paper)
Martin Lostak, TESCAN, a.s. (Czech Republic)

Curiously, the coherence-controlled holographic microscopy (CCHM) was brought into the world owing to the endeavor of Chmelik’s team at Brno University of Technology to avoid scanning in confocal microscopy. As coherence gating seemed to be the way, the Leith & Upatnieks proposal of incoherent holography had been considered attractive. Their method made interference system free from strict dependence on both spatial and temporal coherence. Off axis holographic system proposed on such basis has been proved capable of coherence based depth discrimination in single wide-field shot in reflected-light arrangement. Consequently, extremely low-coherence holographic imaging had been found highly contributive also to the image quality depriving it from coherence artefacts and improving its transversal resolution. This is why CCHM promised high precision of quantitative phase imaging (QPI) in transmitted light set up that was realized for cell biology. However the cost of necessarily complicated optical design and need of very precise mechanics forced the University team to search for a company capable of mastering the instrument.

It was TESCAN ORSAY the highly successful scanning electron microscopes producer that finally took charge of the commercial design. Long-term collaboration of the company with the University made possible both the CCHM technology successful transfer up to Q-PHASE scope production as well as the company Light microscopy division reinforcement. This contribution merges views of CCHM technology author and the head of the TESCAN development team.

9718-78, Session PMon
Automatic tissue segmentation of breast biopsies imaged by QPI
Hassaan Majeed, Tan H. Nguyen, Mikhail E. Kandel, Univ. of Illinois at Urbana-Champaign (United States); Virgilia Macias, Univ. of Illinois at Chicago (United States); Minh N. Do, Univ. of Illinois at Urbana-Champaign (United States); Andre Kajdacsy-Balla, Univ. of Illinois at Chicago (United States); Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

The current tissue evaluation method for breast cancer would greatly benefit from higher throughput and less inter-observer variation. Since quantitative phase imaging (QPI) measures physical parameters of tissue, it can be used to find quantitative markers, eliminating observer subjectivity. Furthermore, since the pixel values in QPI remain the same regardless of the instrument used, classifiers can be built to segment various tissue components without need for color calibration. In this work we use a texton-based approach to segment QPI images of breast tissue into various tissue components (epithelium, stroma, etc.). A tissue microarray comprising of 900 unstained cores from 400 different patients was imaged using Spatial Light Interference Microscopy. The training data were generated by manually segmenting the images for 50 cores and labelling each pixel (epithelium, stroma etc.). For each pixel in the data, a response vector was generated by the Leung-Malik (LM) filter bank and these responses were clustered using the k-means algorithm to find the centers (called textons). A random forest classifier was then trained to find the relationship between a pixel's label and the histogram of these textons in that pixel's neighborhood. The segmentation was carried out on the validation set by calculating the texton histogram in a pixel's neighborhood and generating a label based on the model learnt during training. Segmentation of the tissue into various components is an important step toward efficiently computing parameters that are markers of disease. Automated segmentation, followed by diagnosis, can improve the accuracy and speed of analysis leading to better health outcomes.

9718-79, Session PMon
Study of erythrocyte membrane fluctuation using light scattering analysis
Hoyoon Lee, Korea Univ. (Korea, Republic of); Sangyun Lee, YongKeun Park, KAIST (Korea, Republic of); Sehyun Shin, Korea Univ. (Korea, Republic of)

We present a light scattering analysis of number of red blood cells (RBCs) to investigate their rheological characteristic of the membrane. We obtained the phase information of scattered light and analyzed the scattered light intensity though multiple RBCs with fluctuating membrane. The light scattering analyses were compared between healthy controls and artificially hardened RBCs and these results are further compared with conventional measurements of RBC deformability.

9718-80, Session PMon
The study on RBC characteristic in paroxysmal nocturnal hemoglobinuria (PNH) patients using common path interferometric quantitative phase microscopy (CPIQPM)
Byung Jun Park, Youngjae Won, Byungyeon Kim, Seungrag Lee, Osong Medical Innovation Foundation (Korea, Republic of)
We have studied the RBC membrane fluctuations between a normal RBC and a RBC in Paroxysmal nocturnal hemoglobinuria (PNH) patient using common path interferometric quantitative phase microscopy (CPIQPM). CPIQPM system has provided the subnanometer optical path length sensitivity on a millisecond. We have measured the dynamic thickness fluctuations of a normal RBC membrane and a RBC membrane in PNH patient over the whole cell surface with CPIQPM. PNH is a rare and serious disease of blood featured by destruction of red blood cells (RBCs). This destruction happens since RBCs show the defect of protein which protects RBCs from the immune system. We have applied CPIQPM to study the characteristic of RBC membrane in PNH patient. We have shown the morphological shape, volume, and projected surface for both different RBC types. The results have showed both RBCs had the similar shape with donut, but membrane fluctuations in PNH patient was shown to reveal the difference of temporal properties compared with a normal RBC. In order to demonstrate the practical tool of the CPIQPM technique, we have also obtained the time series thickness fluctuation outside a cell. We have minimized the effect of the small lateral movement in the RBC membrane fluctuation map to obtain more accurate RBC membrane properties.

9718-81, Session PMon
Digital-micromirror device-based quantitative phase imaging
Renjie Zhou, Massachusetts Institute of Technology (United States); Cuifang Kuang, Zhejiang Univ. (China); Zahid Yaqoob, Peter T. C. So, Massachusetts Institute of Technology (United States)

Driven by the need in high-resolution and 3D label-free quantitative phase imaging (QPI), several new techniques recently have been developed to engineer the illumination beam. Here, we propose the use of a digital micromirror device (DMD) to modulate the illumination beam angle for QPI applications. Compared with the galvo-mirror angle scanning, DMD is compact in size and has more flexibility in selecting the illumination angles. In our system, the DMD is imaged to the back aperture of a condenser lens, enabling numerous imaging modalities. We first demonstrate its capability in performing Fourier ptychographic microscopy (FPM), a recently developed computational QPI method, with a 532 nm laser as the illumination. By coding the on/off states of the micromirrors on the DMD, the incident plane wave angle is sequentially varied. A sequence of images, corresponding to different illumination angles, are used to recover the high-resolution phase and amplitude image. Unlike the previously demonstrated LED-based FPM systems, laser illumination provides sufficient photons on the detector, thus significantly improves the imaging sensitivity. We tested our system using a USAF resolution target as well as a plant tissue paper, demonstrating its high-resolution imaging capability. Currently, we are studying selected biological samples including red blood cells and HeLa cells. Taking the advantage of DMD speed (potentially up to 32 kHz), we envision this technique to be widely used for the real-time live imaging in biology and medicine.

9718-83, Session PMon
Quantitative measurement of displacement in photopolymer layers during holographic recording using phase shifting electronic speckle pattern interferometry
Mohesh Moothanchery, Nanyang Technological Univ. (Singapore); Viswanath Bavigadda, Dublin Institute of Technology (Ireland); Paul Kumar Upputuri, Manojit Pramanik, Nanyang Technological Univ. (Singapore); Vincent Toal, Izabela Naydenova, Dublin Institute of Technology (Ireland)

The ability to measure in-plane and out-of-plane displacements independently of each other makes Electronic speckle pattern interferometry (ESPI) a suitable technique for determining surface deformations. In addition the displacement at each pixel in the image of an object is determined by phase shifting technique, thus a complete displacement profile of the object can be obtained. A phase shifting ESPI system has been developed to determine changes in photopolymer layer’s surface profile due to shrinkage during holographic recording. A beam reflected from the surface of a photopolymer layer and a beam partially reflected from a glass plate attached to a piezo electric transducer (PZT) are allowed to interfere and fall on to a CMOS camera. The layer is not photosensitive to the wavelength of these two beams. Phase shifted specklegrams of the photopolymer layer were captured initially and a five frame algorithm was used to obtain a wrapped phase map. The layer was then polymerised using another beam. Phase shifted specklegrams corresponding to the polymerised layer were also captured and a wrapped phase map of the layer was obtained as before. The two wrapped phase maps before and after photopolymerisation were then unwrapped inorder to get a smooth phase map. The two unwarppped phase maps maps before and after photopolymerisation were subtracted from one another in order to obtain a 3D displacement map due to shrinkage of the photopolymer during holographic recording. The study provided information about the dependence of the shrinkage of the photopolymer on exposure time and polymerizing beam intensity.

9718-82, Session PMon
Phase retrieved optical projection tomography for 3D imaging through scattering layers
Daniele Ancora, Foundation for Research and Technology-Hellas (Greece) and Univ. of Crete (Greece); Diego Di Battista, Stylianos Psycharakis, Foundation for Research and Technology-Hellas (Greece); Georgia Giasafaki, Foundation for Research and Technology-Hellas (Greece) and Univ. of Crete (Greece); Athanasios Zacharopoulos, Giannis Zacharakis, Foundation for Research and Technology-Hellas (Greece)

Recently great progress has been made in biological and biomedical imaging by combining non-invasive optical methods, novel adaptive light manipulation and computational techniques for intensity-based phase recovery and three dimensional image reconstruction. In particular and in relation to the work presented here, Optical Projection Tomography (OPT) is a well-established technique for imaging mostly transparent absorbing biological models such as C. Elegans and Danio Rerio. On the contrary, scattering layers like the cocoon surrounding the Drosophila during the pupae stage constitute a challenge for three dimensional imaging through such a complex structure. However, recent studies [Nature 491, 232-234 (2012)] enabled image reconstruction through scattering curtains up to few transport mean free paths via phase retrieval iterative algorithms [App. Opt. 21, No. 15, 2758-2769 (1982)] allowing to uncover objects hidden behind complex layers. By combining these two techniques via adaptive optical illumination, using a Spatial Light Modulator to control the phase of the illuminating speckled-wavefront, we perform a three dimensional image reconstruction of a fluorescent object embedded between scattering layers without compromising its structural integrity. Dynamical cross correlation registration was implemented for the registration process due to translational and flipping ambiguity of the phase retrieval problem, in order to provide the correct aligned set of data to perform the back-projection reconstruction. We have thus managed to reconstruct a fluorescent complex object by rotating it between static scattering curtains and compared with the effective tomographic reconstruction to fully understand the process before the in vivo biological implementation.
Conference 9718: Quantitative Phase Imaging II

9718-84, Session PMon

**Single-shot and 4-step phase shifting digital holographic microscopy using a 2D grating**

Taeseok D. Yang, Hyung-Jin Kim, Beop-Min Kim, Kyoung-Jin Lee, Youngwoon Choi, Korea Univ. (Korea, Republic of)

We demonstrate an on-axis digital holographic microscope based on a 4-step phase shifting algorithm, but featured with a single-shot measurement. A standard Mach-Zehnder interferometric microscope is used as a collinear configuration for acquiring phase images of an object. In order for the single-shot measurement, a 2-D grating is positioned in the Fourier plane of the detection port of the microscope. The grating generates multiple copies of a same object onto an image plane and four of them are taken by a single camera. By adjusting the position of the grating along two lateral directions, relative phases of 0, π/2, π, 3π/2 are introduced to each duplicated image. Four different interference images are acquired simultaneously and are processed using a 4-step phase shifting algorithm to produce a single phase image.

Since each copy of the object image fits into a quadrant of the detector, the number of pixels required for single independent phase information can be minimized. Comparing to the off-axis configuration, which requires at least 878 pixels to obtain one phase information, our approach needs only 272, 16 times less pixels, to attain the equivalent information. Thus, potentially, we can utilize an image sensor for quantitative phase imaging with much higher information density without any loss of acquisition speed. The proposed method will serve as a tool for studying dynamics of biological specimens.

9718-85, Session PMon

**Color-coded LED microscopy for multi-contrast and quantitative phase imaging**

Donghak Lee, DaeSeong Jung, Soocheol Kim, Chulmin Joo, Yonsei Univ. (Korea, Republic of)

Bright-field (BF), dark-field (DF) and phase-contrast microscopes represent the most common and widely employed label-free imaging methods. BF microscopy provides images by mapping light absorption of a specimen. However it may not be suitable for translucent samples such as cells, as they do not exhibit strong attenuation at visible light. DF microscopy offers high-contrast images of specimen boundaries, being sensitive to the edges of the samples. Phase contrast microscopy provides images by transforming the optical phase delay of samples into intensity distribution. Although these imaging methods are complementary, simultaneous acquisition of these images is not feasible in conventional microscopes, as each modality requires a distinct optical arrangement and dedicated optical elements.

Here, we present a simple strategy for multi-contrast microscopy capable of tri-modal imaging in a single shot. Our method, termed color-coded LED microscopy (cLEDscope), employs color-coded illumination with a LED array so that each color corresponds to a different illumination angle on specimen. Specimen image is recorded by a color image sensor, which is then separated into the images of each color and computed to generate BF, DF and differential phase-contrast images. Quantitative phase imaging is also achieved by sequential acquisition and computation with the images obtained based on two different LED patterns. We describe operation and implementation of cLEDscope, and demonstrate its real-time imaging capability by presenting trimodal images of various transparent specimens and dynamic behavior of Caenorhabditis elegans. Quantitative imaging capability of our setup is also validated by imaging a calibration sample and biological cells.

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9718-86, Session PMon

**White light phase shifting interferometry and color fringe analysis for the detection of contaminants in water**

Vishesh Dubey, Veena Singh, Azeem Ahmad, Gyanendra Singh, Dalip Singh Mehta, Indian Institute of Technology Delhi (India)

We report white light phase shifting interferometry in conjunction with color fringe analysis for the detection of contaminants in water such as Escherichia coli (E.coli), Campylobacter coli and Bacillus cereus. The experimental setup is based on a common path interferometer using Mirau interferometric objective lens. White light interferograms are recorded using a 3-chip color CCD camera based on prism technology. The 3-chip color camera have lesser color cross talk and better spatial resolution in comparison to single chip CCD camera. A piezo-electro transducer (PZT) phase shifter is fixed with the Mirau objective and they are attached with a conventional microscope. Five phase shifted white light interferograms are recorded by the 3-chip color CCD camera and each phase shifted interferogram is decomposed into the red, green and blue constituent colors, thus making three sets of five phase shifted interferograms for three different colors from a single set of white light interferogram. This makes the system less time consuming and have lesser effect due to surrounding environment. Initially 3D phase maps of the bacteria are reconstructed for red, green and blue wavelengths from these interferograms using MATLAB, from these phase maps we determines the refractive index (RI) of the bacteria. Experimental results of 3D shape measurement and RI at multiple wavelengths will be presented. These results might find applications for detection of contaminants in water without using any chemical processing and fluorescent dyes.

9718-87, Session PMon

**Intravital quantitative phase imaging for quantitative analysis of blood flow in live mouse mesentery**

Kyoohyun Kim, Kibaek Choe, Pihlan Kim, YongKeun Park, KAIST (Korea, Republic of)

Quantitative phase imaging (QPI) has been an invaluable tool for imaging live biological cells and tissues. By measuring two-dimensional (2-D) optical phase maps and 3-D refractive index (RI) distribution, QPI provides quantitative biochemical and structural information of biological samples with high resolution including protein concentration, cell dry mass, cell volume, and membrane fluctuation. So far, most studies using QPI are conducted in vitro by culturing biological samples on microscopic chambers which mimics natural physiological conditions of the cells. Since cellular behaviors are significantly influenced by many factors including temperature, pH, and CO2 concentration, in vivo imaging of intact biological samples has been highly required for studying cell biology. However, the in vivo application of QPI has been unexplored yet because complex optical fields of biological samples are severely degraded after passing through stacks of highly scattering tissues.

Here, we present an intravital QPI technique which can measure 2-D phase maps and 5-D RI distribution of individual RBCs flowing inside of microvasculature of live mouse mesentery. We measured complex optical fields of microvasculature via Mach-Zehnder interferometry, from which multiple light scattering occurring at the interface of microcapillary and adipocytes was effectively removed by selection of time-varying optical phase delay components. We experimentally demonstrate that 2-D intravital QPI can investigate the fluid dynamics of individual RBCs in capillaries of mouse mesentery and 3-D intravital optical diffraction tomography (ODT) measures chemical and morphological parameters of RBCs in capillaries.
Optical diffraction tomography for inspection of mobile phone lenses

Kyoohyun Kim, Jonghee Yoon, YongKeun Park, KAIST (Korea, Republic of)

The mobile phone industry has been grown rapidly for decades, and technologies for manufacturing mobile phone camera, one of the key components of mobile phones, have been developed continuously. In mobile phone cameras, optical plastic lenses fabricated by injection molding process have been widely used since the optical plastic lenses can achieve high imaging performance from aberration-corrected freeform geometry with low production cost. During injection molding process, however, plastic materials are inherently oriented and aggregated which may cause inhomogeneous optical properties of the lenses. To inspect the imaging quality of the lenses, various techniques have been developed including topography measurements and wavefront measurements. Previous techniques, however, have limited access on measuring three-dimensional (3-D) internal structural distribution of plastic lenses which is essential for the inspection of plastic lens fabrication as well as the enhancement of fabrication processes with reduced failure.

Here, we present a novel technique measuring 3-D refractive index (RI) distributions of plastic lenses. Complex optical fields of lenses with various orientations were measured by Mach-Zehnder interferometer, from which 3-D RI distributions of the lenses were reconstructed via optical diffraction tomography. For demonstrating the proof of principle, we first demonstrated the present method by reconstructing 3-D RI distribution of solid-glass beads with known RI value, and then applied on measuring tomograms of plastic lenses. The present technique could determine optical inhomogeneity and defects within optical plastic lenses with high spatial resolution and high RI sensitivity.

Non-invasive optical detection method for food spoilage using light speckle correlation

Jonghee Yoon, KyeoReh Lee, YongKeun Park, KAIST (Korea, Republic of)

Microorganisms such as bacteria, yeast, and mold make food products harmful to humans by food poisoning. To detect the microorganisms in food products, several methods such as DNA and protein based assay, immunological techniques, and spectroscopic analysis have been developed and utilized. While these methods are capable of measuring low-level of microorganisms in foods, they usually require expensive and complex equipment and skilled technicians, which limit personalized applications of food inspection methods.

In this work, we developed simple and non-invasive optical methods for detecting microorganisms in food products. Transmitted (or reflected) coherent light through the optically inhomogeneous sample would form the ‘speckle pattern’ due to the multiple light scattering. In spite of the complexity in light speckle distributions, the speckle formation is a deterministic process. Thus, light transmitted (or reflected) through a static sample forms a deterministic result. However, we found that the spontaneous movement of microorganisms in food products significantly varies the light speckle patterns transmitted (or reflected) through food products. Therefore, temporal analysis of the light speckle pattern can effectively measure the quantity of the microorganisms in food products, and be utilized for the food spoilage investigation.

High-speed quantitative phase imaging with line-field swept-source phase microscopy using a 1D array detector

Soon-Woo Cho, Hyung-Seok Lee, Gyeong Hun Kim, Nam-Soo Park, Chang-Seok Kim, Pusan National Univ. (Korea, Republic of)

Spectral domain phase microscopy (SDPM) for quantitative phase imaging (QPI) has been studied by using a broadband light source and a spectroscopic detector with limited response time. For quantitative phase imaging by SDPM, a complex spectrometer system is absolutely required to obtain the A-scan because the wavelength information is necessary for depth-resolved phase information. Recently, to reduce the scanning dimension at the sample arm, a line-field illumination method has been reported using a 2D area detector with several kHz frame rate of 2-D phase imaging. Since one axis of 2D area detector is assigned for the spatial information and the other axis for the wavelength information, each depth-information of line beam is simultaneously acquired within the detector response time.

In this work, we suggest a line-field swept source phase microscopy (LF-SSPM) with a 1D array detector which has the faster response time. Since the depth-resolved phase information can be obtained by wavelength scanning of the swept laser, the 1D array detector is enough to acquire the 2-D phase imaging using line-field illumination. Therefore, the 2-D quantitative phase imaging can be easily obtained with 36 kHz frame rate which corresponds to 36 fps of 3-D phase imaging. To demonstrate the stability of the high speed 3-D imaging setup, phase sensitivity is also measured within sub-nanometer. Thus, we propose the simple interferometric setup to demonstrate the feasibility of nano-scale 3-D surface imaging with higher speed.

Measurements of the membrane fluctuations of sickle cell trait red blood cells by a quantitative phase imaging unit in Tanzania

JaeHwang Jung, KAIST (Korea, Republic of); Lucas E. Matemba, National Institute for Medical Research (Tanzania, United Republic of); Dong-Jin Kim, Nelson Mandela African Institute of Science and Technology (Tanzania, United Republic of); YongKeun Park, KAIST (Korea, Republic of)

Sickle cell disease (SCD) is an inherited blood disorder caused by a point mutation in the beta-globin gene to produce self-assembling sickle hemoglobin which elongation in the deoxygenated condition dramatically damages the membrane of red blood cells (RBCs). While sickle genic homozygous individuals exhibit fatal symptoms including vaso-occlusion and organ damages, heterozygous individuals, called sickle cell trait, do not display the severe symptoms of SCD, and it is a still controversial debate whether RBCs in sickle cell traits have different physiology compared to that of normal individuals. Due to the limited access of biomedical instruments in sub-Saharan countries where SCD is spread, simple and cost-effective imaging tools are necessary for in situ study of SCD and sickle cell traits.

In this work, we measured the membrane fluctuation of RBCs collected from sickle genic homozygous and heterozygous individuals at Morogoro Regional Hospital in Tanzania using a quantitative phase imaging unit (QPIU), a hand-held sized module that can easily convert a conventional bright-field microscope into an align-free quantitative phase microscope. We measured over 500 RBCs and categorized into 4 groups: from non-sickle individuals (AA); sickle heterozygotes (SA); and from sickle homozygotes with sickle shapes (SS+), and without sickle shapes (SS-). The membrane fluctuations of sickle gene related groups (SS+, SS-, and...
Investigation of ethanol effects on morphological and biochemical properties of human red blood cells

SangYun Lee, HyunJoo Park, KAIST (Korea, Republic of); Catherine Best-Popescu, Univ. of Illinois at Urbana-Champaign (United States); Seongsoo Jang, Univ. of Ulsan (Korea, Republic of) and Asan Medical Ctr. (Korea, Republic of); YongKeun Park, KAIST (Korea, Republic of)

We systematically investigate the effects of ethanol on morphological, biochemical, and biomechanical characteristics of human red blood cells (RBCs). We employ common-path diffraction tomography to quantitatively and non-invasively measure the properties of individual RBCs. The 3-D refractive index distributions and the membrane fluctuations of human erythrocytes under various ethanol exposures from healthy (0.0% v/v) to lethal (0.5% v/v) conditions were measured, from which the morphological (volume, surface area, and sphericity), biochemical (Hemoglobin (Hb) concentration and Hb content), and biomechanical (membrane fluctuation) indices of red cells were sequentially obtained at the individual cell level. The correlative relations among these retrieved red cell indices were also considered to find unique alcohol-inducing mechanisms acted on human erythrocytes.

We observed that the erythrocytes at lethal alcoholic condition exhibit slightly lower cellular volumes than those of control cells and it seems to be related to the hemolysis or echinocytosis of large RBCs by ethanol. Meanwhile, the Hb decreases in RBCs were also observed, which is consistent with the previous reports using an infrared Raman spectroscopy. It was also shown that the amplitude of RBC membrane fluctuations were significantly elevated under ethanol exposure, implying the enhancements of RBC deformability, and this result also accords with the precedent researches based on an electron paramagnetic resonance and a laser diffraction ektacytometry.

Field of view extension in quantitative phase microscopy with broadband illumination

Pinhas Girshovitz, Irena Frenklach, Natan T. Shaked, Tel Aviv Univ. (Israel)

We review our new module, which uses the technique of interferometry with double-imaging area (DIA) and designed to operate with existing interferometric phase microscope setups illuminated by broadband illumination sources. This new configuration is attached at the output port of the off-axis interferometer, and optically creates a multiplexed interferogram on the digital camera. This is done by optically projecting two different fields-of-view with orthogonal interference fringes on the camera. The orthogonal interference fringes encode two wave-fronts into empty areas in the spatial frequency domain so that the two fields of view can be extracted from a single off-axis interferogram. We demonstrate using this module with a diffractive phase microscopy interferometer illuminated by a broadband light source, for imaging microscopic diatom shells cancer cells, and flowing blood cells.

Quantitative morphological and biochemical studies on human downy hairs using 3D quantitative phase imaging

SangYun Lee, Kyoohyun Kim, KAIST (Korea, Republic of); Yuhyun Lee, Sungjin Park, Heejae Shin, Jongwon Yang, Kwanhong Ko, Daeyeon Dongsin Science High School (Korea, Republic of); HyunJoo Park, YongKeun Park, KAIST (Korea, Republic of)

We optically measure the morphologies and 3-D refractive index (RI) distributions of individual human downy hairs in quantitative and non-invasive manners using a Mach-Zehnder type laser interferometric microscopy. The complex optical fields of downy hairs with varying laser incidence angles are systematically obtained by employing a dual-axis galvanometer mirror, from which the 2-D high-resolution synthetic aperture images, emulated differential interference contrast images, and 3-D RI distributions of downy hair edges are retrieved. From reconstructed 3-D RI distributions of the hair edges, the morphological parameters including 20-um-length hair volumes, cylindrical and effective radii are calculated to find common features shared by downy hairs. The effects of hydrogen peroxide (H2O2), most renowned hair bleaching agent, on fine hair structures are also investigated by measuring same downy hair edges before and after 24 hr H2O2 treatments.

Substrate stiffness influence on melanoma cell growth studied by QPI

Shamira Sridharan, Yanfen Li, Mikhail E. Kandel, Natalya Bapst, Kristopher A. Kilian, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Substrates of varying stiffness are used to mimic the microenvironment that cells encounter in the body. Studying cell proliferation in vitro commonly use cell counters or nucleotide incorporation assays, which make it difficult to simultaneously quantify individual cell growth and migration on substrates of different stiffness. In our experiments, we used B16 melanoma cells, of varying metastatic potential, and cultured them on polyacrylamide gels of different stiffness. The substrate was stamped with fibronectin, an extracellular matrix protein, which promotes cells adhesion. We used gels with Young’s modulus of 5kPa, 40kPa and 100kPa to mimic the microenvironment in the brain, muscle, and pre-calcified bone respectively. Cell growth was measured using spatial light interference microscopy (SLIM) for 24 hours. Our results show that metastatic and non-metastatic cells have comparable growth rates on the 40kPa and 100kPa substrates. By contrast, in the 5kPa substrate, the metastatic cells had a significantly higher growth rate than non-metastatic cells and also displayed decreased circularity, and better ability to spread.
9718-96, Session PMon

Validation of stromal optical anisotropy as marker for prostate cancer recurrence

Shamira Sridharan, Univ. of Illinois at Urbana-Champaign (United States); Virgilia Macias, Univ. of Illinois at Chicago (United States); Krishnarao V. Tangella, Presence Covenant Medical Ctr. (United States); Jonathan Melamed, New York Univ. Langone Medical Ctr. (United States); Andre Kajdacsy-Balla, Univ. of Illinois at Chicago (United States); Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

The risk for biochemical recurrence after radical prostatectomy for treatment of prostate cancer is estimated to be 17–33%. We developed a tool that uses spatial light interference microscopy to extract anisotropy of light scattering associated with the stromal layer to predict prostate cancer recurrence. Previously, using a nested case-control study design we demonstrated the added value of our technique for identifying recurrence among patients for whom current clinical methods fail. Here, we validate this biomarker on a general population of consecutive 192 patients and confirm that anisotropy predicts recurrence with an area under the receiver-operating characteristic curve (AUC) of 0.74, which is comparable to current clinical methods, CAPRA-S and Gleason score. However, among patients with intermediate risk of recurrence (Gleason histology grading score 7), anisotropy outperforms CAPRA-S. Among patients with high risk of recurrence (Gleason 8-10), anisotropy shows better specificity than CAPRA-S and could thus reduce the number of patients who are currently being over-treated.

9718-97, Session PMon

Morphological evaluation of sperm cells using quantitative phase microscopy

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In this study, we compared label-free interferometric phase microscopy (IPM) to label-free and label-based bright-field microscopy (BFM) in analyzing sperm cell morphology, in order to evaluate the potential of label-free IPM for clinical sperm analysis. For the evaluation, the same 350 normal and pathologic sperm cells from healthy donors were imaged using the three imaging systems and evaluated using the World Health Organization (WHO) criteria for men fertility. The sperm cells were evaluated based on their morphology, i.e. length and width of the sperm head and mid-piece, size and width of the acrosome, head, mid-piece and tail configuration, and general normality of the cell. Based on the WHO criteria, we found no statistical difference between the results measured using IPM and label-based BFM. In addition, we showed that the sensitivity and specificity of IPM were higher than label-free BFM.

9718-98, Session PMon

Improvement of reconstructed phase distribution of fast moving phase object in digital holographic microscope

Peng Xia, Kouichi Nitta, Osamu Matoba, Kobe Univ. (Japan); Yasuhiro Awatsuji, Kyoto Institute of Technology (Japan)

For defect detection or unwanted object in commercial products, it is required to develop a fast measurement system that can obtain three-dimensional distribution of surface of the opaque medium such as metal or inside of the transparent medium. For this purpose, we fabricated a digital holographic microscope (DHM) using a fast image sensor when the phase object is put on a fast movable stage. In the fabricated system, an image sensor operated at maximum frame rate of 2000 fps and a movable stage operated at maximum speed of 300 mm/s are introduced. Under the continuous wave illumination, motion-blurred phase object is reconstructed. By using numerical processing such as deconvolution filter, the reconstructed phase distribution is much improved. Numerical and experimental results are presented. In the numerical evaluation, a phase object with a Gaussian profile is used. Numerical results indicated that the deconvolution filter can improve the reconstructed phase distribution. In the experiments, a USAF test chart and beads with a diameter of 4 μm are used for the evaluation. We also discuss how to determine the depth position of an unknown three-dimensional phase object. The reconstructed position along the propagation direction is a critical issue. The automatic detection of the motion-blurred phase object is discussed and then we propose a method for detecting the depth position from the evaluated focus value from both amplitude and the phase distributions.

9718-99, Session PMon

Optical phase analysis in cortical drilled porcine bones using digital holographic interferometry

Cesar G. Tavera, Manuel H. De la Torre-Ibarra, Jorge M. Flores-Moreno, Juan M. Luna, Manuel de Jesus Briones Reyes, Fernando Mendoza Santoyo, Ctr. de Investigaciones en Óptica, A.C. (Mexico)

A study in porcine femoral bones with and without the presence of cortical drilling is presented. An out of plane digital holographic interferometer is used to retrieve the optical phase during the controlled compression tests. These tests try to simulate physiological deformations in postmortem healthy bones and compare their mechanical response with those having a cortical hole. The cortical drilling technique is widely used in medical procedures to fix plaques and metallic frames to a bone recovering from a fracture. Several materials and drilling techniques are used for this purpose. In this work we analyze the superficial variations of the bone when different drilling interspace and diameter are used. By means of the optical phase it is possible to recover the superficial deformation of the tissue during a controlled deformation with a high resolution. This information could give a better understand about the micro structural variations of the bone instead of a bulk response. As proof of principle, several tests were performed to register the modes and ranges of the displacements for compressive loads. From these tests notorious differences are observed between both groups of bones, having less structural stiffness the drilled ones as expected. However, the bone’s characteristic to absorb and adjust itself due the load is also highly affected according to the number of holes. Results from different kind of samples (undrilled and drilled) are presented and discussed in the manuscript.

9718-100, Session PMon

Study of inhomogeneity within PMMA samples using a 3D-SOCT system

Manuel de Jesus Briones Reyes, Manuel H. De la Torre-Ibarra, Jorge M. Flores-Moreno, Cesar G. Tavera, Juan M. Luna Hernandez, Fernando Mendoza Santoyo, Ctr. de Investigaciones en Óptica, A.C. (Mexico)

The industrial applications of embedded materials have been increasing in the last years and the study of their mechanical properties is of interest.
as well as their homogeneity due the uniformity in their mechanical responses which can represent a significant improvement or decay for the intended application. The 3D-SOCT system proposed here can show the internal micro structure and the displacements of the volume. This optical system uses a 2D camera sensor array to record with a single shot the tomographic image of the sample and using a second state of the same layer, the optical phase can be achieved. The system uses a linear motion stage to obtain a full 3D (consecutive B-Scans) tomographic and optical phase information avoiding the use of expensive tilting mirrors. The results show simultaneously the 3D tomographic micro reconstruction of the samples and the 3D optical phase information that identifies the inhomogeneities regions within the volume. A series of different deformations were proposed to enhance the detection of the uniform or non-uniform internal deposition of the micro particles.

9718-101, Session PMon

The substructure imaging method of a multimedium cell based on two orthogonal phase images

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It is very important to image the substructure of cells in lots of science researching fields and clinical application. In order to rebuild a substructure image of a cell’s sample by digital phase imaging (DPI), a multimedium entropy tomographic method is proposed, in which, body of the sample is segmented into more cubes that with same size and some unknown refractive index, upon the two orthogonal phase images of the sample (that are extremely related with the refractive index and thickness of the sample) and the maximum entropy theory, it can be iterative calculated that the optimized refractive index of each cube by scanning one by one, thus the information about 3D refractive index distribution is gotten. The substructure surface can be determined by constituting the mutation dots of the refractive index for all cubes. Finally, the 3D structure image of the sample is reconstructed based on these surface and the size of the cubes. As demonstrating examples, some multimedium models of blood cells are built that looks as a ball included two nucleus (or one nucleus) which with different diameter and refractive index considering on the substructure feature of these blood cells, the substructure images of the models are rebuilt by simulation experiment based on our method, the rebuilding results show that the volume of it, size of nucleus, distribution of refractive index and substructure position are highly accordance with the model. It may help the substructure imaging of cells to go into a new way.

9718-102, Session PMon

Portable Hilbert phase profilometry (HPP) for imaging dynamic mesoscopic objects

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Fringe projection profilometry is one of the popular non-contact topography methods, which can yield surface profiles by single-shot imaging to achieve high-throughput. We developed HPP, a portable fringe projection system which consists of a LED pocket projector and a cell phone. The pocket projector casts a sinusoidal grating onto the measured object surface and the cell phone camera detects the resulting deformed grating image. We developed an Android application that processes the fringe image via a Hilbert transform to obtain the surface profile in real-time. We envision that this portable system will have many applications in the consumer (e.g., profilometry of 3D objects for printing) as well as biomedical application space. To illustrate the biomedical use, we imaged the microvasculature a live chicken embryos. The experimental results demonstrate the system can obtain the accurate vascular surface profile and dynamics measurements.

9718-103, Session PMon

Osmolality modulation to decouple erythrocyte measurements by diffraction phase microscopy

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In many Quantitative Phase Imaging (QPI) studies with human red blood cells (erythrocytes), it is assumed that the refractive index inside the cell is homogeneous and constant over time. However, it is known that changes in the surrounding medium (e.g. temperature, concentration) produce a change in the refractive index, which in turn, can result in inaccurate cell property calculations obtained from phase measurements. To overcome this limitation, we have developed a new theoretical and experimental technique to obtain 2D maps of phase differences. These maps contain coupled information about the integral refractive index and thickness of the cell. In order to decouple these quantities, we induce osmolality variations in a carrier fluid to modulate the refractive index of the medium surrounding the red blood cells. By using laser Diffraction Phase Microscopy (DPM), we can now accurately compute cell properties from phase information. In addition, being based on common path interferometry, this non-invasive technique exhibits advantages over other methods including fast image acquisition speeds with high spatial and temporal stability.

9718-104, Session PMon

Quantitative study of the effects of spatiotemporal coherence of illumination to speckle noise reduction

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Speckle noise degrades the imaging quality of coherent imaging systems including optical coherence tomography, synthetic aperture radar, ultrasound imaging, and quantitative phase imaging. The speckle noise is caused by multiple reflections and unwanted diffraction from dust particles and other samples located in defocused planes. Over the last decade, several studies have been reported to utilize spatially or temporally incoherent sources for speckle noise reduction. However, the quantitative relation between the effect of spatiotemporal coherence of illumination and speckle noise reduction has not been studied yet.

In this work, we quantitatively investigated the effects of spatiotemporal coherence of illumination to speckle noise reduction. We changed the spatiotemporal frequencies of illumination by employing a rotating galvanoscanner and a custom-made wavelength-swept source, and measured optical fields with various spatiotemporal frequencies, and was quantified by calculating spatial and temporal coherence lengths. The speckle noise was calculated as the spatial phase fluctuation of the synthesized optical fields, and the results show that the speckle noise was proportional to the spatial and temporal coherence length. The speckle noise was reduced by 85.8, 41.7% with decreasing spatial and temporal coherence lengths from 11.8, 63.3 μm to 0.34, 1.4 μm, respectively. The present study suggests that the effect of spatiotemporal coherence of illumination to speckle noise is predictable, which can be implemented for designing partially incoherent quantitative phase microscopes.
Coherence-controlled holographic microscopy for live-cell quantitative phase imaging in turbid media

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In this work we present the coherence controlled holographic microscopy (CCHM) and its ability to image the living cells in turbid media. CCHM provides quantitative phase imaging (QPI), a label-free method suitable for long-term observations of living cells. Thanks to the fact that CCHM is based on off-axis interferometer the whole QPI can be reconstructed just from single hologram without the need of scanning and thus CCHM can image even rapid processes in living cells. CCHM in combination with other common optical microscopy techniques enables to fully exploit the advantages of QPI and simultaneously to identify the observed structures by well-established imaging methods. One of the main advantages of CCHM is the fact that it uses low-coherent light. This leads to images which are free of speckles and parasitic coherence effects. Moreover the use of low-coherent light leads to coherence-gating effect, which enables to image through turbid media. In our experiments we use biologically active phospholipids (BAPs) as a cytopathic turbid emulsion. First image quality obtained with sources of different coherence-lengths was compared and then reactions of cells to treatment by BAPs were studied. It was shown that BAPs cause cell death by necrosis and QPI time-lapse observations revealed changes of cell morphology, cell behavior and cell fate that would otherwise remain hidden behind turbid media. Part of this work is already accepted for publication.

Multi-modal digital holographic microscopy for wide-field fluorescence and 3D phase imaging

Xiangyu Quan, Peng Xia, Osamu Matoba, Kouichi Nitta, Kobe Univ. (Japan); Yasuhiro Awatsuji, Kyoto Institute of Technology (Japan)

A simultaneous detection of 3D phase images and fluorescence images is carried out by means of multi-modal digital holographic microscopic system. The proposed system combines transmission-type geometry of a Mach-Zehnder interferometer for three-dimensional phase imaging and a reflective-type optical fluorescence microscopy for wide-field fluorescence imaging. Two cameras are utilized and correlated for simultaneous recordings of those two types of information from a single phenomenon. The wavelengths of transmitted light for phase imaging and the excited fluorescence are specifically chosen, then carefully controlled to separate them from a single light path by using a wavelength sensitive polarization beam splitter and a high-pass filter. The system is applicable in various bio-research fields by fast acquisition of both imaging field from a single phenomenon. Experiment on simple structured beads and complex cells are demonstrated. The samples are moving in three dimensions and each light fields are captured individually in real time. Reconstruction results is shown as movies and the results show high performance of multi-modal digital holographic microscope in its simultaneous acquisition ability in real time fashion. Experiments results are implementing practical applications towards many science researches.

Lens-free synthetic optical holography

Andrea Di Donato, Simone Torquati, Univ. Politecnica delle Marche (Italy); Tiziana Pietrangelo, Univ. degli Studi G. d’Annunzio Chieti Pescara (Italy); Marco Farina, Univ. Politecnica delle Marche (Italy)

Synthetic optical holography (SOH) is an imaging technique, recently introduced in scanning microscopy to record amplitude and phase of
typically on the order of 100 micron for biological tissues. As a consequence, light scattering. The signal strength from the target objects decays
Optical imaging of objects embedded within scattering media such as Synthetic aperture imaging of objects in parallel, can handle a large dynamic range of particle sizes (40 nm – 100s nm. This family of approaches can individually size more than 10^5 particles
phase quantitatively corresponds to particle size with an accuracy of +/-11 images reconstructed from captured holograms. The magnitude of the non-scattered and single-scattered light contributions to the final intensity can be reconstructed by using the standard Fourier Transform (FT) filtering, combined with a demodulation of the pattern and an inverse FT. Thanks to the presence of a low-coherence source, the system allows to acquire multiple holograms at different wavelengths, realizing amplitude and phase imaging at multi-wavelengths with just one scan, paving the way toward synthetic holographic spectral imaging for biological applications.

9718-45, Session 6
High-throughput nanoparticle sizing using lensfree holographic microscopy and liquid nanolenses (Invited Paper)
Euan McLeod, The Univ. of Arizona (United States)
The sizing of individual nanoparticles and the recovery of the distributions of sizes from populations of nanoparticles provide valuable information in virology, exosome analysis, air and water quality monitoring, and nanomaterials synthesis. Conventional approaches for nanoparticle sizing include those based on costly or low-throughput laboratory-scale equipment such as transmission electron microscopy or nanoparticle tracking analysis, as well as those approaches that only provide population-averaged quantities, such as dynamic light scattering. Some of these limitations can be overcome using a new family of alternative approaches based on quantitative phase imaging that combines lensfree holographic on-chip microscopy with self-assembled liquid nanolenses. In these approaches, the particles of interest are deposited onto a glass coverslip and the sample is coated with either pure liquid polyethylene glycol (PEG) or aqueous solutions of PEG. Due to surface tension, the PEG self-assembles into nano-scale lenses around the particles of interest. These nanolenses enhance the scattering signatures of the embedded particles such that individual nanoparticles as small as 40 nm are clearly visible in phase images reconstructed from captured holograms. The magnitude of the phase quantitatively corresponds to particle size with an accuracy of +/-11 nm. This family of approaches can individually size more than 10^5 particles in parallel, can handle a large dynamic range of particle sizes (40 nm – 100s of microns), and can accurately size multi-modal distributions of particles. Furthermore, the entire approach has been implemented in a compact and cost-effective device suitable for use in the field or in low-resource settings.

9718-46, Session 6
Synthetic aperture imaging of objects embedded within scattering media
Pilsung Kang, Wonshik Choi, Korea Univ. (Korea, Republic of)
Optical imaging of objects embedded within scattering media such as biological tissues suffers from the strong background noise due to multiple light scattering. The signal strength from the target objects decays exponentially at the length scale of the scattering mean free path, which is typically on the order of 100 micron for biological tissues. As a consequence, targets located at a depth of just a few scattering mean free paths lose their fine details.

9718-47, Session 6
Some advances in design and calibration of limited angle optical diffraction tomography system for biological applications
Arkadiusz Kus, Piotr L. Makowski, Malgorzata Kujawinska, Warsaw Univ. of Technology (Poland)
Optical diffraction tomography has been steadily proving its potential to study one of the hot topics in modern cell biology – 3D dynamic changes in cells’ morphology represented with refractive index values. In this technique digital holography is combined with tomographic reconstruction and thus it is necessary to provide projections acquired at different viewing directions. Usually the Mach-Zehnder interferometer configuration is used and while the object beam performs scanning, the reference beam is in most cases stationary. This approach either limits possible scanning strategies or requires additional mechanical movement to be introduced in the reference beam. On the other hand, spiral or grid scanning is possible in alternative common-path or Michelson configurations. However, in this case there is no guarantee that a specimen is sparse enough for the object to interfere with an object-free part of the beam. In this paper we present a modified version of Mach-Zehnder interferometer-based tomographic microscope, in which both object and reference beam are subject to scanning using one scanning device only thus making any scanning scenario possible. This concept is realized with a custom-built optical system in the reference beam and is appropriate for mechanical as well as with optical scanning. Usually, the tomographic reconstruction setups and algorithms are verified using a microscope phantom, which is not enough to verify the distribution of the projections. In this work we propose a more complex calibration object created with laser lithography.

9718-48, Session 6
3D differential phase contrast microscopy
Michael Chen, Lei Tian, Laura Waller, Univ. of California, Berkeley (United States)
In this study, we present a 3D complex index of refraction reconstruction using differential phase contrast (DPC) optical microscopy. By replacing the source of a commercial brightfield microscope with a programmable LED array, asymmetric illumination with respect to different axes are generated and the corresponding images are collected. Traditional brightfield images can be achieved by summing up the recorded images, while DPC microscopy is computationally generated by the difference between one image and the other under the opposite asymmetric illuminations. With the first Born approximation, multiple scattering effects are ignored, while the non-scattered and single-scattered light contributions to the final intensity
Quantitative phase imaging is commonly used with coherent illumination for semi-transparent biological samples. Quantitative phase imaging (QPI) [1] to make tridimensional reconstruction of biological samples and cell growth using quantitative phase imaging

Shamira Sridharan, Matthew T. Leslie, Natalya Bapst, John Smith, H. Rex Gaskins, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Quantitative phase imaging has been used in the past to study the dry mass of cells and study cell growth under various treatment conditions. However, the relationship between cellular redox and growth rates has not yet been studied in this context. This study employed the recombinant Glrx-roGFP2 redox biosensor targeted to the mitochondrial matrix or cytosolic compartments of A549 lung epithelial carcinoma cells. The Glrx-roGFP2s biosensor consists of a modified GFP protein containing internal cysteine residues sensitive to the local redox environment. The formation/ dissolution of sulfide bridge contorts the internal chromophore, dictating corresponding changes in fluorescence emission that provide direct measures of the local redox potential. Combining 2-channel fluorescent imaging of the redox sensor with quantitative phase imaging allowed observation of redox homeostasis alongside measurements of cellular mass during full cycles of cellular division. The results indicate that mitochondrial redox showed a stronger inverse correlation with cell growth than cytoplasmic redox states; although redox changes are restricted to a 5% range. We are now studying the relationship between mitochondrial redox and cell growth in an isogenic series of breast cell lines built upon the MCF-10A genetic background that vary both in malignancy and metastatic potential.

9718-52, Session 7

Studying the relationship between redox and cell growth using quantitative phase imaging

Shamira Sridharan, Matthew T. Leslie, Natalya Bapst, John Smith, H. Rex Gaskins, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Quantitative phase imaging has been used in the past to study the dry mass of cells and study cell growth under various treatment conditions. However, the relationship between cellular redox and growth rates has not yet been studied in this context. This study employed the recombinant Glrx-roGFP2 redox biosensor targeted to the mitochondrial matrix or cytosolic compartments of A549 lung epithelial carcinoma cells. The Glrx-roGFP2s biosensor consists of a modified GFP protein containing internal cysteine residues sensitive to the local redox environment. The formation/ dissolution of sulfide bridge contorts the internal chromophore, dictating corresponding changes in fluorescence emission that provide direct measures of the local redox potential. Combining 2-channel fluorescent imaging of the redox sensor with quantitative phase imaging allowed observation of redox homeostasis alongside measurements of cellular mass during full cycles of cellular division. The results indicate that mitochondrial redox showed a stronger inverse correlation with cell growth than cytoplasmic redox states; although redox changes are restricted to a 5% range. We are now studying the relationship between mitochondrial redox and cell growth in an isogenic series of breast cell lines built upon the MCF-10A genetic background that vary both in malignancy and metastatic potential.

9718-53, Session 7

Label-free three dimensional reconstruction of biological samples

Sherazade Aknoun, PHASICS S.A. (France); Pierre Bon, Institut d’Optique Graduate School (France); Julien Savatier, Serge Monneret, Institut Fresnel (France) and Aix-Marseille Univ. (France); Benoît F. Wattellier, PHASICS S.A. (France)

We describe the use of spatially incoherent illumination combined with quantitative phase imaging (QPI) [1] to make tridimensional reconstruction of semi-transparent biological samples. Quantitative phase imaging is commonly used with coherent illumination for the relatively simple interpretation of the phase measurement. We propose
to use spatially incoherent illumination which is known to increase lateral and axial resolution compared to classical coherent illumination. The goal is to image thick samples with intracellular resolution [2].

The 3D volume is imaged by axially scanning the sample with a quadri-wave lateral shearing interferometer used as a conventional camera while using spatially incoherent white-light illumination (native microscope halogen source) or NIR light. We use a non-modified inverted microscope equipped with a Z-axis piezo stage. A z-stack is recorded by objective translation along the optical axis.

The main advantages of this approach are its easy implementation, compared to the other state-of-the-art diffraction tomographic setups, and its speed which makes even label-free 3D living sample imaging possible. A deconvolution algorithm is used to compensate for the loss in contrast due to spatially incoherent illumination. This makes the tomographic volume phase values quantitative. Hence refractive index could be recovered from the optical slices.

We will present tomographic reconstruction of cells, thick fixed tissue of few tens of micrometers using white light, and the use of NIR light to reach deeper planes in the tissue.

9718-54, Session 7

Holographic microscopy for 3D tracking of bacteria

Jay L. Nadeau, California Institute of Technology (United States) and McGill Univ. (Canada); Yong Bin Cho, California Institute of Technology (United States); Marwan El-Kholy, McGill Univ. (Canada); Manuel Bedrossian, Stephanie Rider, California Institute of Technology (United States); Christian A. Lindensmith, James K. Wallace, Jet Propulsion Lab. (United States) and California Institute of Technology (United States)

Understanding when, how, and if bacteria swim is key to understanding critical ecological and biological processes, from carbon cycling to infection. Imaging motility by traditional light microscopy is limited by focus depth, requiring cells to be constrained in z. Holographic microscopy offers an instantaneous 3D snapshot of a large sample volume, and is therefore ideal in principle for quantifying unconstrained bacterial motility. However, resolving and tracking individual cells is difficult due to the low amplitude and phase contrast of the cells; the index of refraction of typical bacteria differs from that of water only at the second decimal place. In this work we present a combination of optical and sample-handling approaches to facilitating bacterial tracking by holographic phase imaging. The first is the design of the microscope, which is an off-axis design with the optics along a common path, which minimizes alignment issues while providing all of the advantages of off-axis holography. Second, we use anti-reflective coated etalon glass in the design of sample chambers, which reduce internal reflections. Improvement seen with the antireflective coating is seen primarily in phase imaging, and its quantification is presented here. Finally, dyes may be used to increase phase contrast according to the Kramers-Kronig relations. Results using three test strains are presented, illustrating the different types of bacterial motility characterized by an enteric organism (Escherichia coli), an environmental organism (Bacillus subtilis), and a marine organism (Vibrio alginolyticus). Data processing steps to increase the quality of the phase images and facilitate tracking are also discussed.

9718-55, Session 7

Deciphering the internal complexity of living cells with quantitative phase microscopy: a multi-scale approach

Cristina E. Martinez-Torres, Bastien Laperrousaz, Lotfi}

Berguiga, Elise Boyer-Provera, Ecole Normale Supérieure de Lyon (France); Juan Elezgaray, Institut European de Chimie et Biologie (France); Franck E. Nicolini, Ctr. Hospitalier Univ. de Lyon (France); Véronique Maguer-Satta, Institut de Recherche en Cancérologie de Lyon (France); Alain Arneodo, Francoise Argoul, Ecole Normale Supérieure de Lyon (France)

During the last decades, identification of the physical properties of single living cells has been a subject of considerable interest for diagnoses. Interestingly, quantitative optical microscopic methods have shown that the refractive index (RI) of a cell can be used as an indicator for cell transformation in pathological situations. Quantitative phase microscopy (QPM) is well suited for uncovering the intracellular organization non-intrusively. However, the interpretation of quantitative phase images captured from living cells remains a difficult task because (i) we still have very little knowledge of the impact of its internal macromolecular complexes on the local refractive index and (ii) phase changes produced by light propagation through the sample are mixed with diffraction effects by the internal cell bodies. We propose the implementation a 2D wavelet-based contour chain detection method, to distinguish internal boundaries thanks to their greatest optical path difference gradients. These contour chains correspond to the highest image phase contrast and follow the local refractive index inhomogeneities linked to the intracellular structural intricacy. Their statistics and spatial distribution are morphological indicators for distinguishing cells of different origins and/or to follow their transformation in pathologic situations. We use this method to compare non-adherent blood cells from primary and laboratory culture origins, in healthy and pathological situations (chronic myelogenous leukaemia). In a second part of this presentation, we concentrate on the temporal dynamics of the phase contour chains and we discuss the spectral decomposition of their dynamics in healthy and pathological situations.

9718-56, Session 7

3D measurements of live cells via digital holographic microscopy and terahertz spectroscopy

Sean Norbury, Dorian Oser, Jun Yong Park, Alexander T. Khalmaladze, Anna V. Sharikova, Univ. at Albany (United States)

A combined digital holography/terahertz spectroscopy technique for live cell cultures is presented. Both methods are non-invasive and label-free. Digital holographic microscopy (DHM) records an interference pattern between an object and reference waves, so that the computationally reconstructed holographic image contains both amplitude and phase information. When the phase is mapped across the sample and converted into height information for each pixel, a 3D image is obtained. The measurement of live cell cultures by digital holographic microscopy yields information about cell cycle and cell death mechanisms, since these processes are correlated with individual cell volume and shape. Terahertz (THz) is the “fingerprint” region, containing rotational and vibrational molecular transitions, as well as intermolecular vibrations. Therefore, terahertz spectroscopy can provide complementary information about cells, such as protein, lipid and nucleic acid content, and, particularly, the spectral signatures associated with structural changes in molecules. The cell cultures are kept in a temperature-controlled environmental chamber during the experiment, which allows monitoring over multiple cell cycles. The DHM system is made in-house, and combines a visible (red) laser source with a conventional microscope base, and LabVIEW-run data processing. The THz spectroscopy system uses a low-cost laser diode to pump THz antennas in a coherent detection setup. We analyze and compare cell culture information obtained by these two methods.
Multi-color phase imaging and sickle cell anemia

Poorya Hosseini, Renjie Zhou, Zahid Yaqoob, Peter T. C. So, Massachusetts Institute of Technology (United States)

Quantitative phase measurements at multiple wavelengths has created an opportunity for exploring new avenues in phase microscopy such as enhancing imaging-depth (1), measuring hemoglobin concentrations in erythrocytes (2), and more recently in tomographic mapping of the refractive index of live cells (3). To this end, quantitative phase imaging has been demonstrated both at few selected spectral points as well as with high spectral resolution (4,5). However, most of these developed techniques compromise imaging speed, field of view, or the spectral resolution to perform interferometric measurements at multiple colors. In the specific application of quantitative phase in studying blood diseases and red blood cells, current techniques lack the required sensitivity to quantify biological properties of interest at individual cell level. Recently, we have set out to develop a stable quantitative interferometric microscope allowing for measurements of such properties for red cells without compromising field of view or speed of the measurements. The feasibility of the approach will be initially demonstrated in measuring dispersion curves of known solutions, followed by measuring biological properties of red cells in sickle cell anemia.

References:

Hilbert phase dynamometry (HPD) for real-time measurement of cell generated forces

Shamira Sridharan, Yanfen Li, Basanta Bhaduri, Hassan Majeed, Univ. of Illinois at Urbana-Champaign (United States); Paul Dupenloup, Alex Levine, Univ. of California, Los Angeles (United States); Kristopher A. Kilian, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Traction force microscopy is the most widely used technique for studying the forces exerted by cells on deformable substrates. However, the method is computationally intense and cells have to be detached from the substrate prior to measuring the displacement map. We have developed a new method, referred to as Hilbert phase dynamometry (HPD), which yields real-time force fields and, simultaneously, cell dry mass and growth information. HPD operates by imaging cells on a deformable substrate that is patterned with a grid of fluorescent proteins. A Hilbert transform is used to extract the phase map associated with the grid deformation, which provides the displacement field. By combining this information with substrate stiffness, an elasticity model was developed to measure forces exerted by cells with high spatial resolution. In our study, we prepared 10kPa gels and them with a 2-D grid of FITC-conjugated fibronogen/fibronectin mixture, an extracellular matrix protein to which cells adhere. We cultured undifferentiated mesenchymal stem cells (MSC), and MSCs that were in the process of undergoing adipogenesis and osteogenesis. The cells were measured over the course of 24 hours using Spatial Light Interference Microscopy (SLIM) and wide-field epi-fluorescence microscopy allowing us to simultaneously measure cell growth and the forces exerted by the cells on the substrate.

Research of chromatin conformation in interphase nuclei using quantitative phase imaging

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Application of the newest methods of optical bioimaging has been one of the main reasons for the explosive development of the scientific field in the last decade. Quantitative phase imaging (QPI) can be considered as a potential tool to extract important information on the refractive index of the cellular and subcellular structures. Interphase chromatin is an original biosensor, a detector of early changes in morphofunctional cell condition. Location of decondensed chromosomes within the interphase nucleus is strictly regulated (not chaotic). These compact formations were called “chromosome territories”. Their nuclear refractivity reflecting the features of the nuclear proteins conformation and chromatin decondensation degree may be an objective quantitative criterium of their dynamics in norm and pathology. The authors presented a technology of densitometric segmentation based on the quantitative phase microscopy and computed analysis of the changes in the optic density of interphase chromatin as a biosensor. Our studies were performed on a computer phase-interference microscope (Westrade LTD, Moscow, Russia): height accuracy 0,5 nm, coordinate accuracy 10 nm, image area 256x256 pixels, optical magnification 1000. The advantages of the technology include the opportunity of non-invasive high-sensitivity and high-speed studying bio-objects’ condition, that allows close following dynamics of intracellular processes. We will discuss application QPI for dynamic characterization of interphase nuclei. Using the interphase chromatin as a promising biosensor opens up new possibilities in the diagnosis of diseases. Research was fulfilled with financial support of Ministry of education and science according to governmental task 17.2608.2014/K.
Medical in-vivo diagnostics of biological tissues can strongly profit from non-invasive optical imaging techniques. In many applications additional information about the depth of an object is required. Measuring this quantity is particularly challenging due to strong scattering. Fluorescence microscopy enables the efficient suppression of scattered excitation light, but coherence between excitation and detected light is lost which prohibits the quantitative phase evaluation by interference with a reference wave. This work shows that shearing interferometry can be applied to the quantitative wave front reconstruction of fluorescent light. The depth information is encoded in the wave front curvature. In order to improve the signal quality and lateral resolution, the excitation of fluorescence can be restricted to a small volume by using structured illumination with two interfering beams of a white-light laser.

Complementary to the experimental setup, methods for modelling the propagation of light in scattering media are presented. This includes the angular spectrum of plane waves method, which locally allows the analytical modeling of the light propagation based on solutions of Maxwell equations, as well as numerical Monte Carlo methods as solution of the radiative transfer equation. Scattering losses are taken into account and the background in the interference signal due to multiple scattering can be quantitatively estimated.

The experimental and numerical methods are validated by measurements in scattering phantoms with properties similar to dental enamel containing fluorescing particles and layers in known depths. The setup is characterized in terms of the maximal penetration depth, lateral and vertical resolution and depth uncertainty.

9718-61, Session 8
Quantitative phase contrast imaging using aNomarski microscope with variable shear distance
Claas Falldorf, Bremer Institut für angewandte Strahltechnik GmbH (Germany); Mostafa Agour, Bremer Institut für angewandte Strahltechnik GmbH (Germany) and Aswan Univ. (Egypt); Ralf B. Bergmann, Bremer Institut für angewandte Strahltechnik GmbH (Germany) and Univ. Bremen (Germany)

We present a Nomarski microscope with variable shear distance that allows for determining the full quantitative phase contrast of a specimen, rather than only its differential phase contrast. The system preserves all beneficial properties of the Nomarski setup with respect to coherence and stability. Hence, we can measure the quantitative phase contrast under Köhler illumination with full condenser aperture and without vibration isolation.

The setup is based on a 4f-arrangement with an electronically addressable spatial light modulator (SLM) in the corresponding Fourier domain. The SLM is used to generate a diffractive grating with two diffraction orders. Thus, light entering the 4f-arrangement on either side will be split up into two waves. Both, illuminating and recording light will be directed over the SLM, thereby inherently ensuring spatial coherence. The only coherence requirement is that the difference between the two optical path lengths is smaller than the coherence length of the light.

Since we can adjust the magnitude and the orientation of the lateral shift between the two waves by means of the SLM, the system resembles a lateral shear interferometer with tailored illumination. We can therefore evaluate several measurements with varying lateral shifts in combination and use methods known from Computational Shear Interferometry (CoSI) to calculate the quantitative phase contrast. Additionally, since the methods also allow recovering of the corresponding amplitude, we can refocus the images subsequent to the measurement. We will explain the system, give an analysis of the coherence requirements and provide examples from the field of cell biology.

9718-62, Session 8
Dual wavelength digital holographic imaging of embedded layered structures
Jun Yong Park, Xinzhong Chen, Anna V. Sharikova, Alexander T. Khmaladze, Univ. at Albany (United States)

We present a three-dimensional microscopic technique based on simultaneous dual wavelength digital holography. In digital holographic microscopy, interference patterns produced by an object and reference waves are recorded by a camera. Then, computationally reconstructed holographic images contain the information of both amplitude and phase of the light reflected from the object. Phase is then mapped across the sample and is converted into a height information for each pixel. This technique was applied to imaging of electrodes embedded into glass substrates, which allowed three-dimensional reconstruction of their structure. Holographic imaging of the embedded layered structures, where each layer can be separated from the others by axial distances exceeding multiple wavelengths of imaging light, is difficult, because software phase unwrapping is practically impossible. The use of two wavelengths enables accurate axial measurements of multiple layers by comparing the phase maps produced by each individual wavelength. We demonstrated that the correct choice of wavelengths maximizes the axial range, at which an unambiguous 3D imaging can be performed. This provides not just three-dimensional structure of each layer, but also allows for height differentiation of layers. By employing wavelength edge filters, we were able to obtain the phase maps simultaneously, enabling fast measurements.

We also developed a background removal technique, based on the quality of interference fringe pattern, which suppresses low intensity signal, when no reliable phase information can be extracted. We showed that this is especially useful for multilayered embedded electrode structures, where each sample consist of both high and low reflectivity features.

9718-63, Session 8
Unlimited field-of-view optofluidic quantitative phase imaging
Vittorio Bianco, Melania Paturzo, Valentina Marchesano, Pietro Ferraro, Istituto di Scienze applicate e Sistemi Intelligenti (Italy)

The study of biological specimens onboard compact Lab-on-a-Chip (LoC) platforms with embedded label-free, quantitative, 3D imaging functionalities is highly demanded. Here we introduce a novel imaging modality, named Space-Time Scanning Interferometry (STSI), which synthesizes space-time interferograms with intriguing features. Indeed, a single linear sensor array is sufficient to build up a synthetic interferogram with unlimited Field of View (FoV) along the scanning direction and reduced noise. If a small subset of lines of the detector is selected, synthetic interferograms can be obtained, shifted each other of the desired phase step allowing phase estimation. The STSI principle is well-suited to be applied in all the cases where the object motion is an intrinsic feature of the system, e.g. in case of microfluidics, so that the advantages of STSI have no cost associated with. Starting from these considerations, we applied the STSI method to in-flow on-chip microscopy of biological samples. Out-of-focus recordings are performed using a single line detector and polymeric micro-lenses embedded onboard chip, in order to synthesize a Space-Time Digital Hologram (STDH) carrying full-field, 3D information of the flowing samples. We discuss the method and prove that a STDH still maintains all the advantageous capabilities of DH microscopy. The throughput of the imaging system is dramatically increased as STDH provides unlimited FoV, refocusable imaging of samples flowing inside a liquid volume with no need for hologram stitching. Thus, it is possible to move a huge step toward the integration of the imaging functionalities onboard chip for high-throughput rapid diagnostics.
Computational optical imaging by correcting wavefronts and aberrations in phase-resolved optical coherence tomography systems (Invited Paper)

Stephen A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Advances in optical imaging have leveraged computational approaches and integrated hardware and software algorithms in innovative ways to optimize imaging performance, correct aberrations, or extract more meaningful information from the optical data. Using a stable phase-resolved optical coherence tomography (OCT) system, it is possible to extend the depth-of-focus and yield spatially-invariant transverse resolution using interferometric Synthetic Aperture Microscopy (ISAM). With similar processing, it is also possible to correct optical aberrations induced by the optical system or sample, a form of Computational Adaptive Optics (CAO). Computational adaptive optics using coherent imaging offers the potential to augment or replace hardware adaptive optics systems for imaging the living human retina. As an application of these computational optical imaging approaches, we demonstrate compensation of the resolution-degrading effects of human ocular aberrations.

Fast quantitative retardance imaging of biological samples using quadri-wave interferometry

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We describe the use of polarized spatially coherent illumination to perform linear retardance imaging and measurements of semi-transparent biological samples using a quantitative phase imaging technique [1]. Quantitative phase imaging techniques [2-5] are used in microscopy for the imaging of semi-transparent samples and gives information about the optical path difference (OPD). The strength of those techniques is their non-invasive (the sample is not labelled) and fast approach. However, this high contrast is non-specific and cannot be linked to specific properties of the sample.

To overcome this limitation, we propose to use polarized light in combination with QPI. Indeed, anisotropy has been used to reveal ordered fibrous structures in biological samples without any staining or labelling with polarized light microscopy [6-8]. Recent studies have shown polarimetry as a potential diagnostic tool for various dermatological diseases on thick tissue samples [9]. Particularly, specific collagen fibers spatial distribution has been demonstrated to be a signature for the optical diagnosis and prognosis of cancer in tissues [10].

In this paper, we describe a technical improvement of our technique based on high-resolution quadri-wave lateral shearing interferometry (QWLSI) and liquid crystal retarder to perform quantitative linear birefringence measurements on biological samples. The system combines a set of quantitative phase images with different excitation polarizations to create birefringence images. These give information about the local retardance and orientation of biological anisotropic components.

We propose using a commercial QWLSI [11] (SID4Bio, Phasics SA, Saint Aubin, France) directly plugged onto a lateral video port of an inverted microscope (TE2000-U, Nikon, Japan). We are able to take retardance images in less than 1 second which allows us to record dynamic phenomena (living cells study) and make high speed acquisitions to reconstruct tissues virtual slides. Results on living cells and comparisons between healthy and tumoral 10 µm thick skin tissues will be presented.

Shape measurement of microscopic objects using computational shear interferometry

Mostafa Agour, Claas Falldorf, Ralf B. Bergmann, Bremer Institut für angewandte Strahltechnik GmbH (Germany)

We present an efficient implementation of Computational Shear Interferometry (CoSI). The method is used to recover the three dimensional form of a micro injection molded part from a set of shear measurements. For this purpose, ten recordings have been made with the shears varying in orientation and magnitude. Varying the shears’ orientation and magnitude is applied using a setup that consists of a 4f-filter with a reflective phase-only spatial light modulator (SLM) in the corresponding Fourier domain. The SLM is used as an electronically addressable optical diffraction grating with a blazed structure. Based on the birefringent properties of the SLM, two orthogonally polarized diffraction orders, one diffracted and one reflected, appear on the camera sensor and produce shear interferograms. The shift between the images depends on the period and the orientation of the blazed grating. From the shear interferograms, the quantitative phase contrast of light diffracted by the object is reconstructed by means of CoSI. Thus the height map of the object is determined.

In contrast to standard interference based methods, CoSI offers the following advantages:

- It is robust against mechanical distortions, since the superposed wave fields travel the same path.
- It has low demands with respect to the coherence of the illuminating light. This enables the usage of eye-safe and cheap light sources, e.g. light emitted from LED,
- It suppresses image distortions that are independent of the shear.

These advantages make CoSI very suitable to be integrated in a production platform.

High-sensitivity quantitative phase microscopy

Renjie Zhou, Massachusetts Institute of Technology (United States); Cuifang Kuang, Zhejiang Univ. (China); Poorya Hosseini, Massachusetts Institute of Technology (United States); Ravi Chowdhary, Univ. of Illinois at Urbana-Champaign (United States); Zahid Yaqoob, Peter T. C. So, Massachusetts Institute of Technology (United States)

In the past decade, various quantitative phase microscopy (QPM) techniques have emerged, driven by the need to study biological samples non-invasively. However, the fundamental limit for phase noise is scarcely discussed in the literature. In a typically off-axis phase microscope system, the phase noise is limited to a few milliradians using a moderate camera. Common-path QPMs offer much reduced phase noise compared to typical Mach-Zehnder-based systems. However, further scaling down the phase noise becomes difficult. Here we propose a high-sensitivity common-path QPM that promises to reduce the phase noise by a factor of 10 (assuming the mechanical noise is negligible). This is achieved by a specifically designed signal filter, leaving only the subtle phase fluctuations coming from the dynamics sample scattering. By working at photon shot-noise limited detection, we can magnify the subtle phase contrast which is proportional to the camera well depth. We expect this system to have the
height sensitivity similar to an atomic force microscopy, while measuring
biological structures with a full field of view in a single-shot. We plan to
use this system to study cell dynamics, particularly lamellipodial height
fluctuations as well as stiffer cell membrane fluctuations.

9718-69, Session 9
**GPC for QPI (Invited Paper)**
Jesper Glückstad, Technical Univ. of Denmark (Denmark)

Generalized Phase Contrast (GPC) is a light efficient method for generating
speckle-free contiguous optical distributions using binary-only or analog
phase levels. It has been used in applications such as optical trapping
and manipulation, active microscopy, structured illumination, optical
security, parallel laser marking and labelling and recently in contemporary
biophotonics applications such as for adaptive and parallel two-photon
optogenetics and neurophotonics. We will present our most recent GPC
developments geared towards these applications. First, a compact GPC
Light Shaper implementation based on our latest theoretical derivations
is used to demonstrate the benefits for typical applications where lasers
have to be actively shaped into particular light patterns. We then show the
potential of GPC for biomedical and multispectral applications where we
experimentally demonstrate the active light shaping of a supercontinuum
laser over most of the visible wavelength range. Finally, we discuss how GPC
can be advantageously applied for Quantitative Phase Imaging (QPI).

9718-70, Session 9
**Extended synthetic wavelength phase imaging by multiwavelength digital
holography**
David C. Clark, Myung K. Kim, Univ. of South Florida
(United States)

We present a technique of visible spectrum quantitative phase imaging with
ten millimeter range without phase ambiguity while achieving better than
ten micrometer precision. Significantly extended synthetic wavelengths can
be achieved using two wavelengths with a very small difference (e.g. less
than 100 pm); however, the phase noise of the resulting image is magnified
by the same factor as the extended range. This amplified phase noise is then
reduced by an iterative optical and algorithm based process involving, in
the present case, two additional source wavelengths. These wavelengths are
selected to systematically reduce the synthetic wavelength of the previous
step. The result of this process has the advantages of both the unambiguous
range of the largest synthetic wavelength and the precision of the smallest
synthetic wavelength. We demonstrate the application by phase imaging
and three-dimensional reconstruction of various appropriately sized objects.
Additionally, we demonstrate that our four source wavelengths can be
acquired from a single laser diode source by realizing the wavelength output
response to fine temperature control.

9718-71, Session 9
**Gradient light interference microscopy (GLIM) for imaging thick specimens**
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Illinois at Urbana-Champaign (United States)

Compared to the Phase Contrast, Differential Interference Contrast (DIC)
has been known to give higher depth sectioning as well as a halo-free
images when investigating transparent specimens. Thanks to relying
on generating two slightly shifted replicas with a small amount of shift, within
the coherence area, DIC is able to operate with very low coherence light.
More importantly, the method is able to work with very large numerical
aperture of the illumination, which offer comparable sectioning capability
to bright field microscopy. However, DIC is still a qualitative method, which
limits potential applications of the technique. In this paper, we introduce
a method that extends the capability of DIC by combining it with a phase
shifting module to extract the phase gradient information. A theoretical
model of the image formation is developed and the possibility of integrating
the gradient function is analyzed. Our method is benchmarked on imaging
embryos during their 7-day development, HeLa cells during mitosis, and
control samples.
that a gradient-index (GRIN) lens (effective focal length of 300 µm) can be 
used to magnify the Fourier transform of the zero order to the point 
where the DC component fills the camera sensor. We show that the resulting 
Magnified Object Spectrum Interference Microscopy (MOSIM) system can 
successfully reconstruct quantitative phase images, without the need for 
tedious alignment. Because it conserves the common path geometry, 
MOSIM is characterized by 11 nm spatiotemporal pathlength noise. Since it is 
single shot, we demonstrated 400 frames/s acquisition. We anticipate that 
this new method can potentially lead to a more robust and less vibration- 
sensitive QPI instrument for carrying out biological studies at various 
spatiotemporal scales.

9718-74, Session 9

Confocal reflectance quantitative phase microscopy system for 3D refractive index 
mapping
Vijay Raj Singh, SMART-Singapore MIT Alliance for Research & Technology (Singapore); Peter T. C. So, 
Massachusetts Institute of Technology (United States)

Quantitative phase microscopy (QPM), used to measure the refractive index, 
provides the optical path delay measurement at each point of the specimen 
under study and becomes an active field in biological science. In this work we 
present development of confocal reflection phase microscopy system to 
provide depth resolved quantitative phase information for investigation of 
intracellular structures and other biological specimen. The system hardware 
development is mainly divided into two major parts. First, creates a pinhole 
array for parallel confocal imaging of specimen at multiple locations 
simultaneously. Here a digital micro mirror device (DMD) is used to generate 
pinhole array by turning on a subset micro-mirrors arranged on a grid. 
Second is the detection of phase information of confocal imaging foci by 
using a common path interferometer. With this novel approach, it is possible to 
measure the nucle membrane fluctuations and distinguish them from the 
plasma membrane fluctuations. Further, depth resolved quantitative phase 
can be correlated to the intracellular contents and 3D map of refractive 
index measurements.

9718-75, Session 10

Quantitative thermal phase imaging with a low-coherence illumination
Jaeduck Jang, Taeho Shin, Changhoon Jung, Soohwan Sul, Samsung Advanced Institute of Technology (Korea, 
Republic of)

Probing temperature on sub-micrometer scales is of fundamental interest 
in many areas of modern science and technology as well as industrial 
applications, for example, in thermal management of integrated circuits. It has 
been, however, a technically challenging task to investigate thermal-
induced phenomena on the sub-micron scale in real time. This is simply 
due to the lack of a reliable means for a sensitive temperature probe, 
which can be in wide use, just as an alcohol-in-glass thermometer. Recent 
developments in quantitative phase imaging (QPI) has enabled us to 
visualize, without introducing labels, biological cells, nano-materials, and 
defects in semiconductors. Utilizing QPI with a wavefront analyzer as a 
sensitive temperature probe has been recently introduced for non-invasive 
and label-free thermal imaging of metallic nano- and micro-structures. In 
this paper, we present an optical thermal imaging technique with a 
high temperature sensitivity using common-path type diffraction phase 
microscopy (DPM). For a better thermal sensitivity, we utilize a low-
coherence light illumination in DPM, which dramatically reduces speckle 
noise or unwanted fringe patterns inevitably present with a monochromatic 
laser. This noise reduction, in turn, enhances the temperature sensitivity 
shortening overall data collection time in thermal imaging. The coherent 
length of typical single-mode HeNe laser is ca. 100 m and that of ultrashort 
Ti:Sapphire laser is on the order of tens of micrometers. We compare the 
direct effect of the coherence length on thermal images obtained with 
continuous-wave HeNe laser and ultrashort pulsed laser.

9718-76, Session 10

Through-the-objective holographic surface plasmon resonance imaging for 
quantitative measurement of thin film thickness
Biagio Mandracchia, Vito Pagliarulo, Melania Paturzo, Pietro Ferraro, Istituto di Scienze applicata e Sistemi 
Intelligenti (Italy)

We built and tested a Holographic Surface Plasmon Resonance (HoloSPR) 
objective-based microscope for simultaneous amplitude-contrast and 
phase-contrast Surface Plasmon Resonance imaging (SPRI). Thanks to the 
complementary nature of these two contrast methods, we show that 
it possible to measure quantitatively thin film thickness without need of 
angular scanning.

SPRI is a widely spread tool for label-free detection of changes in refractive 
index and concentration, as well as mapping of thin films. Currently, most 
of the SPR sensors rely on the detection of amplitude or phase changes of 
light. Despite the high sensitivities achieved so far, each technique alone has 
a limited detection range with optimal sensitivity.

Recently, a prism-based configuration for simultaneous measurements of 
amplitude and phase contrast SPR images using Digital Holography was 
proposed [1]. Unfortunately, the physical constraint of the prism limits both 
numerical aperture (NA) and magnification of an imaging system. Hence, 
it yields poor spatial resolution compared to that achievable with optical 
microscopy. Moreover, resolution in prism-based imaging is mainly governed 
by geometrical aberrations, which distort SPR images [2].

Here we use a high numerical aperture objective that avoids all the 
limitations due to the use of prism-based configuration, yielding highly 
magnified and distortion-free images. We also present holographic 
reconstructions of SPR images and real-time kinetic measurements to show 
the capability of HoloSPR to provide a versatile imaging method for high-
throughput SPR detection.

tools for targeted drug delivery and are currently used as contrast agents for sonography. We measured size, shell thickness and refractive index for hundreds of polymeric microbubbles showing that shell thickness displays a large variation that is strongly correlated with its refractive index and thus with its composition.

On the other hand we demonstrate that DHM can be used for accurate 3D tracking and sizing of a holographically trapped colloidal probe in a diamond anvil cell (DAC). Polystyrene beads were trapped in water up to Gigapascal pressures while simultaneously recording in-line holograms at 1 KHz frame rate. This technique may potentially provide a new method for spatially resolved pressure measurements inside a DAC.
9719-2, Session 1

**Photo-induced binding studies on human serum albumin and protoporphrins: ZnPPIX and hemin**

Jie Hu, Ryan Allen, Lorenzo Brancalone, The Univ. of Texas at San Antonio (United States)

In this study, we present a new methodology that allows to study the physophysical properties of the interaction of human serum albumin (HSA) with Zn-protoporphyrin IX - Zn(II)PPIX and Hemin - Fe(III)PPIX without the "interference" from aggregates of the porphyrins. This new method enables us to retrieve more accurate binding constants using the Benesi-Hildebrand analysis of the porphyrin emission (or absorption) and the fluorescence quenching (i.e. Stern-Volmer analysis). Moreover, more accurate results are obtained from fluorescence lifetime that showed the clear occurrence of FRET between Trp214 and each porphyrin ligand. This yielded a more accurate model for the location of the binding site but also improved our characterization of the conformational effects prompted by the irradiation of the ligand.

9719-3, Session 1

**Biological inspiration in optics and photonics: harnessing nature's light manipulation strategies for multifunctional optical materials (Invited Paper)**

Mathias Kolle, Joseph D. Sandt, Sara N. Nagelberg, Lauren D. Zarzar, Massachusetts Institute of Technology (United States); Moritz Kreysing, Max-Planck-Institut für molekulare Zellbiologie und Genetik (Germany); Peter Vukusic, Univ. of Exeter (United Kingdom)

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 that consist 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. Biomimetic and bio-inspired approaches for the manufacture of photonic systems rely on self-assembly and bottom-up growth techniques often combined with conventional top-down manufacturing. In this regard, we can benefit in several ways from highly sophisticated material solutions that have convergently evolved in various organisms. We explore design concepts found in biological photonic architectures, seek to understand the mechanisms underlying morphogenesis of bio-optical systems, aim to devise viable manufacturing strategies that can benefit from insight in biological formation processes and the use of established synthetic routines alike, and ultimately strive to realize new photonic materials with tailor-made optical properties.

This talk is focused on the identification of biological role model photonic architectures, a brief discussion of recently developed bio-inspired photonic structures, including mechano-sensitive color-tunable photonic fibers and reconfigurable fluid micro-lenses. Potentially, early-stage results in studying and harnessing the structure-forming capabilities of living cells that lie at the origin of many species’ ability to grow photonic materials will also be presented.

9719-4, Session 1

**Near-real time monitoring of live to dead bacterial cell ratios**

Fang Ou, Rachel Guo, Cushla McGoverin, The Univ. of Auckland (New Zealand) and The Dodd-Walls Ctr. for Photonic and Quantum Technologies (New Zealand); Simon Swift, The Univ. of Auckland (New Zealand); Frédérique Vanholsbeeck, The Univ. of Auckland (New Zealand) and The Dodd-Walls Ctr. for Photonic and Quantum Technologies (New Zealand)

The antibiotic pipeline is running dry; there is a need to develop new antibiotics, maximize the efficacy of antibiotics currently available and to minimize the risk of exposure. All these endeavors benefit from the assessment of live to dead bacterial ratios within a sample. Live to dead bacterial ratios are determined by fluorescence measurements of samples stained with two dyes of different specificities for live and dead cells (e.g. SYTO 9 and propidium iodide). Fluorescence microscopy and flow cytometry have been used extensively to quantify the live/dead cell fluorescence signals, however, both these methods of detection are relatively expensive. We have developed an all fibre based fluorometer for the measurement of SYTO 9 and propidium iodide staining within bacterial solutions.

Escherichia coli solutions with differing ratios of live to dead cells were stained with SYTO 9 and propidium iodide. After method optimisation a 1:12 molar ratio of SYTO 9 to propidium iodide was used, and bacterial solutions were first mixed with propidium iodide, then SYTO 9. Fluorescence spectra were collected from samples using an all fibre based fluorometer consisting of a 473 nm solid state laser, a photodiode for continuous measurement of incident laser intensity and a CCD spectrometer. The bound SYTO 9 signal was ratioed to the bound propidium iodide signal and correlated with live to dead bacterial cell ratios as determined by flow cytometry (R2 = 0.88). Preliminary results are promising and indicate this may be a method of measurement adaptable to a wide range of environments.

9719-5, Session 1

**Cherenkov imaging during volumetric modulated arc radiation therapy for real-time beam tracking and treatment response monitoring**

Jacqueline M. Andreozzi, Dartmouth College (United States); Rongxiao Zhang, Harvard Medical School (United States); David J. Gladstone, Lesley A. Jarvis, Dartmouth Hitchcock Medical Ctr. (United States); Brian W. Pogue, Dartmouth College (United States)

**Purpose:** The purpose of this study was to investigate the suitability of Cherenkov imaging during highly dynamic radiotherapy, specifically volumetric modulated arc therapy (VMAT) of head and neck cancer patients. Potential for real-time beam tracking on the patient, as well as viability of employing detected Cherenkov emission intensity as a re-planning metric were investigated.

**Methods:** Building on previous studies of Cherenkov imaging during...
whole-breast radiotherapy, two patients undergoing VMAT for head and neck cancer were imaged during treatment using a clinically developed Cherenkov imaging system. A PIMax4 1024i ICCD camera synchronized to the radiation treatment beam captured the Cherenkov signal emitted by patient tissue, even with room lights on at a modest level for patient safety and comfort. Software was developed to collect background non-Cherenkov images in between active Cherenkov emission images during the treatment. For both patients, multiple treatment fractions were imaged to allow day-to-day comparison of Cherenkov intensity throughout the duration of the radiation treatment. Comparison with the simulated treatment plan was also conducted.

Results: Alternating active beam-on images with background images was necessary to limit the noise introduced by the moving linear accelerator gantry, and resulted in an effective frame-rate of up to 4fps. The patient immobilization mask did not inhibit the ability of the system to detect the dynamic beam shape and movement.

Conclusion: Cherenkov imaging of VMAT patients was conducted for the first time, allowing visualization of the radiation treatment beam on the surface of two patients in real-time, and supporting viability in clinical applications.

9719-6, Session 1

Optical trapping for optogenetics: otoliths manipulation

Itia A. Favre-Bulle, Ethan Scott, Halina Rubinsztein-Dunlop, The Univ. of Queensland (Australia)

Otoliths play an important role in Zebrafish in terms of hearing and sense of balance. Many studies have been conducted to understand its structure and function, however the encoding of its movement in the brain remains unknown. Here we developed a noninvasive system capable of manipulating the otolith using optical trapping while we image its behavioral response and brain activity. We’ll also present our tools for behavioral response detection and brain activity mapping.

Acceleration is sensed through movements of the otoliths in the inner ear. Because experimental manipulations involve movements, electrophysiology and fluorescence microscopy are difficult. As a result, the neural codes underlying acceleration sensation are poorly understood. We have developed a technique for optically trapping otoliths, allowing us to simulate acceleration in stationary larval zebrafish. By applying forces to the otoliths, we can elicit behavioral responses consistent with compensation for perceived acceleration. Since the animal is stationary, we can use calcium imaging in these animals’ brains to identify the functional circuits responsible for mediating responses to acceleration in natural settings.

9719-7, Session 1

Phase sensitive signal analysis for bi-tapered optical fibers

Amit Ben Harush Negari, Univ. of Dayton (United States); Daniel Jauregui Vazquez, Juan M. Sierra-Hernandez, Univ. of Dayton (United States) and Univ. de Guanajuato (Mexico); Diego F. Garcia Mina, Branden J. King, Ighodalo U. Idehenre, Peter E. Powers, Karolyn M. Hansen, Joseph W. Haus, Univ. of Dayton (United States)

This study focuses on the characterization of bi-tapered optical fibers, specifically signal analysis for sensing applications. Molecular binding to the fiber surface changes the refractive index and thickness of the biolayer, which interacts with propagating light, causing a measureable phase shift in the output.

Coating the tapered fiber region with biomolecular recognition elements provides a biosensing capability: binding of analyte to the recognition layer results in a molecular conformational change that is detected as a change in the light propagation pattern, that is, the refractive index. Single mode fibers were tapered biconically different waist lengths and diameters. Optical fibers with 9/125µm core/cladding diameter, solution refractive indices ranged from 1.2220-1.3405 and tunable laser (1480-1550nm) were used.

The tapered fibers work by the interaction of (at least) two modes in the taper region: a high order mode associated with the cladding and the fundamental core mode. These modes generate an oscillatory response of the optical fiber sensor as the wavelength is scanned. Our signal analysis detected refractive index variations of order 0.0005RIU and found that a nearly linear wavelength shift of the interference patterns occurs. The main new element is that the data analysis can resolve extremely small variations in the refractive index. In our analysis we decompose the signal data into Fourier components and use the dominant oscillation frequency for further analysis. The signal analysis enables improved high-resolution detection and this protocol will now be applied to bio-functionalized bi-tapered fibers for the detection of real-world analytes in aqueous solutions.

9719-8, Session 2

Crowding, dynamics and transcription (Invited Paper)

Igal Szleifer, Northwestern Univ. (United States)

Biophotonic studies based on partial wave spectroscopy have shown that early carcinogenesis is characterized by a change in the nanoscale molecular organization of the cell nuclei. These findings suggest that cancer is associated with change in macromolecular crowding. In this presentation we will discuss a recent approach that we have developed to incorporate molecular scale information into a systems based approach to study the role of macromolecular crowding on different phenomena ranging from protein diffusion to gene transcription. Macromolecular crowding affects both dynamics and equilibrium properties. We will show that transcription is a non-monotonic function of crowders concentration in the cell nuclei. Furthermore, we will show how changes in macromolecular crowding in the nuclei and in the cytoplasm lead to different changes in the oscillatory behavior on NF-κB upon stimuli. Our results show the important regulatory role that non-specific interactions play in biological systems.

9719-9, Session 2

A macromolecular crowding study of RNA folding and activity: polymer pore size matters!

Richard Börner, Erica Fiorini, Univ. Zürich (Switzerland); Bishnu Paudel, David Rueda, MRC Clinical Sciences Ctr. (United Kingdom); Roland K. O. Sigel, Univ. Zürich (Switzerland)

Catalytic RNAs, like the group IIb intron ribozyme of S. cerevisiae, require a high magnesium(II) concentration to show folding and function in vitro [1]. In contrast, in vivo conditions are characterized by a highly crowded cellular environment and much lower ion concentration. Molecular crowding agents are a widespread tool to mimic cellular crowding [2]. However, particular physical/chemical properties explaining the crowders influence are mostly not understood. In this study, we gain new insights on how polymer properties like viscosity, pore size etc. influence the activity and folding of a large RNA.

We combined bulk activity assays and single-molecule Förster Resonance Energy Transfer experiments. screening the PEG volume fraction (%) and molecular weight (MW). Our results revealed that upon the influence of crowding agents, a compaction of the underlying structure depends on the PEG % and the presence of different PEG MW and % unveiled an optimal pore size in terms of catalytic activity. In summary, an increasing density of
The application of low angle light scattering to evaluate qualitatively and quantitatively the dynamics of formation of oligomers in heme protein sensors


The aim of this study is to investigate the structural organization and oligomerization properties of the sensory kinase protein DevS using low-angle light scattering (LALS) and high performance liquid chromatography (HPLC). In addition, the structural characteristics of FixL and BSA were investigated and compared with DevS to better elucidate LALS. DevS is a direct and specific O2 sensing protein in M. tuberculosis and acts as an activator of the transcription factor protein DevR that triggers the latency state of tuberculosis cells under hypoxic conditions1–4. DevS structure in the on and off conformation has been evaluated in order to better understand its behavior in solution in different conditions of pH, ionic force, and with and without O2. LALS and HPLC analysis were performed right after DevS expression and purification process. Preliminary LALS results showed a hydrodynamic radius (Rh) varying from 6.6-7.3 nm for DevS protein that is in agreement with previous results applying other light scattering methods5. Possible oligomer equilibrium is involved of tetramer (~250 KDa) and octamer (~500 KDa). The Debye plot varying the concentration of DevS from 0.2-1.4 mg/ml pointed out a molecular mass value of approximately 500 KDa. The results of LALS for BSA has proven to be highly reliable with a Rh value of c.a. 3.6 nm. Considering BSA a globular protein, the molecular weight estmative, using LALS is near 67 KDa, which is reasonable with the value reported in the literature. HPLC results for DevS indicated the presence of multiple oligomers, mainly tetramers, hexamers and octamers.

9719-12, Session 3

Recovering refractive index correlation function from measurement of tissue scattering phase function (Invited Paper)

Jeremy D. Rogers, Univ. of Wisconsin-Madison (United States)

 Numerous methods have been developed to quantify the light scattering properties of tissue. These properties are of interest in diagnostic and screening applications due to sensitivity to changes in tissue ultrastructure and changes associated with disease such as cancer. Tissue is considered a weak scatterer because that the mean free path is much larger than the correlation length. When this is the case, all scattering properties can be calculated from the refractive index correlation function $Bn(r)$.

Direct measurement of $Bn(r)$ is challenging because it requires refractive index measurement at high resolution over a large tissue volume. Instead, a model is usually assumed. One particularly useful model, the Whittle-Matern function includes several realistic function types such as mass fractal and exponential. Optical scattering properties for weakly scattering media can be determined analytically from $Bn(r)$ by applying the Rayleigh-Gans-Debye (RGD) or Born Approximation, and so measured scattering properties are used to fit parameters of the model function. Direct measurement of $Bn(r)$ would provide confirmation that the function is a good representation of tissue or help in identifying the length scale at which changes occur.

The RGD approximation relates the scattering phase function to the refractive index correlation function through a Fourier transform. This can be inverted without approximation, so goniometric measurement of the scattering can be converted to $Bn(r)$. However, geometric constraints of the measurement of the phase function, angular resolution, and wavelength result in a band limited measurement of $Bn(r)$. These limits are discussed and example measurements are described.

9719-13, Session 3

Modelling refractive index changes due to molecular interactions

Manoj M. Varma, Indian Institute of Science (India)

There are a large number of sensing techniques which use optical changes to monitor interactions between molecules. In the absence of fluorophores or other labels, the basic signal transduction mechanism relies on refractive index changes arising from the interactions of the molecules involved. A quantitative model incorporating molecular transport, reaction kinetics and optical mixing is presented which reveals important insights concerning the optimal detection of molecular interactions optically. Although conceptually
simple, a comprehensive model such as this has not been reported anywhere. Specifically, we investigate the pros and cons of detecting molecular interactions in free solution relative to detecting molecular interactions on surfaces using surface bound receptor molecules such as antibodies. The model reveals that the refractive index change produced in surface based sensors is 2-3 orders of magnitude higher than that from interactions in free solution. On the other hand, the model also reveals that it is indeed possible to distinguish specific molecular interactions from non-specific ones based on free-solution bulk refractometry without any washing step necessary in surface based sensors. However, the refractive index change for free solution interactions predicted by the model is smaller than 10^-7 RIU, even for large proteins such as IgG in sufficiently high concentrations. This value is smaller than the typical 10^-6 RIU detection limit of most state of the art optical sensing techniques therefore requiring techniques with substantially higher index sensitivity such as Back Scattering interferometry.

9719-14, Session 3

The role of membrane dynamics in electrical and infrared neural stimulation

Erk K. Moen, The Univ. of Southern California (United States); Hope T. Beier, Bennett L. Ibye, Air Force Research Lab. (United States); Andrea M. Armani, The Univ. of Southern California (United States)

We recently developed a nonlinear optical imaging technique based on second harmonic generation (SHG) to identify membrane disruption events in live cells. This technique was used to detect nanoporation in the plasma membrane following nanosecond pulsed electric field (nsPEF) exposure. It has been hypothesized that similar poration events could be induced by the thermal gradients generated by infrared (IR) laser energy. Optical pulses are a highly desirable stimulus for the nervous system, as they are capable of inhibiting and producing action potentials in a highly localized but non-contact fashion. However, the underlying mechanisms involved with infrared neural stimulation (INS) are not well understood. The ability of our method to non-invasively measure membrane structure and transmembrane potential via Two Photon Fluorescence (TPF) make it uniquely suited to neurological research. In this work, we leverage our technique to understand what role membrane structure plays during INS and contrast it with nsPEF stimulation. We begin by examining the effect of IR pulses on Cho hM1 cells before progressing to NG108 neuroblastomas and finally primary neurons. This allows us to identify nanoporation during the pulse in a wide array of conditions. Additionally, we study membrane order as result of cholesterol content, a key contributor to neuronal function. The transfected Cho cells provide a surrogate for investigating the activation of intracellular signaling pathways as a result of the pulse. They also provide a unique opportunity to directly compare poration as a result of IR pulses to nsPEF exposure.

9719-15, Session 3

Cloud-based Monte Carlo modelling of BSSRDF for the rendering of human skin appearance

Alexander Doronin, Holly E. Rushmeier, Yale Univ. (United States); Igor Meglinski, Alexander V. Bykov, Univ. of Oulu (Finland)

We present a new Monte Carlo based approach for the modelling of Bidirectional Scattering-Surface Reflectance Distribution Function (BSSRDF) for accurate rendering of human skin appearance. The variations of both skin tissues structure and the major chromophores are taken into account correspondingly to the different ethnic and age groups. The computational solution utilizes HTML5, accelerated by the graphics processing units (GPUs), and therefore is convenient for the practical use at the most of modern computer-based devices and operating systems. The results of imitation of human skin reflectance spectra, corresponding skin colours and examples of 3D faces rendering are presented and compared with the results of phantom studies.

9719-16, Session 4

New directions in light sheet imaging (Invited Paper)

Kishan Dholakia, Univ. of St. Andrews (United Kingdom)

in many photonics fields there is a recognition that using the direct form a laser output – the Gaussian beam – is restrictive for a number of applications in biophotonics including manipulation, imaging and beyond. I will describe the use of shaped light fields namely propagation invariant ('non-diffracting') light fields and complex beam shaping. Propagation invariant light fields, as the name suggests retain their transverse intensity profile upon propagation. Bessel light fields and Airy light fields are prime examples of such beams. In terms of imaging, single plane illumination (light sheet) microscopy (SIPI) offers a myriad of unique advantages. Orthogonal detection allows rapid imaging of large, three-dimensional, samples of living tissue. Illumination with a thin sheet of light ensures high contrast by minimizing the fluorescent background. Moreover, by restricting the sample exposure to a single plane, photo-bleaching and damage are minimized. This is crucial when imaging photo-sensitive samples over a larger period of time. Recent enhancements to the original technique attempt to overcome the inherent trade off between axial resolution and field-of-view of conventional light sheet microscopy. Until recently, this was only achieved by compromising on one or more of its key advantages: high contrast, time-resolution, or minimal sample exposure. I will discuss the use of propagation invariant light fields for the enhancement within this imaging modality and furthermore, the combination of optical trapping and manipulation with light sheet imaging.

9719-17, Session 4

Biomechanical cell analysis using quantitative phase imaging (Invited Paper)

Adam Wax, Han Sang Park, William J. Eldridge, Duke Univ. (United States)

Quantitative phase imaging provides nanometer scale sensitivity and has been previously used to study spectral and temporal characteristics of individual cells in vitro, especially red blood cells. Here we extend this work to study the mechanical responses of individual cells due to the influence of external stimuli. Cell stiffness may be characterized by analyzing the inherent thermal fluctuations of cells but by applying external stimuli, additional information can be obtained. The time dependent response of cells due to external shear stress is examined with high speed quantitative phase imaging and found to exhibit characteristics that relate to their stiffness. However, analysis beyond the cellular scale also reveals internal organization of the cell and its modulation due to pathologic processes such as carcinogenesis. Further studies with microfluidic platforms point the way for using this approach in high throughput assays.

9719-18, Session 4

Nanoscale characterization of vesicle adhesion by normalized total internal reflection fluorescence microscopy

Marcelina Cardoso Dos Santos, Cyrille Vézy, Rodolphe Jaffiol, Univ. de Technologie Troyes (France)

Total Internal Reflection Microscopy (TIRFM) is widely used to study the adhesion of living cells (for example by imaging the cytoskeleton or the...
focal adhesion zone. The evanescent wave produced at the glass-medium interface is characterized by an exponential decay of the intensity along the z direction. It provides a selective excitation of fluorescent molecules in the vicinity of the surface. But from the TIRF pictures it is not possible to get quantitative information about the distance between the membrane and the surface, which is an important parameter in cell adhesion study. To get such crucial information, we developed a prismless setup equipped with a rotatable mirror to switch easily between TIRFM and epi-fluorescence microscopy. As a biomimetic system, we used fluorescent Giant Unilamellar Vesicles (GUVs). The normalization of the TIRF images of GUVs by epi-fluorescence ones allows us to determine the absolute distance between GUVs and various substrates with a nanoscale accuracy [1]. We have investigated GUV adhesion by changing the nature and the strength (through chemical functionalization of surfaces) of the interactions between the GUVs and the substrate [2].


9719-19, Session 4

Novel optical approaches for label-free quantification of nano-cytotoxic effects

Sarah Mues, Jan Antunovic, Steffi Ketelhut, Björn Kemper, Jürgen Schnekeburger, Westfälische Wilhelms-Univ. Münster (Germany)

The in vitro cytotoxicity assessment of engineered nanomaterials commonly involves the measurement of different endpoints like the formation of reactive oxygen species, cell viability or cell death. Usually these parameters are determined by optical readouts of enzymatically converted substrates that are often affected by the tested nanomaterials.

Using cell viability (WST-8) and cell death (LDH) assays as parameter we have initially investigated the toxic effects of spherical (NM 300) and rod shaped (NM 302) silver nanomaterials with a matrix of four cell lines representing different functions. In addition, we explored digital holographic microscopy (DHM) for multimodal label-free analysis of nanomaterial toxicity. Quantitative DHM phase images of cells that were incubated with nanomaterials were analyzed for refractive index, volume, density and dry mass. Moreover, we have used a label-free flow cytometer configuration to investigate interactions of particles and macrophages as well as fibroblasts by side scatter signal analysis.

We demonstrate that silver spheres lead to higher cytotoxic effects than rods in all four examined cell lines and both assay. Furthermore, we could correlate these data to a decrease of the cellular refractive index after incubation with NM 300 as well as a decreased dry mass and surface area development indicating reduced cell viability and cell death. Finally, the dose dependent uptake kinetics of both materials that were observed by label-free flow cytometry were compared with toxicity data. Taken together, our results demonstrate the potential of digital holographic microscopy and flow cytometry as novel valuable label-free tools for the analysis nanomaterial toxicity and cell particle interaction studies.

9719-20, Session 4

2D light scattering label-free cytometry using light-sheet illumination

Meiai Lin, Xuantao Su, Shandong Univ. (China)

Flow cytometry that measures optical signals from biological cells has wide application in many fields such as biomedicine. Conventionally the cells are labeled with fluorescent dyes to obtain detailed cellular information, which is complex and may interrupt the cell function. Compared with fluorescent labeling, two-dimensional (2D) light scattering cytometry is an effective label-free technology for cell analysis.

Light-sheet technique has been introduced into fluorescence microscopy in recent years. Here we adopt the light-sheet illumination in 2D light scattering static cytometry. The light-sheet restricts the excitation volume near the scatter, thus reduces background noise. In our cytometer, a cylindrical lens is used to form light-sheet and a sample chamber is fabricated for better excitation of the cells under an inverted microscope. Two-dimensional light scattering patterns are obtained by a complementary metal oxide semiconductor (CMOS) detector via a low numerical aperture (NA 0.4) objective lens.

The thickness of the light-sheet measured in fluorescent solution is about 10 μm. Two-dimensional light scattering patterns of single polystyrene microspheres with 3.87 μm in diameter are obtained. The experimental patterns characterized with fringe structure agree well with the Mie theory simulated ones. We further demonstrate that the technique developed here can be used for yeast cell size determination. Our results suggest that the light-sheet illumination is an effective excitation method for label-free cytometry and light-sheet-based 2D light scattering static cytometry has the potential for cell diagnostics.

9719-21, Session 5

Identifying quiescent cancer cell populations in heterogeneous samples with fluorescence lifetime imaging

Tiffany Heaster, Vanderbilt Univ. (United States); Alex J. Walsh, Vanderbilt Univ. (United States) and Air Force Research Lab. (United States); Melissa C. Skala, Vanderbilt Univ. (United States)

Quiescent cancer cell populations introduce a high degree of cellular heterogeneity within a tumor, presenting challenges including evasion from drug treatment and increased likelihood of tumor recurrence. Changes in cellular metabolism control transitions between proliferative and quiescent states. Fluorescence lifetime imaging (FLIM) exhibits sensitivity to intracellular activity of metabolic co-enzymes NAD(P)H and FAD and thus provides a platform to study the metabolism of cells in these two states. To address the issue of heterogeneity, we first characterized metabolism of proliferating and quiescent cell populations by measuring the optical redox ratio (NAD(P)H fluorescence intensity divided by FAD intensity), as well as NAD(P)H and FAD fluorescence lifetimes using two-photon microscopy and time-correlated single photon counting. Our hypothesis that quiescent populations can be metabolically distinguished from proliferating populations was supported upon comparison of optical metabolic properties of Kasumi-1 human acute myeloid leukemia myeloblasts treated with DMSO (proliferative cells) and JQ1, an inhibitor of cell cycle progression (quiescent cells). Proliferative and quiescent cell populations displayed significant differences in measurements of redox ratio (p<0.05), NAD(P)H lifetime (p<0.05), and FAD lifetime (p<0.05). Additionally, we observed metabolic separation between proliferating and quiescent sub-populations within heterogeneous samples. Cell suspensions with varying proportions of proliferating and quiescent cells were prepared to generate heterogeneous populations. Individual populations were distinguished by fitting single-cell multiphoton FLIM data to a Gaussian mixture model, which achieved low errors in extracted proportions of proliferating and quiescent populations. These results establish a threshold for differentiating mixed populations of proliferating and quiescent cells and may allow further characterization of tumor progression and drug resistance mechanisms influenced by this heterogeneity.
ISOCT study of effects of enzymatic crosslinking of collagen in field carcinogenesis

Graham Spicer, Scott T. Young, Ji Yi, Northwestern Univ. (United States); Lonnie D. Shea, Univ. of Michigan (United States); Vadim Backman, Northwestern Univ. (United States)

The role of extracellular matrix modification and signaling in cancer progression is an increasingly recognized avenue for the progression of the disease. Previous study of field effect carcinogenesis with inverse Spectroscopic Optical Coherence Tomography (ISOCT) has revealed pronounced changes in the nanoscale-sensitive mass fractal dimension D measured from field effect tissue when compared to healthy tissue. However, the origin of this difference in tissue ultrastructure in field effect carcinogenesis has remained poorly understood.

Here, we present findings supporting the idea that enzymatic crosslinking of the extracellular matrix is an effect that presents at the earliest stages of carcinogenesis. We use a model of collagen gel with crosslinking induced by lysyl oxidase (LOXL4) to recapitulate the difference in D previously reported from healthy and cancerous tissue biopsies. Furthermore, STORM imaging of this collagen gel model verifies the morphologic effects of enzymatic crosslinking at length scales as small as 40 nm, close to the previously reported lower length scale sensitivity threshold of 35 nm for ISOCT. Analysis of the autocorrelation function from STORM images of collagen gels and subsequent fitting to the Whittle-Matern correlation function shows a similar effect of LOXL4 on D from collagen measured with ISOCT and STORM. We extend this to mass spectrometric study of tissue to directly measure concentrations of collagen crosslink residues. The validation of ISOCT as a viable tool for non-invasive rapid quantification of collagen ultrastructure lends it to study other physiological phenomena involving ECM restructuring such as atherosclerotic plaque screening or cervical ripening during pregnancy.

A model for oxygen-dependent backscattering spectroscopic contrast from single red blood cells

Rongrong Liu, Ji Yi, Siyu Chen, Hao F. Zhang, Vadim Backman, Northwestern Univ. (United States)

The oxygen-dependent absorption of hemoglobin provides the fundamental contrast for all label-free techniques measuring blood oxygenation. When hemoglobin is packed into red blood cells (RBCs), the structure of the cells creates light scattering which also depends on the absorption based on the Kramers-Kronig relationship. Thus a proper characterization of the optical behaviors of blood has been a key to any accurate measurement of blood oxygenation, particularly at the capillary level where RBCs are deoxygenated and deoxygenated single RBCs with different sizes for standard and possible deformed cell geometries in blood flow, all which suggested similar backscattering spectroscopic contrast and were confirmed by Mie Theory and experiments using visible Optical Coherence Tomography (visOCT). As long as the cell size satisfies Gaussian distribution with a coefficient variance (C.V.) large enough, there is clear absorption contrast between the backscattering spectra of oxygenated and deoxygenated single RBCs calculated by this model, so oxygen saturation can then be characterized. Thus, this theoretical model can be extended to extract absorption features of other scattering particles as long as they satisfy Born Approximation.

Label-free in-vivo measurement of lymph flow velocity using Doppler optical coherence tomography

Cedric Blatter, Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States); Eelco F. J. Meijer, Massachusetts General Hospital (United States); Ahhyun S. Nam, Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States); Dennis Jones, Timothy P. Padera, Massachusetts General Hospital (United States); Benjamin J. Vakoc, Massachusetts General Hospital (United States) and Wellman Ctr. for Photomedicine (United States)

Alterations in lymphatic network function contribute to the lymphedema development, cancer progression and impairment in regional immune function. However, there are limited tools available to directly measure lymphatic vessel function and transport in vivo. Existing approaches such as fluorescence recovery after photo-bleaching (FRAP) require injection of exogenous labels which intrinsically alter the physiology of the local lymphatic network. A label-free approach to imaging lymph flow in vivo would provide direct and unaltered measurements of lymphatic vessel transport and could catalyze research in lymphatic biology. Here, we demonstrate and validate the use of Doppler optical coherence tomography (DOCT) to measure lymph flow in vivo at speeds as low as 50μm/s. Compared to blood, lymph is relatively acellular (under normal conditions), but contains similar soluble components to blood plasma. We demonstrate that the small but detectable scattering signal from lymph can be used to extract fluid velocity using a dedicated algorithm optimized for Doppler analysis in low signal-to-noise settings (0 to 6 dB typical). We demonstrate the accuracy of this technique by comparing DOCT to FRAP measurements, using an intralipid lymph proxy in microfluidic devices and in vivo in the mouse ear. Finally, we demonstrate the label free measurement of lymph speed in the hind-limb of mice with a temporal resolution of 0.25s that agree well with prior literature reports. We anticipate that DOCT can become a powerful new tool in preclinical lymphatic biology research—including the relationship between lymphatic function and metastasis formation—with the potential to later expand also to clinical settings.

Elasticity-based identification of tumor margins using Brillouin spectroscopy

Maria A. Troyanova-Wood, Zhaokai Meng, Andrew J. Traverso, Omar Yusufzai, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Accurate identification of tumor margins is crucial for surgical removal resulting in minimal residual diseased cells. It is possible to discriminate between malignant and healthy tissues on the basis of the difference in their elastic properties. Brillouin spectroscopy is a powerful spectroscopic technique capable of assessment of mechanical properties of the sample. In this work, change of viscoelasticity between tumor and surrounding normal tissue was evaluated to localize the pathology boundary. Two models of skin cancer were used to obtain the measurements. Tumors incised from Sinclair miniature swine were used as animal model of human cutaneous malignant melanoma. However, the observed elasticity of the melanoma compared to the surrounding tissue was dissimilar between different samples, possibly due to different states of spontaneous regression of the melanoma at the time of acquisition. To obtain measurements from a sample with controlled properties, a cell model of a basal cell carcinoma was created by hanging drop technique. The Brillouin measurements on both models were supplemented with chemical information obtained from Raman spectroscopy.
Study of reversible color change of elytron of Aspidimorpha santaecrucis beetle

Ekata H. Ghate, Gauri R. Kulkarni, Savitribai Phule Pune Univ. (India)

Reversible color change properties of Aspidimorpha santaecrucis [Tortoise Beetle] were studied. It was observed that, when beetle specimens were dipped in absolute alcohol, color of the elytra changes to metallic golden color. In dry state, elytron shows dark brown color. Aspidimorpha santaecrucis is a tortoise beetle so named because of its tortoise like overall appearance. It belongs to family Chrysomelidae and subfamily Cassidinae. Many relatives of this species have brilliant green and black and red coloration. Some of these even appear golden colored due to structural colors. These beetles are found in and around Pune, India. In order to investigate whether the surface morphology of the elytron changes in dry and wet state ESEM was used as this technique allows microscopy of the specimens in ‘wet’ state. Metallic golden color of tortoise beetle was characterized in present study. ESEM revealed that the surface morphology of elytral surface of beetle remains unaltered in dry and wet state. TEM revealed broadband multilayer structure. Reflection spectroscopy was used to study reflection properties of the elytral surface in dry and wet state. Morphological characterization of the surface and internal structure of elytron indicated that broadband multilayer structure present in the cuticle is responsible for the metallic golden sheen of the beetle. Reversible color change properties of such beetles has many applications in the field of biomimetics.
9720-1, Session 1

Compressed ultrafast photography at 100 billion frames per second (Invited Paper)
Liang S. Gao, Ricoh Innovations, Inc. (United States)

Video recording of ultrafast phenomena using a detector array based on the CCD or CMOS technologies is fundamentally limited by the sensor’s on-chip storage and data transfer speed. To get around this problem, the most practical approach is to utilize a streak camera. However, the resultant image is normally one-dimensional—only a line of the scene can be seen at a time. Acquiring a two-dimensional image thus requires mechanical scanning across the entire field of view. This requirement poses severe restrictions on the applicable scenes because the event itself must be repetitive.

To overcome these limitations, we have developed a new computational ultrafast imaging method, referred to as compressed ultrafast photography (CUP), which can capture two-dimensional dynamic scenes at up to 100 billion frames per second. Based on the concept of compressed sensing, CUP works by encoding the input scene with a random binary pattern in the spatial domain, followed by shearing the resultant image in a streak camera with a fully-opened entrance slit. The image reconstruction is the solution of the inverse problem of above processes. Given sparsity in the spatiotemporal domain, the original event datacube can be reasonably estimated by employing a two-step iterative shrinkage/thresholding algorithm.

To demonstrate CUP, we imaged light reflection, refraction, and racing in two different media (air and resin). Our technique, for the first time, enables video recording of photon propagation at a temporal resolution down to tens of picoseconds. Moreover, to further expand CUP’s functionality, we added a color separation unit to the system, thereby allowing simultaneous acquisition of a four-dimensional datacube (x,y,t,?) where ? is wavelength, within a single camera snapshot.

9720-2, Session 1

Multi-aperture ultra-high-speed imaging with lateral electric field charge modulators (Invited Paper)
Keiichiro Kagawa, Futa Mochizuki, Min-Woong Seo, Keita Yasutomi, Shoji Kawahito, Shizuoka Univ. (Japan)

The time resolution of charge modulation in CMOS image sensors has entered the sub-nano second regime and is still reducing toward tens of pico-second. The lateral electric field modulators (LEFM) invented at Shizuoka University has significantly contributed to the recent progress in the solid-state time-resolving imaging field. Based on the LEFM technology, we are developing ultra-high-speed CMOS image sensors whose frame rate or time resolution is determined only by the charge modulation speed, although the frame rate of the conventional ultra-high-speed image sensors is defined by the multi-stage charge transfer speed in CCD or the signal transfer speed from the pixel to the column frame memory. Our method is a fusion of optics, electronics, and signal processing. Unlike the conventional ultra-high-speed image sensors, our sensor has no frame memory. However, the pixel works as a memory, and the optical signal is temporally multiplexed on the focal plane. In other words, multiple-exposure with focal-plane electronic shutter is the key to achieve ultra-high frame rate. Here, the compressive sampling with random temporal shutters is exploited to reproduce more frames than the apertures in the camera.

9720-3, Session 1

Pixel super-resolution of time-stretch imaging by an equivalent-time sampling concept
Antony C. S. Chan, Edmund Y. Lam, Kevin K. Tsia, The Univ. of Hong Kong (Hong Kong, China)

Optical time-stretch imaging relies on conversion of spectrally-encoded spatial information to the serial temporal waveform by group velocity dispersion. Limited to the optical attenuation, the space-to-time conversion is generally limited to 0.05ns/7m or less. This implies that a state-of-the-art digitizer operating at over 20GSa/s is required to resolve the micrometer-scale features for microscopy applications. The acquired image is thus easily corrupted by aliasing if sampled at a lower rate. However, the loss of high resolution can be restored by employing pixel super-resolution (SR) algorithm from multiple subpixel-shifted, low-resolution images. Precise subpixel shifting in optical time-stretch imaging can be realized when the digitizer sampling clock is unlocked from the repetition rate of the laser – a feature common to many time-stretch imaging systems. By harnessing this effect at a slower sampling rate, we extract multiple low-resolution line-scans, each of which is introduced with a subpixel shift automatically. This technique resembles the concept of equivalent-time sampling adopted in sampling oscilloscopes. Unlike any classical pixel SR imaging techniques, this method requires no additional hardware for active subpixel-shift control. We here present the general system design rules and the algorithm for realizing pixel SR time-stretch imaging. We also show a proof-of-concept experiment with adaptive subpixel-registration and image restoration algorithms. At a lower sampling rate (50Sa/s), we are able to restore the image size of 2.7m from the original aliased image with a pixel size of 3.67m. We also demonstrate that such technique facilitates the morphological classification of phytoplankton culture.
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Scan-less, line-field confocal microscopy by combination of wavelength/space conversion with dual optical comb

Takeshi Yasui, Eiji Hase, Shuji Miyamoto, Yi-Da Hsieh, Takeo Minamikawa, The Univ. of Tokushima (Japan); Hirot sugu Yamamoto, Utsunomiya Univ. (Japan)

Optical frequency comb (OFC) has attracted attentions for high accuracy, high resolution, and broadband spectroscopy in visible and infrared regions because the mode-resolved OFC spectrum can be used as a precise frequency ruler due to both characteristics of broadband radiation and narrow-line CW radiation. Furthermore, the absolute accuracy of all frequency modes in OFC is secured by phase-locking a repetition frequency frep and a carrier-envelope-offset frequency fceo to a frequency standard. However, application fields of OFC other than spectroscopy are still undeveloped.

Another aspect of OFC except for the frequency ruler is optical carrier having a huge number of discrete frequency channels because OFC is composed of a series of frequency spikes regularly separated by frep in the broad spectral range. If a certain quantity to be measured is superimposed on each comb mode separately by dimensional conversion, a huge number of data for the measured quantity can be obtained from a single mode-resolved spectrum of OFC.

In this paper, we superimpose the confocal microscopic line-image of a sample onto the mode-resolved OFC spectrum by the dimensional conversion between wavelength and 1D-space, and then the resulting modulated OFC spectrum is acquired by dual comb spectrometer. Finally, the line image of the sample is decoded from the spectral amplitude of the mode-resolved OFC spectrum. The combination of OFC with the dimensional conversion enables to establish both confocal imaging and line-field imaging under the scan-less condition.

A mask-aided shrinkage/thresholding (MAST) algorithm to improve reconstructed image quality in compressed ultrafast photography

Yujia Chen, Liren Zhu, Jinyang Liang, Liang S. Gao, Cheng Ma, Lihong V. Wang, Washington Univ. in St. Louis (United States)

As the fastest receive-only camera in the world, the single-shot compressed ultrafast photography (CUP) camera has pushed the imaging speed to an unprecedented level. However, the reconstructed image quality in the CUP camera still can be improved. In the current CUP paradigm, no external information about the scene is used in reconstructing the image. As a result, the two-step iterative shrinkage/thresholding (TwIST) algorithm estimates a dynamic scene with only limited image quality. In this paper, we introduce a mask-aided shrinkage/thresholding (MAST) algorithm, which suppresses background artifacts and improves image reconstruction quality. Based on the TwIST algorithm, our MAST algorithm incorporates external information from two sources. First, a CCD camera installed in the CUP system takes an unshaped time-integrated image of the dynamic scene. This image is then transformed into a binary mask by thresholding to define an active area, only in which the image reconstruction takes place. Second, in the TwIST algorithm, we incorporate a non-negative threshold constraint to mitigate artifacts in weak intensity. To evaluate the reconstructed image quality, we choose different thresholds and calculate the mean square error (MSE) between the time-projected reconstructed datacube and the CCD image. We then select the threshold value corresponding to the minimum MSE error. In a simulation, we used a temporally flashing Shepp-Logan phantom.
Laura Waller, Univ. of California, Berkeley (United States)

Field datasets

9720-10, Session 3

3D imaging through scattering with light field datasets (Invited Paper)

Laura Waller, Univ. of California, Berkeley (United States)

Invited talk

9720-11, Session 3

Gigapixel imaging with microlens arrays

Antony Orth, RMIT Univ. (Australia); Ethan F. Schonbrun, The Rowland Institute at Harvard (United States)

Developing novel pharmaceuticals is a data-intensive task involving molecular, cellular and whole-animal screens. Cellular scale screens are often implemented via image-based assays wherein millions of cells are imaged and their morphology digitized. Similar screens are performed in a clinical setting for circulating tumour cell detection and immune profiling. The amount of image data recorded in image-based assays is enormous: a 96-well plate contains nearly 18 gigapixels at 0.5μm/pixel, however widefield high-content screening microscopes image at 5 megapixels/sec, or an hour per plate. Hundreds of hours are required for a large screen with hundreds of plates and several image channels. Speeding up high-content imaging would increase the speed of drug discovery and diagnosis, benefiting patients and pharmaceutical companies alike. To this end, we have developed the microlens microscope, a high-throughput microscope for image-based assays.

Instead of a microscope objective, the microlens microscope uses a cm-scale microlens array as a massively parallelized point scanning microscope. A laser illuminates the microlens array, creating a focal spot array at the sample plane. Each of the ~100,000 microlenses in the array operates as a separate epi-fluorescent confocal microscope. Fluorescence captured by each microlens is sent through a common pinhole and re-imaged onto a high speed camera. The parallelization of this design allows for a 3-fold throughput increase over traditional high-content microscopes. We demonstrate gigapixel-scale brightfield, fluorescent and hyperspectral imaging. As a proof-of-principle clinical experiment, we discuss CD15/16 expression in a 100,000 white blood cell dataset, followed by morphological analysis using digital image processing.

9720-12, Session 3

All-IP-Ethernet architecture for real-time sensor-fusion processing

Kei Hiraki, Mary Inaba, Hiroshi Tezuka, Hisanobu Tomari, Kenichi Koizumi, Shuya Kondo, The Univ. of Tokyo (Japan)

Serendipiter is a device that distinguishes and selects very rare particles and cells from huge amount of population. We are currently designing and constructing information processing system for a Serendipiter. The information processing system for serendipiter is a kind of sensor-fusion system but with much more difficulties:

(a) Real-time processing in short period with strict deadline. Because continuous processing in a serendipiter is required, deadline of sensing, processing and classification for a particle or a cell is about 10ms.

(b) Arriving rate of particles or cells is about 100 times larger than the required deadline. Therefore, 100-stage pipelined processing and buffering is required.

(c) Very high-bandwidth data from visual sensors (STEAM etc.) and other detectors. Currently we estimate about 8 GB/s total bandwidth to support particle rate described above.

(d) Machine learning, especially deep learning is necessary to distinguish rare particles and cells from background population.

To fulfill these requirements, we adopt following basic architecture.

Basic architecture of an All IP-Ethernet based data processing system is as follows:

* Conversion of dedicated sensor/detector data to IP-Ethernet packet stream.

* All the data are combined into a single Ethernet/TCP/IP stream by a L2 100Gbps Ethernet switch.

* An FPGA board with 100Gbps Ethernet I/F is connected to the switch and a Xeon based server. Circuits in the FPGA include 100Gbps Ethernet MAC,

* FPGA boards have dedicated FPGA-FPGA interconnection using high-speed general purpose I/O signals from FPGA.

9720-13, Session 3

**A computational approach to real-time image processing for serial time-encoded amplified microscopy**

Minoru Oikawa, Daisuke Hiyama, Ryuji Hirayama, Satoki Hasegawa, Yutaka Endo, Takahisa Sugie, Norimichi Tsumura, Mai Kuroshima, Masanori Maki, Genki Okada, Chiba Univ. (Japan); Cheng Lei, Yasuyuki Ozeki, Keisuke Goda, The Univ. of Tokyo (Japan); Tomoyoshi Shimobaba, Chiba Univ. (Japan)

High-speed optical imaging is an indispensable technique for identifying or evaluating fast-moving objects. As one of the unique high-speed imagers, serial time-encoded amplified microscopy (STEAM) enables us to capture images at a few orders of magnitude higher frame rate than that of conventional methods based on CCD and CMOS image sensors without sacrificing sensitivity due to its optical image amplifier. Unfortunately, the continuous operation of STEAM is hindered by the massive amount of data that is generated by its ultrahigh frame rate, which subsequently limits its performance in real-time systems such as flow cytometry. In this talk, we present a computational approach to continuously processing this extremely large amount of image data in real time for high-throughput image cytometry. Specifically, we use an analog-to-digital converter with 7-GS/s digitization rate and 8-bit resolution for digitizing the photodetector signal in which gray-scale STEAM images are encoded, meaning that STEAM continuously generates 7 Gbytes/s of image data. We employ a field-programmable gate array (FPGA) device a digital signal pre-processor for constructing 2D images and finding objects flowing in a microchannel with high sensitivity. We also use a graphics processing unit (GPU) device to help accelerate the calculations in the FPGA device. We show the performance of our prototype system that builds on STEAM with the FPGA and GPU devices in real-time object identification using microparticles as virtual biological cells in a microfluidic environment.

9720-14, Session 4

**Mapping biological tissues with hyperspectral coherent Raman scattering microscopy (Invited Paper)**

Eric O. Potma, Alba Alfonso Garcia, Univ. of California, Irvine (United States)

We discuss the implementation of wavelength-swept coherent Raman scattering (CRS) microscopy for the rapid acquisition of hyperspectral datacubes. We highlight two multivariate analysis approaches for efficiently generating spectroscopic maps from the acquired data: principal component analysis (PCA), which is a popular method for extracting information from multidimensional datasets, and vertex component analysis (VCA), which has previously been successfully used for the analysis of spontaneous Raman microscopy data. Through several biomedical imaging examples, we discuss the advantages and disadvantages of these approaches for CRS microscopy.

9720-15, Session 4

**Instrumentation and data management for high-speed spectroscopic coherent Raman imaging (Invited Paper)**

Marcus T. Cicerone, Charles H. Camp Jr., Young J. Lee, Peter Bajcsy, Petru S. Manescu, National Institute of Standards and Technology (United States)

I will provide some background and a brief history of coherent Raman imaging methods, with a particular focus on broadband coherent anti-Stokes Raman scattering (BCARS) microscopy. Spectroscopic coherent Raman imaging can generate data streams of approximately 8MB/s over extended periods, resulting in large data sets containing significant amounts of information. I will discuss approaches to retrieving reproducible, instrument independent spectral data, and to extracting information from our spectroscopic images. I will also give some illustrative examples of data handling and management challenges and discuss the approach we have taken to meeting these challenges.

9720-16, Session 4

**Label-free chemical imaging of live euglena gracilis by high-speed stimulated Raman scattering spectral microscopy**

Yoshifumi Wakisaka, Yuta Suzuki, Kyoya Tokunaga, The Univ. of Tokyo (Japan); Misa Hirose, Ryota Domon, Rina Akaho, Mai Kuroshima, Norimichi Tsumura, Tomoyoshi Shimobaba, Chiba Univ. (Japan); Osamu Iwata, Kengo Suzuki, Ayaka Nakashima, euglena Co., Ltd. (Japan); Keisuke Goda, The Univ. of Tokyo (Japan) and Univ. of California (United States) and Japan Science and Technology Agency (Japan); Yasuyuki Ozeki, The Univ. of Tokyo (Japan)

Microbes, especially microalgae, have recently been of great interest for developing novel biofuels, drugs, and biomaterials. Imaging-based screening of live cells can provide high selectivity and is attractive for efficient bio-production from microalgae. Although conventional cellular screening techniques use cell labeling, labeling of microbes is still under development and can interfere with their cellular functions. Furthermore, since live microbes move and change their shapes rapidly, a high-speed imaging technique is required to suppress motion artifacts. Stimulated Raman scattering (SRS) microscopy allows for label-free and high-speed spectral imaging, which helps us visualize chemical components inside biological cells and tissues. Here we demonstrate high-speed SRS imaging, with temporal resolution of 0.14 seconds, of intracellular distributions of lipid, polysaccharide, and chlorophyll concentrations in rapidly moving Euglena gracilis, a unicellular phytoflagellate. Furthermore, we show that our method allows us to analyze the amount of chemical components inside each living cell. Our results indicate that SRS imaging may be applied to label-free screening of living microbes based on chemical information.

9720-17, Session 4

**High-throughput light-sheet Raman microscope based on full field Fourier transform spectrometry**

Dushan N. Wadduwage, Vijay R. Singh, SMART-Singapore MIT Alliance for Research & Technology (Singapore); Paul T. Matsudaira, National Univ. of Singapore (Singapore);
A CMOS image sensor using high-speed lock-in pixels for stimulated Raman scattering spectroscopy

De Xing Lioe, Kamel Mars, Taishi Takasawa, Keita Yasutomi, Keiichiro Kagawa, Shizuoka Univ. (Japan); Mamoru Hashimoto, Osaka Univ. (Japan); Shoji Kawahito, Shizuoka Univ. (Japan)

A CMOS image sensor using high-speed lock-in pixels for stimulated Raman scattering (SRS) spectroscopy is presented in this paper. The effective SRS signal from the stimulated emission of SRS mechanism is very small, which is in the ratio of 10^-4 to 10^-5 to the offset of a probing laser source. Current technology of SRS detection using a single photodiode and a lock-in amplifier is not suitable for multi-point parallel measurement of SRS signal. In order to address this issue, we propose a CMOS image sensor using lock-in pixels which include functions of a photo detector, a demodulator and a lock-in amplifier. The CMOS image sensor is intended to be operated with a laser pulse at high repetition rate of 80MHz and modulation frequency of 20MHz. A lateral electric field charge modulator (LEFM) capable to operate at high speed is designed and utilized instead of the conventional pinned photodiode to demodulate the SRS signal which is modulated at high-frequency of 20MHz. A prototype chip is implemented using 0.11µm CMOS image sensor technology. Characterization of the image sensor shows the capability of power ratio detection up to 10^-5. Further measurement results will be presented at the conference.
4D megahertz optical coherence tomography: imaging and live display beyond 1 gigavoxel/sec (Invited Paper)
Robert A. Huber, Univ. zu Lübeck (Germany); Wolfgang Draxinger, Wolfgang Wieser, Ludwig-Maximilians-Univers. München (Germany) and Optores GmbH (Germany); Jan Philip Kolb, Univ. zu Lübeck (Germany); Tom Pfeiffer, Univ. zu Lübeck (Germany); Sebastian N. Karpf, Ludwig-Maximilians-Univers. München (Germany); Matthias Eibl, Univ. zu Lübeck (Germany); Thomas Klein, Optores GmbH (Germany)

Over the last 20 years, optical coherence tomography (OCT) has become a valuable diagnostic tool in ophthalmology with several 10,000 devices sold today. Other applications, like intravascular OCT in cardiology and gastrointestinal imaging will follow. OCT provides 3-dimensional image data with microscopic resolution of biological tissue in vivo. In most applications, off-line processing of the acquired OCT-data is sufficient. However, for OCT applications like OCT aided surgical microscopes, for functional OCT imaging of tissue after a stimulus, or for interactive endoscopy an OCT engine capable of acquiring, processing and displaying large and high quality 3D OCT data sets at video rate is highly desired.

We developed such a prototype OCT engine and demonstrate live OCT with 25 volumes per second at a size of 320x320x320 pixels. The computer processing load of more than 1.5 TFLOPS was handled by a GTX 690 graphics processing unit with more than 3000 stream processors operating in parallel. In the talk, we will describe the optics and electronics hardware as well as the software of the system in detail and analyze current limitations. The talk also focuses on new OCT applications, where such a system improves diagnosis and monitoring of medical procedures. The additional acquisition of hyperspectral stimulated Raman signals with the system will be discussed.

Fiber-Bragg-grating-array MHz range optical coherence tomography
Roman V. Kuranov, Joseph Price, Nishant Mohan, Vincente Valdez, Jeffrey Soohoo, Michael Sullivan, Wasatch Photonics, Inc. (United States)

Optical Coherence Tomography (OCT) is a powerful imaging tool that is used for multiple diagnostic applications. Its potential is limited by relatively slow (100 Ka-scans/s) acquisition rate of current commercial systems. An OCT system with faster than 1 million A-scans/second acquisition rate removes motion artifacts, increases imaging area, facilitates functional OCT and enables real-time 3D imaging. Recently few OCT systems with MHz A-scan rate including Fourier Domain Mode Locking (FDML) OCT have been demonstrated. The mechanical scanning of the Fabry-Perot cavity in the FDML laser sets the restrictions on data acquisition rate. In addition, use of moving parts and long fiber cavity makes FDML laser potentially complex and expensive and hence limiting its use.

Here we introduce 1.2 MHz A-scan rate Fiber-Bragg-Grating-Array (FBGA) OCT. The 1.2 MHz rate is selected for the proof-of-concept purpose, while the system can be scaled up to multi-MHz A-scan rate. Our new and simple MHz OCT solution has no mechanically moving parts in the light source and do not require k-space resampling. The FBGA-OCT is operated at 1300 nm central wavelength with 75 nm bandwidth. We achieved 15 lfm axial resolution and a peak sensitivity of 89 dB with an average sensitivity reduction of 5 dB/mm down to 6 mm imaging depth. The system sensitivity is limited by amplified spontaneous emission (ASE) from semiconductor optical amplifier (SOA). Several ways to increase sensitivity of FBGA-OCT are suggested. In conclusion, FBGA-OCT may become new ultra-fast OCT paradigm by improving current and opening doors for new applications.

Selective-plane illumination microscopy for high-content volumetric biological imaging
Ryan McGorty, Univ. of San Diego (United States); Bo Huang, Univ. of California, San Francisco (United States)

We have designed and built a light-sheet or selective-plane illumination microscope (SPIM) capable of high-content volumetric imaging. SPIM has emerged in recent years as a powerful optical-sectioning technique well suited to studying developmental biology. We have configured a light-sheet microscope, which we refer to as an open-top SPIM, that can image samples placed on a planar glass substrate. Therefore we can image samples prepared in formats suitable to high-throughput imaging like multi-well plates or in planar microfluidic devices.

Light-sheet microscopy typically employs two objectives orthogonal to one another and with the sample in the common focal plane. One objective (the illumination objective) creates a thin sheet of light that lies within the focal plane of the second, imaging objective. We place these two objectives underneath the sample mounted atop a glass surface, such as a coverslip or multi-well plate. Therefore, we must image the sample across a glass interface that is tilted 45 degrees relative to the optical axis. This introduces large aberrations that we lessen by introducing additional optical elements plus, when using high numerical-aperture objectives, a deformable mirror.

We have used our open-top SPIM to image dozens of developing Drosophila embryos loaded into a planar microfluidic device. We have shown that...
I will discuss the current and past studies on a mammalian circadian clock as a wide range of temperature. Mammalian circadian clock system is such a complex and dynamic system identified structure and observed dynamics. It shows various dynamic behaviors including i) endogenous oscillation with about 24-hour period, ii) entrainment to the external environmental changes and (3) design and implementation of artificial networks of validation based on quantitative measurement and perturbation of network behavior, and to perform functional imaging of the early nervous system in fruit fly and zebrafish. I will furthermore present strategies for systematically image the early development of entire fruit fly, zebrafish and mouse embryos and to perform functional imaging of the early nervous system using the early development of entire fruit fly, zebrafish and mouse embryos and to perform functional imaging of the early nervous system in fruit fly and zebrafish. I will further present new strategies for automated, efficient and robust large-scale image processing of these multi-terabyte light-sheet microscopy data sets, including methods for multi-view data processing, cell segmentation and cell tracking. This combined experimental and computational framework allows us to perform whole-organism functional imaging and to quantitatively analyze developmental lineages and their interrelationships in the entire animal. The higher bandwidth requires higher signal levels to avoid the higher contribution of shot noise and amplifier noise to the fluorescence signal. The higher signal detection efficiency is limited by poor spatial mode-matching of the target fluorophore rather than the imaging system itself. The higher bandwidth and better signal detection efficiency work synergistically because higher bandwidth requires higher signal levels to avoid the contribution of shot noise and amplifier noise to the fluorescence signal. Due to its unprecedentedly high-speed performance, our method has a wide variety of applications in cancer detection, drug discovery, and regenerative medicine.

References

9720-24, Session 6
High-speed light sheet imaging of in-vivo brain function with scape microscopy (Invited Paper)
Elizabeth M. Hillman, Columbia Univ. (United States)

Abstract invited by Kevin Tsia and Keisuke Goda

9720-25, Session 6
Whole-animal imaging with high spatio-temporal resolution (Invited Paper)
Raghav Chhetri, Fernando Amat, Yinan Wan, Burkhard Höckendorf, William Lmeon, Philipp Keller, Howard Hughes Medical Institute (United States)

I will present our recent advances in light-sheet fluorescence microscopy, an emerging imaging technology that achieves high imaging speed and signal-to-noise ratio while minimizing light exposure of the specimen. This powerful combination of capabilities makes light-sheet microscopes indispensable for developmental [1] and functional [2] in vivo imaging of complex biological systems at high spatio-temporal resolution. We develop advanced implementations of light-sheet microscopy, such as our SIMView and h-SIMView frameworks for simultaneous multi-view imaging, to systematically image the early development of entire fruit fly, zebrafish and mouse embryos and to perform functional imaging of the early nervous system in fruit fly and zebrafish. I will further present new strategies for automated, efficient and robust large-scale image processing of these multi-terabyte light-sheet microscopy data sets, including methods for multi-view data processing, cell segmentation and cell tracking. This combined experimental and computational framework allows us to perform whole-organism functional imaging and to quantitatively analyze developmental lineages and their interrelationships in the entire animal. The higher bandwidth requires higher signal levels to avoid the higher contribution of shot noise and amplifier noise to the fluorescence signal. The higher signal detection efficiency is limited by poor spatial mode-matching of the target fluorophore rather than the imaging system itself. The higher bandwidth and better signal detection efficiency work synergistically because higher bandwidth requires higher signal levels to avoid the contribution of shot noise and amplifier noise to the fluorescence signal. Due to its unprecedentedly high-speed performance, our method has a wide variety of applications in cancer detection, drug discovery, and regenerative medicine.

References

9720-26, Session 6
Towards organisms-level systems biology (Invited Paper)
Hiroki R. Ueda, The Univ. of Tokyo (Japan)

The logic of biological networks is difficult to elucidate without (1) comprehensive identification of network structure, (2) prediction and validation based on quantitative measurement and perturbation of network behavior, and (3) design and implementation of artificial networks of identified structure and observed dynamics. Mammalian circadian clock system is such a complex and dynamic system consisting of complicatedly integrated regulatory loops and displaying the various dynamic behaviors including i) endogenous oscillation with about 24-hour period, ii) entrainment to the external environmental changes (temperature and light cycle), and iii) temperature compensation over the wide range of temperature. I will discuss the current and past studies on a mammalian circadian clock as an example of molecule-to-cell-level systems biology, and also discuss the challenges and opportunities towards the organism-level systems biology. Especially, I will introduce the current update on the whole-brain and whole-body imaging with single-cell resolution as well as its biological applications.

References

9720-27, Session 7
Enhanced speed in fluorescence imaging using beat frequency multiplexing (Invited Paper)
Hirofumi Kobayashi, Hideharu Mikami, The Univ. of Tokyo (Japan); Yisen Wang, The Univ. of Tokyo (Japan) and Tianjin Univ. (China); Syed Hamad, Yasuyuki Ozeki, The Univ. of Tokyo (Japan); Keisuke Goda, The Univ. of Tokyo (Japan) and Univ. of California, Los Angeles (United States)

Fluorescence imaging using radiofrequency-tagged emission (FIRE) is an emerging technique that enables higher imaging speed (namely, temporal resolution) in fluorescence microscopy compared to conventional fluorescence imaging techniques such as confocal microscopy and image-sensor-based wide-field microscopy. It works based on the principle that it uses multiple intensity-modulated fields in an interferometric setup as excitation fields and appies frequency-division multiplexing to fluorescence signals. Unfortunately, despite its high potential, FIRE has limited imaging speed due to two practical limitations: signal bandwidth and signal detection efficiency. The signal bandwidth is limited by that of an acousto-optic deflector (AOD) employed in the setup, which is typically 100-200 MHz for the spectral range of fluorescence excitation (400-600 nm). The signal detection efficiency is limited by poor spatial mode-matching between two interfering fields to produce a modulated excitation field. Here we present a method to overcome these limitations and thus to achieve higher imaging speed than the prior version of FIRE. Our method achieves an increase in signal bandwidth by a factor of two and nearly optimal mode matching between two interfering fields to produce a modulated excitation field. Here we present a method to overcome these limitations and thus to achieve higher imaging speed than the prior version of FIRE. Our method achieves an increase in signal bandwidth by a factor of two and nearly optimal mode matching between two interfering fields to produce a modulated excitation field. Here we present a method to overcome these limitations and thus to achieve higher imaging speed than the prior version of FIRE. Our method achieves an increase in signal bandwidth by a factor of two and nearly optimal mode matching between two interfering fields to produce a modulated excitation field. Here we present a method to overcome these limitations and thus to achieve higher imaging speed than the prior version of FIRE. Our method achieves an increase in signal bandwidth by a factor of two and nearly optimal mode matching between two interfering fields to produce a modulated excitation field. Here we present a method to overcome these limitations and thus to achieve higher imaging speed than the prior version of FIRE. Our method achieves an increase in signal bandwidth by a factor of two and nearly optimal mode matching between two interfering fields to produce a modulated excitation field.

References

9720-28, Session 7
A light sheet confocal microscope for image cytometry with a variable linear slit detector
Joshua A. Hutcheson, Foyal Z. Khan, Amy J. Powless, Devin Benson, Courtney J. Hunter, Ingrid Fritsch, Timothy J. Muldoon, Univ. of Arkansas (United States)

We present a light sheet confocal microscope capable of high-resolution
imaging of cell suspensions in a microfluidic environment. In lieu of conventional pressure-driven flow or mechanical translation of the samples, we have employed a novel method of fluid transport, redox-magnetohydrodynamics (MHD). This method achieves fluid motion by inducing a small current into the suspension in the presence of a magnetic field via electrodes patterned onto a silicon chip. This on-chip transportation requires no moving parts, and is coupled to the remainder of the imaging system. The microscopy system comprises a 450 nm diode 20 mW laser coupled to a single mode fiber and a cylindrical lens that converges the light sheet into the back aperture of a 10x. 0.3 NA objective lens in an epi-illumination configuration. The emission pathway contains a 150 mm tube lens that focuses the light onto the linear slit detector at the conjugate image plane. The linear sensor (ELiXIA+ 8k/4k) has three lateral binning modes which enables variable detection aperture widths between 5, 10, or 20 µm, which can be used to vary axial resolution. We have demonstrated redox-MHD-enabled light sheet microscopy in suspension of fluorescent polystyrene beads as well as human leukocytes and oral squamous cells. This approach has potential as a high-throughput image cytometer with myriad cellular diagnostic applications.

9720-29, Session 7

High-speed, high-sensitivity infrared spectroscopy using mid-infrared swept lasers


Infrared spectroscopy is a highly attractive read-out technology for compositional analysis of biomedial specimens because of its unique combination of high molecular sensitivity without the need for exogenous labels. Traditional techniques such as FTIR and Raman have suffered from comparatively low speed and sensitivity however recent innovations are challenging this situation. Direct mid-IR spectroscopy is being speeded up by innovations such as MEMS-based FTIR instruments with very high mirror speeds and supercontinuum sources producing very high sample irradiation levels. Here we explore another possible method – external cavity quantum cascade lasers (EC-QCL’s) with high cavity tuning speeds (mid-IR swept lasers).

Swept lasers have been heavily developed in the near-infrared where they are used for non-destructive low-coherence imaging (OCT). We adapt these concepts in two ways. Firstly by combining mid-IR quantum cascade gain chips with external cavity designs adapted from OCT we achieve spectral acquisition rates approaching 1 kHz and demonstrate potential to reach 100 kHz. Secondly we show that mid-IR swept lasers share a fundamental sensitivity advantage with near-IR OCT swept lasers. This makes them potentially able to achieve the same spectral SNR as an FTIR instrument in a time x N shorter (N being the number of spectral points) under otherwise matched conditions. This effect is demonstrated using measurements of a PDMS sample.

The combination of potentially very high spectral acquisition rates, fundamental SNR advantage and the use of low-cost detector systems could make mid-IR swept lasers a powerful technology for high-throughput biomedical spectroscopy.

9720-30, Session 7

Ultra-wideband fiber optical parametric amplifier for spectrally-encoded microscopy

Xiaoming Wei, The Univ. of Hong Kong (Hong Kong, China); Sisi Tan, The Univ. of Hong Kong (China); Arnaud Mussot, Alexandre Kudlinski, Lab. de Physique des Lasers, Atomes et Molécules, Univ. des Sciences et Technologies de Lille (France); Kevin K. Tsia, Kenneth K. Wong, The Univ. of Hong Kong (China)

Fiber optical parametric amplifier (FOPA) has gained its popularity in the telecommunication systems at the 1.5-um window for its gain, bandwidth etc. Unfortunately, its practical application at the bio-favorable window, i.e. 1.0 um, still requires substantial efforts. Thus, here we report a versatile all-fiber optical parametric amplifier for life-science (OPALS) at 1.0 um as an add-on module for optical imaging system. The parametric gain fiber (photonic-crystal fiber (PCF), 110 m in length) is specially designed to reduce the longitudinal dispersion fluctuation, which yields a superior figure of merit, i.e. a total insertion loss of ~2.5 dB and a nonlinear coefficient of 34 /(W?km). Our OPALS delivers a superior performance in terms of gain (~158,000), bandwidth (>100 nm) and gain flatness (< 3-dB ripple).

Experimentally, we show that: 1) a wavelength-varying quasi-monochrome pump achieves a 52-dB gain and 160-nm bandwidth, but at the expense of a larger gain-spectrum ripple, i.e. a bell-shaped; 2) the birefringence of the parametric gain medium, i.e. PCF in this case, can be utilized to improve the gain-spectrum flatness of OPALS by 10.5 dB, meanwhile a 100-nm bandwidth can be guaranteed; 3) the gain-spectrum flatness of OPALS can be further flattened by using a high-speed wavelength-sweeping pump, which exhibits a 110-nm flat gain spectrum with ripple less than 3 dB. Finally, we employ this versatile all-fiber OPALS as an add-on module to enhance the sensitivity of a spectrally-encoded microscope by 47 dB over an ultra-wide spectral range.

9720-31, Session 8

High-throughput time-stretch microscopy with morphological and chemical specificity

Cheng Lei, Masashi Uegawa, Taisuke Nozawa, Takuro Ideguchi, The Univ. of Tokyo (Japan); Dino Di Carlo, Univ. of California, Los Angeles (United States); Sadao Ota, Yasuyuki Ozeki, Keisuke Goda, The Univ. of Tokyo (Japan)

Particle analysis is an effective method in analytical chemistry for sizing and counting microparticles such as emulsions, colloids, and biological cells. However, conventional methods for particle analysis fall into two extreme categories: Sieving and Coulter counting are capable of analyzing particles with high throughput, but due to their lack of detailed information such as morphological and chemical characteristics, they can only provide statistical results with low specificity. On the other hand, CCD or CMOS image sensors can be used to analyze single microparticles with high content, but due to their slow charge download, the frame rate (hence, the throughput) is significantly limited. Here by integrating a time-stretch optical microscope with a three-color fluorescent analyzer on top of an inertial-focusing microfluidic device, we demonstrate an optofluidic particle profiler with a sub-micrometer spatial resolution down to 780 nm and a high throughput of 10,000 particles/s. In addition to its morphological specificity, the particle profiler provides chemical specificity to identify chemical expressions of particles via fluorescence detection. Our results indicate that we can identify different species of microparticles and blood cells with high specificity without sacrificing throughput. Our method holds promise for high-precision statistical particle analysis in chemical industry and pharmaceutics.

9720-32, Session 8

Compressive high speed flow microscopy with motion contrast

Bryan Bosworth, Jasper R. Stroud, Dung N. Tran, Trac D. Tran, Johns Hopkins Univ. (United States); Sang Chin, Johns Hopkins Univ. (United States) and Boston Univ.
9720-33, Session 8
Quantitative asymmetric-detection time-stretch optical microscopy (Q-ATOM) for ultrafast quantitative phase imaging flow cytometry

Andy K. S. Lau, Anson H. L. Tang, Bob M. F. Chung, Kwok Yeung Tsang, Antony C. S. Chan, Xiaoming Wei, Kenneth K. Wong, Edmund Y. Lam, Kathryn S. E. Cheah, Anderson H. C. Shum, Kevin K. Tsia, The Univ. of Hong Kong (Hong Kong, China)

Based on the interferometric or holographic approaches, recent QPM techniques provide quantitative-phase information, e.g. cell volume, dry mass and optical scattering properties for label-free cellular physical phenotyping. These approaches generally rely on iterative phase-retrieval algorithms to obtain quantitative-phase information, which are computationally intensive. Moreover, current QPM techniques can only offer limited image acquisition rate by using CMOS/CCD image sensors, these two limitations hinder QPM for high-throughput quantitative image-based single-cell analysis in real-time. To this end, we demonstrate an interferometry-free quantitative phase microscopy developed on a new generation of time-stretch microscopy, asymmetric-detection time-stretch optical microscopy (ATOM), which is coined quantitative ATOM (Q-ATOM) - featuring an unprecedented cell measurement throughput together with the asserted intrinsic optical phenotypes (e.g. angular light scattering profile) and the derived physical properties of the cells (e.g. cell size, dry mass density etc.). Based on a similar concept to Schlieren imaging, Q-ATOM retrieves quantitative-phase information through multiple off-axis light-beam detection at a line-scan rate of >10 MHz - a speed unachievable by any existing QPM techniques. Phase retrieval in Q-ATOM relies on a non-iterative method, significantly reducing the computational complexity of the technique. It is a particularly important feature which facilitates real-time continuous label-free single-cell analysis in Q-ATOM. With the use of a non-interferometric configuration, we demonstrate ultrafast Q-ATOM of mouse chondrocytes and hypertrophic chondrocytes in ultrafast microfluidic flow with sub-cellular resolution at an imaging throughput equivalent to ~100,000 cells/sec without image blur. This technique shows a great potential for ultra-high throughput label-free image-based single-cell biophysical phenotyping.

9720-35, Session 8
Ultrafast quantitative time-stretch imaging flow cytometry of phytoplankton

Queenie T. K. Lai, Andy K. S. Lau, Anson H. L. Tang, Kenneth K. Wong, Kevin K. Tsia, The Univ. of Hong Kong (Hong Kong, China)

Comprehensive quantification of phytoplankton abundance, sizes and other parameters, e.g. biomasses, has been an important, yet daunting task in aquatic sciences and biofuel research. It is primarily because of the lack of effective tool to image and thus accurately profile individual microalgae in a large population. The phytoplankton species are highly diversified and heterogeneous in terms of their sizes and the richness in morphological complexity. This fact makes time-stretch imaging, a new ultrafast real-time optical imaging technology, particularly suitable for ultrascale taxonomic classification of phytoplankton together with quantitative image recognition and analysis. We here demonstrate quantitative imaging flow cytometry of single phytoplankton based on quantitative asymmetric-detection time-stretch optical microscopy (Q-ATOM) - a new time-stretch imaging modality for label-free quantitative phase imaging without interferometric implementations. Sharing the similar concept of Schlieren imaging, Q-ATOM accesses multiple phase-gradient contrasts of each single phytoplankton, from which the quantitative phase profile is computed. We employ such system to capture, at an imaging line-scan rate of 11.6MHz, high-resolution images of two phytoplankton populations (scenedesmus and chlamydomonas) in ultrafast microfluidic flow (3 m/s). We further perform quantitative taxonomic screening analysis enabled by this technique. More importantly, the system can also generate quantitative phase images of single phytoplankton. This is especially useful for label-free quantification of biomasses (e.g. lipid droplets) of the particular species of interest – an important task adopted in biofuel applications. Combining machine learning for automated classification, Q-ATOM could be an attractive platform for continuous and real-time ultralarge-scale single-phytoplankton analysis.
A programmable Raman spectroscopic imaging technique

Shuo Chen, Quan Liu, Nanyang Technological Univ. (Singapore)

Raman spectroscopy has demonstrated great potential in biomedical applications. But slow data acquisition due to weak Raman signals has prevented its use in measuring samples with time constraint, especially in an imaging setup. Our previous studies have shown the success of the approach of narrow-band Raman measurements followed by spectral reconstruction towards fast Raman imaging, in which commercially available interference filters were used to acquire multiple narrow-band Raman images simultaneously. However, one significant disadvantage of the approach is that optimized filters required to achieve high accuracy frequently are not commercially available. Moreover, these optimal filters vary from one set of samples to another. We propose a programmable Raman imaging system based on digital micro-mirror device (DMD), which can realize any arbitrary transmittance spectrum corresponding to optimized filters. The Raman technique is evaluated in phantoms consisting of the mixture of multiple Raman scatterers. The proposed technique dramatically enhances the versatility of Raman imaging to adapt to different sets of samples, in which no filter replacement is needed.

Analysis of bandwidth limitation in time-stretch compressive sampling imaging system

Hongwei Chen, Zhiliang Weng, Qiang Guo, Minghua Chen, Sigang Yang, Shizhong Xie, Tsinghua Univ. (China)

Compressive sampling (CS) has attracted considerable attention in recent years because it can sample sparse signals far below the Nyquist rate yet reconstruct them faithfully. Traditional single-pixel camera based on CS theory has been demonstrated with the key element of digital micromirror device (DMD). Recently, time-stretch compressive imaging single-pixel imaging technique overcomes the speed limitation of digital micromirror device (DMD). This technique is based on dispersive Fourier transformation in optical fiber. Different sections of fiber act as time lens which perform frequency to time conversion. A fast electro-optic modulator is used for the random measurement pattern loading as the DMD function but with much higher speed. The former works have proved that this method can reach imaging speed up to 1 MHz. Because this method uses ultra-short pulses as the source for active imaging and also the carrier of random patterns, the bandwidth of optical detector will have great impact to the system performance. We analyze the bandwidth limitation in time-stretch compressive sampling imaging system by simulation. Various pulse compression ratio and detector bandwidth have been induced in the system. The mean square error is used to evaluate quality of reconstructed images. The results show that the system performance decreases with the detection bandwidth which is mainly because of the pulse distortion induced by the limited bandwidth. While the pulse compression ratio impact the system much gentler than the detection bandwidth.

A study on the characteristics of the Analog Mean Delay (AMD) method for high-speed Fluorescence Lifetime Imaging Microscopy (FLIM)

Byungyeon Kim, Byungjun Park, Seungrak Lee, Youngjae Won, Oソン Medical Innovation Foundation (Korea, Republic of)

The analog mean-delay (AMD) method is a new alternative method used in determining the lifetime of a fluorescence molecule. Due to its powerful advantages of accurate lifetime determination, photon economy and a high photon-detection rate, the AMD method is very suitable for the realization of high-speed confocal fluorescence lifetime imaging microscopy (FLIM). For a proper use of the AMD method in various FLIM applications, we present a study of the characteristics of the AMD method. The optimum integration window size that satisfies accurate lifetime extraction was estimated in a simple simulation and was experimentally demonstrated using Cy5 and Alexa fluor 633. Photon economy for lifetimes of 1, 3.2, 5 and 8 ns was also evaluated via a Monte-Carlo simulation (MCS). We confirmed that the photon economy of the AMD method is not degraded for longer lifetimes even when the applied integration window size is increased. By an extension of MCS, the photon economy with respect to different designs of the Gaussian low-pass filter (GLPF) used in the AMD setup was also studied. When a GLPF with the highest cutoff frequency of 100 MHz is applied, the most effective photon economy performance is achieved for lifetimes of 1, 3.2, 5, and 8 ns.

Multispectral spatial frequency domain imaging for quantitative and accurate separation of absorption and scattering by utilizing analytical solutions of the RTE

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It was shown that quantitative Spatial Frequency Domain Imaging (SFDI)
of the absorption ($\mu_a$) and reduced scattering coefficient ($\mu_s'$) facilitates the investigation of different kinds of tissue changes and biological processes, e.g. blood perfusion, neuronal death and brain cellular composition changes. However for an accurate and absolute determination of e.g. drug concentrations, spectrally resolved measurements are generally inevitable.

Moreover structural and morphological changes of tissue mostly result in a change of the scattering spectra.

For this purpose sequential measurements with different wavelengths are normally performed, leading to the problem that temporal changes during acquisition can produce errors in the obtained tissue parameters. A new contact free and rapid method for measuring absorption and reduced scattering coefficient spectra from 500nm to 1000 nm, for a maximum of 640 points along a line on the sample, is presented. Therefore a push-broom spectral imager (InnoSpec, Germany) is used to map each point of a line parallel to a sinusoidal spatial intensity modulation spectrally resolved onto a CCD sensor. Every pixel row of the CCD then holds the spectrum of one point on the sample and each pixel column the sinusoidal spatial intensity modulation along the imaged line at a certain wavelength. This allows to measure the radianc for one spatial frequency spectrally resolved for each point on a imaged line with only one set of three phase projections shifted by $2\pi/3$ respectively. In the end, for obtaining a complete spectrum of $\mu_a$ and $\mu_s'$ for every point on the imaged line a minimum of three images have to be acquired. For solving the inverse problem a c++ implemented nonlinear least squares regression, which applies analytical solutions of the Radiative Transfer Equation (RTE), is used. The accuracy of the presented method is verified by measurements of different turbid and absorbing phantoms based on intralipid and epoxy resin.

9720-41, Session PSun

**Acquiring a two-dimensional cross-sectional image in two nanoseconds**

Nanguang Chen, Zaineb A. T. Al-Gazwini, Kalpesh Mehta, National Univ. of Singapore (Singapore)

A prototype ultrafast OCT system has been developed. Parallel illumination and parallel detection are combined to acquire a cross-sectional image in a single snapshot. The shortest frame acquisition time is two nanoseconds. A super-continuum light source delivers pulsed illumination with a very broad spectrum (450 – 2000 nm). The wavelength range of 650-850 nm is used for our OCT setup, which results in an axial resolution around 1.24 microns in air and 0.9 microns in water. The lateral resolution is about 7.6 microns, which is limited by the numerical aperture of the objective lens (-0.06). A high-speed CMOS camera is used to capture interferograms in parallel and one-dimensional inverse Fourier transform is employed to convert the raw images into cross-sectional sample images.

9720-42, Session PSun

**Entropy analysis of OCT signal for automatic tissue characterization**

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Optical coherence tomography (OCT) has great potential in surgical guidance, because information-rich OCT signal can provide microscopic characterization of biological tissue and assist intraoperative decision making. However, raw OCT data is noisy and complicated. It is challenging to extract information directly related to the pathological status of tissue through visual inspection on huge volume of OCT data streaming from the high-speed OCT engine. Therefore, it is critical to discover concise, comprehensible information from massive OCT data through novel signal analysis strategy. In this study, we perform Shannon entropy analysis on OCT signal for automatic skin tissue characterization, which will be applied in intraoperative tumor margin delineation for surgical excision of cancerous tissue. The principle of this technique is based on the fact that normal tissue is usually more structured with higher entropy value, compared to pathological tissue such as cancer tissue. For example, OCT image of normal skin tissue usually shows distinct horizontal layers (epidermis, dermis, hypodermis and underneath structures) and pathological skin tissue usually does not have layered structure. As a result, entropy which quantifies how structured an image is can be used to characterize skin tissue. Briefly, we obtained 2D OCT images from normal and pathological skin tissue in vivo. We applied Canny edge detection to the denoised image to generate a binary image (I) showing the layer structure of skin tissue. We then calculated the entropy of image I to assess tissue’s pathological status. Our results clearly showed different entropy values for different types of skin tissues.

9720-43, Session PSun

**Development of an intravital multi-plane multiphoton microscopy platform for functional cellular imaging in living mice**

Erik Bélanger, Feng Wang, Ctr. de Recherche de l’Institut Univ. en Santé Mentale de Québec (Canada); Sylvain Côté, Daniel C. Côté, Yves De Koninck, Pierre Marquet, Ctr. de Recherche de l’Institut Univ. en Santé Mentale de Québec (Canada)

Pain sensation is propagated from the periphery to the central nervous system by dorsal root ganglion (DRG) neurons. These neurons detect different stimuli and convey information to the dorsal horn where they form synapses. The project focuses on the development of an intravital multi-plane multiphoton microscopy platform for functional cellular imaging in living mice, to study calcium dynamics of DRG neurons stimulated peripherally. We image DRG neurons labelled with the Ca2+ indicator GCaMP6s directly through a laminectomy and stimulate these with feedback-controlled thermal and mechanical stimulators applied to the paw. Subsequent Ca2+ responses are identified using a home-made software to find responsive neurons in real-time. The platform we developed allows online adjustments of the digital zoom level, image size, spatial sampling, acquisition speed and to trigger or be triggered by external devices; all essential capabilities for live animals' physiological experiments. The sensory-evoked activity from DRG neurons stimulated peripherally is recorded at video-rate, and multi-plane capability is ensured by mounting the objective on a piezoelectric actuator allowing nearly whole-DRG recording. Because animals' movement can seriously degrade the optical image, we register the resulting movies offline using graphic card acceleration. Also, we developed an automated pipeline for data processing and visualization in addition to a database to organize and filter experimental results, all of these in the context of Big Data. The system we built is therefore tailored to fit the specific needs of in vivo whole-organ functional microscopy.

9720-45, Session PSun

**Fluorescent Talbot microscopy using incoherent illumination**

Yangyang Sun, Shuo Pang, CREOL, The College of Optics and Photonics, Univ. of Central Florida (United States)

Longitudinal imaging can provide valuable information for many key biological processes in living cells. A large field-of-view (FOV) is often desirable in such imaging application to capture a large population of migrating and dividing cells. However, conventional fluorescence microscope objective lenses have a limited FOV less than 1 mm2. In order to increase the
In this paper, we developed scanless, full-field confocal microscope by combination of wavelength/1D-space conversion with line-imaging configuration. The broadband light was line-focused onto a confocal slit along the vertical direction with a cylindrical lens to generate a line light source. After collimating with another cylindrical lens, each component of wavelength was spatially dispersed at different angles along the horizontal direction by a diffraction grating. After passing through the relay lenses, the optical beam was focused onto a sample as 2D illumination with an objective lens. Vertical and horizontal coordinates in this 2D illumination were related with the line image and the spectral components, respectively. The reflected beam passed thorough the relay lenses and the diffraction grating inversely, and then each component of wavelength was spatially overlapped. After passing through another confocal slit, the spectrum of this line beam was measured by a multichannel spectrometer equipped with 2D CCD camera. The resulting spectral line image directly reflected the confocal 2D image of sample. In this way, the confocal 2D image was obtained without the need for any mechanical scanning.

**Continuous high-speed compressed sensing OCT**

Jasper R. Stroud, Bryan Bosworth, Dung N. Tran, Sang Chin, Trac D. Tran, Mark A. Foster, Johns Hopkins Univ. (United States)

Optical coherence tomography (OCT) is an invaluable tool in medical diagnostics, allowing straightforward assessment of the progress of macular degeneration, multiple sclerosis, and glaucoma. Without the need for a scanning reference arm Fourier domain OCT enables fast imaging rates. However, typical FDOCT methods use a CCD/CMOS sensor to record the spectral interference signal, limiting the A-scan speed of FDOCT to that of the sensor array. Here we utilize compressed sensing to reduce the dimension of the FDOCT signal such that data is compressed in real time and read out serially by a high speed photodetector and ADC. We achieve this using dispersion for time to wavelength mapping of a pseudorandom binary sequence (PRBS) onto the spectrum of ultrafast mode locked laser (MLL) pulses, resulting in unique PRBS spectral patterns on each laser pulse. The pulses are sent into a common path OCT system with balanced detection such that each ADC sample synchronized to the MLL repetition-rate measures the inner product between the FDOCT signal and the respective PRBS spectral pattern. Experimentally, we demonstrate 300 features per pattern with 11.53 GHz optical bandwidth per feature, yielding 4.4-mm imaging range. The MLL provides 25 nm of optical bandwidth at the center wavelength of 1580 nm corresponding to 42-um axial resolution. The measurement rate is acquired in a single-shot at a 90-MHz rate. The OCT images are reconstructed from the measurement vector and the sampling matrix using just a few percent of the samples needed for traditional Nyquist sampling.

**Wide-band and fast wavelength-swept optical parametric oscillator with a photonic crystal fiber based on dispersion tuning technology at 1?m**

Jin Chen, Sigang Yang, Hongwei Chen, Minghua Chen, Shizhong Xie, Tsinghua Univ. (China)

High speed and wide band wavelength swept optical source around 1 ?m wavelength band is significative as the optical source in new generation OCT systems. A high speed, wide band wavelength-swept fiber optical parametric oscillator (FOPO) around 1?m wavelength band is demonstrated in this paper. Firstly, a continuous-wave single-longitudinal-mode ytterbium-doped fiber laser with passive multiple-ring cavity configuration around 1-um wavelength band, is built up as the seed source. Subsequently, the light source is modulated through a LNBo3 Mach-Zehnder modulator to generate pulse train with low repetition rate. The pulse source is amplified with a two-stage ytterbium doped fiber amplifiers (YDFA) and subsequently a high power YDFA to peak power higher than 60 W. A 50-m homemade photonic crystal fiber (PCF) which provides the optical parametric gain is pumped by this high power pulsed source. A swept fiber optical parametric oscillator is built up via time-dispersion tuning technique driven by a frequency swept electric signal. The electric scanning operation in the proposed wavelength-swept oscillator replaces the traditional mechanical scanning operation in the conventional wavelength-swept sources, such as Fourier Domain Mode-Locked (FDML) fiber lasers. Hence the proposed scheme can realize higher swept rate and larger bandwidth. The wide band and fast wavelength-swept optical parametric oscillator at 1?m band is potential to be applied in the new generation OCT system.

**Scanless, full-field confocal microscopy by combination of wavelength/1D-space conversion with line-imaging configuration**

Shuji Miyamoto, Eiji Hase, Ryuji Ichikawa, Takeo Mnamikawa, Takeshi Yasui, The Univ. of Tokushima (Japan); Hirotsugu Yamamoto, Utsunomiya Univ. (Japan)

Confocal microscopy is the imaging modality used most widely in the field of biomedical imaging. Recently, further reduction of image acquisition time is strongly required to visualize dynamics of cells. The conventional confocal microscopy has acquired the 2D image by mechanical scanning of the focused beam spot. However, if the confocal 2D image can be obtained without the need for the mechanical scanning, the image acquisition time will be largely reduced. In this paper, we developed scanless, full-field confocal microscope by combination of wavelength/1D-space conversion with line-imaging configuration. The broadband light was line-focused onto a confocal slit along the vertical direction with a cylindrical lens to generate a line light source. After collimating with another cylindrical lens, each component of wavelength was spatially dispersed at different angles along the horizontal direction by a diffraction grating. After passing through the relay lenses, the optical beam was focused onto a sample as 2D illumination with an objective lens. Vertical and horizontal coordinates in this 2D illumination were related with the line image and the spectral components, respectively. The reflected beam passed thorough the relay lenses and the diffraction grating inversely, and then each component of wavelength was spatially overlapped. After passing through another confocal slit, the spectrum of this line beam was measured by a multichannel spectrometer equipped with 2D CCD camera. The resulting spectral line image directly reflected the confocal 2D image of sample. In this way, the confocal 2D image was obtained without the need for any mechanical scanning.
resembling in that way a spectral version of the knife-edge technique to characterize beam intensity profiles. By differentiating the stack the Raman spectra of the sample structures are retrieved with good spectral resolution. We demonstrate this technique using solvent solutions and composites of polystyrene beads and lipid droplets immersed in agar and by imaging the C–H (2800-3100 cm⁻¹) region in a C. elegans worm. The image acquisition time results in 4 orders of magnitude faster than confocal point scanning Raman systems, allowing the possibility of performing fast spontaneous Raman 3D-imaging on biological samples.

9720-65, Session PSun

**In vivo particle image velocimetry of blood cell flow using high-speed laser scanning confocal microscopy**

Richard M. Boutiller, Sung-hoon Bae, Yoon-joon Ahn, Sang-hoon Choi, Ho Lee, Yong-joong Lee, Kyungpook National Univ. (Korea, Republic of)

Video obtained from our laser scanning microscopy system consists of successive images. Each image shows the split-second distribution of flowing blood cells. PIV analysis is based on identifying individual cells in slightly different positions between pairs of images and calculating their velocity vectors based on the spatial distance and direction between their positions and the elapsed time between frames. If their flow was laminar in nature, the cells' velocity vectors would largely be parallel and PIV analysis would be effective at low frame rates. However, blood vessels are elastic, multi-branched, and constantly changing in shape and diameter, so blood flow is subject to continuous variations in shear stress. Given that red blood cells, in particular, are bi-concave in shape, highly deformable, and susceptible to at least two modes of rotation, their flow is highly turbulent. This makes it difficult to accurately identify particular cells in successive image frames. Thus, our previous video-rate PIV analysis was subject to large error margins in analyzing the blood flow under normal in vivo conditions. In this study, we demonstrate an improvement in the precision of our PIV analysis by using much shorter periods of elapsed time between frames. This was achieved by re-configuring our laser scanning confocal microscopy system to make it capable of reaching up to 180fps. Subsequent PIV analysis revealed an increased precision in the velocity profile during turbulent flow in blood.
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9721-32, Session PMon
Simulation of enhanced intensity and area of plasmon field using trapezoidal nanowire structure
Tae Young Kang, Pusan National Univ. (Korea, Republic of)

As technology manufacturing nanostructure is advanced, we can think and design any shape of structure in nano scale. Unusual nanostructure which has reversed trapezoidal shape is designed and simulated to find enhancement of surface plasmon phenomenon. The simulation is performed with Finite Element Method discretizing Maxwell's equation. The top length of structure is scanned when the bottom length of structure is fixed at specific value. The result of simulation shows enhancement of intensity and area of surface Plasmon field. Also, the tendency of intensity at some specific bottom length is observed. To find the enhancement of sensitivity as a sensor, the angle of incident light is scanned with 0.01 degree interval. Comparing rectangular case and reversed trapezoidal case, the shift of resonance angle is not notably moved. So the structure cannot be a good sensor alone. Using the enhanced intensity and area of surface plasmon field can be co-localized with a Gold particle. So, we simulate the nanostructure attached with Gold nano particles. Due to the enhancement, the co-localized plasmon field on the particle has stronger intensity. Also, the wider area of plasmon field can easily excite the particle located in some distance away from the structure.

9721-33, Session PMon
Experimental system of the full scattering profile of circular phantoms
Idit Feder, Hamootal Duadi, Dror Fixler, Bar-Ilan Univ. (Israel)

Optical methods of sensing physiological tissue state base on light-tissue interaction are non-invasive, inexpensive and simplistic and therefore are very useful. Most of the optical methods are focused on the reflection light from the tissue, which is described as a semi-infinite medium, while very few use the transmitted light. We report, for the first time, an experimental observation of a typical reflected light intensity behavior for a circular structure characterized by the isobaric point. This method allows measuring in a single wavelength with no calibration. We suggest a new theoretically method for measuring the full scattering profile, which is the angular distribution of light intensity, of cylindrical tissues. Furthermore, we found an isobaric point, which is a central angle where the scattered light intensity does not dependence on the tissue’s optical properties and linearly depends on the exact tissue geometry. We present that the experimental results match the simulation results. Furthermore, the experimental work present a new crossover point of the full scattering profiles of subjects with different diameters of the cylindrical tissues.

In addition, the blood vessels in human tissues are the main cause of light absorbing and also scattering. Therefore, the effect of blood vessels on light-tissue interactions is essential for biomedical applications based on optically sensing, such as oxygen saturation, blood perfusion and blood pressure. We present experimental measurements of the full scattering profile of heterogenic cylindrical phantoms which include blood vessels. We show the vessel diameter influence on the full scattering profile, and found higher reflection intensity for larger vessel diameters according to the shielding effect. These findings can be useful for biomedical applications such as non-invasive and simple diagnostic of the fingertip joint, ear lobe and pinched tissues.

9721-34, Session PMon
Quantification of gold nanoparticles in tissue simulating media using spatial frequency domain imaging
Adamo F. G. Monte, Maakekk K. Pronda, Rolf B. Saager, Anthony J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

Gold nanoparticles can be used in a wide range of applications particularly in the biomedical area, where their strong absorption cross-section can enhance contrast in tagged biological tissues for tumor imaging and diagnostics. Efforts to design protocols for an effective in-vivo therapeutic outcome relies on the knowledge of the nanoparticle localization and concentration. However a properly non-invasive in-vivo method to determine nanoparticle concentration or its depth in tissue does not currently exist. We address this issue by demonstrating a new methodology to measure nanoparticle spatial distribution. The methodology is based on the optical absorption maps of the nanoparticles plotted at different depths in a tissue simulating medium. Silicone tissue phantoms are used as a background medium with a priori knowledge of the optical properties of tissues as measured by spatial frequency domain imaging (SFDI). A look-up-table was then created for deducing the optical properties of the embedded nanoparticles at different spatial concentrations and depths at multiple spatial frequencies. From this look-up-table, we have developed an analytical solution to determine the embedded nanoparticle concentration in depth as a function of the measured spatial frequency dependent optical absorption. SFDI was able to spatially localize the nanoparticles with a 3D perspective. These results provide the groundwork for future studies on mapping nanoparticles in-vivo.

9721-35, Session PMon
Conjugated polymer based nanoparticles with enhanced NIR CL for biomedical imaging of hydrogen peroxide
Young Hun Seo, Korea Institute of Science and Technology (Korea, Republic of); Woo-Dong Jang, Yonsei Univ. (Korea, Republic of); Sehoon Kim, Korea Institute of Science and Technology (Korea, Republic of)

As a clinically relevant way of early diagnosis for various inflammatory diseases, the peroxalate chemiluminescence (POCL) is potentially capable of offering extraordinary in vivo sensitivity because it does not involve sources of background noise such as autofluorescence from biological specimen or stray excitation light, unlike fluorescence imaging by photoexcitation. In this study, we devised a rational nanophotonic way of boosting NIR POCL signals without complex design of the emitter structure for proper energy matching. This approach is based on using a new non-conventional bright emitter and ‘nanophotonic energy relay’ to bridge the energy gap between peroxalates and the emitter. First, the emitter was designed to have strong NIR fluorescence by combining the advantageous optical features of low-bandgap polymeric π-conjugation and AIE activity in a single molecular structure. Then, POCL nanoparticles were constructed by co-aggregating AIE-active conjugated polymer with peroxalates, wherein the POCL energy matching is boosted by co-doping another photonic molecule that relays...
9721-1, Session 1

**Innovative nanostructures for highly sensitive vibrational biosensing (Invited Paper)**

Juergen Popp, Leibniz-Institut für Photonische Technologien e.V. (Germany) and Friedrich-Schiller-Univ. Jena (Germany); Thomas Mayerhöfer, Leibniz-Institut für Photonische Technologien e.V. (Germany); Dana Cialla-May, Karina Weber, Leibniz-Institut für Photonische Technologien e.V. (Germany) and Friedrich-Schiller-Univ. Jena (Germany); Uwe Huebner, Leibniz-Institut für Photonische Technologien e.V. (Germany)

Employing vibrational spectroscopy (IR-absorption and Raman spectroscopy) allows for the labelfree detection of molecular specific fingerprints of inorganic, organic and biological substances. The sensitivity of vibrational spectroscopy can be improved by several orders of magnitude via the application of plasmonic active surfaces. Within this contribution we will discuss two such approaches, namely surface enhanced Raman spectroscopy (SERS) as well as surface enhanced IR absorption (SEIRA). It will be shown that SERS using metal colloids as SERS active substrate in combination with a microfluidic lab-on-a-chip (LOC) device enables high throughput and reproducible measurements with highest sensitivity and specificity. The application of such a LOC-SERS approach for therapeutic drug monitoring (e.g. quantitative detection of antibiotics in a urine matrix) will be presented. Furthermore, we will introduce innovative bottom-up strategies to prepare SERS-active nanostructures coated with a lipophilic sensor layer as one-time use SERS substrates for specific food analysis (e.g. quantitative detection of toxic food colorants). The second part of this contribution presents a slat array metamaterial perfect absorber for IR sensing applications consisting of a dielectric layer sandwiched between two metallic layers of which the upper layer is perforated with a periodic array of slits. Light-matter interaction is greatly amplified in the slits, where also the analyte is concentrated, as the surface of the substrate is covered by a thin silica layer. Thus, already small concentrations of analytes down to a monolayer can be detected by refractive index sensing and identified by their spectral fingerprints with a standard mid-infrared lab spectrometer.

9721-2, Session 1

**Highly sensitive protein detection using a plasmonic field effect transistor** *(Invited Paper)*

Hossein Shokri-Kojori, Yiwen Ji, Xu Han, Younghun Paik, Adam Braunschweig, Sung Jin Kim, Univ. of Miami (United States)

Localized surface Plasmon Resonance (LSPR) is a nanoscale phenomenon which presents strong resonance associated with noble metal nanostructures. This plasmon resonance based technology enables highly sensitive detection for chemical and biological applications. Recently, we have developed a plasmon field effect transistor (FET) that enables direct plasmonic-to-electric signal conversion with signal amplification. The plasmon FET consists of back-gated field effect transistor incorporated with gold nanoparticles on top of the FET channel. The gold nanostructures are physically separated from transistor electrodes and can be functionalized for a specific biological application. In this presentation, we report a successful demonstration of a model system to detect Con A proteins using Carbohydrate linkers as a capture molecule. The plasmon FET detected a very low concentration of Con A (0.006 mg/L) while it offers a wide dynamic range of 0.006-50 mg/L. In this demonstration, we used two-color light sources instead of a bulky spectrometer to achieve high sensitivity and wide dynamic range. The details of two-color based differential measurement method will be discussed. This novel protein-based sensor has several advantages such as extremely small size for point-of-care system, multiplexing capability, no need of complex optical geometry.

9721-3, Session 1

**Engineering molecularly-active plasmonic surfaces for disease detection via colorimetry and Raman scattering**

Alasdair W. Clark, Esmaeil Heydari, Jonathan M. Cooper, Univ. of Glasgow (United Kingdom)

We report a novel nanoparticle biosensor platform capable of providing both naked-eye and Raman-scattered detection of multiple genomic disease markers at extreme sensitivities. Combining direct-write lithography, multiplexed DNA nano-patterning, and molecular self-assembly, we create molecularly-active plasmonic nanostructures onto which gold and silver nanoparticles are located due to DNA-hybridisation. Constructing arrays of these structures allows us to build optical surfaces that change state when in contact with specific DNA sequences; shifting the color of the surface while simultaneously generating strong Raman scattering signals. Using dip-pen-nanolithography (DPN) to modify the plasmonic surface with DNA probes, we show that a single colored array can be patterned with sequences relating to multiple diseases. The arbitrary patterning control afforded by DPN allows these modifications to take the form of micro-scale images, lettering or symbols. Completion of a DNA sandwich assay results nanoparticle localisation onto these patterned areas if the specific disease marker for that pattern was present. The resultant interaction between the plasmonic surface and the metallic nanoparticles shifts the plasmonic resonance conditions in these areas, revealing the pattern as a change in surface color. Adding a Raman-active dye to the nanoparticle label allows further detection versatility through multiplexed Raman mapping of the surface. We demonstrate that these molecularly-active plasmonic surfaces hold significant potential in lab-on-a-chip and point-of-care diagnostic tools; delivering dynamic optical surfaces with visual, colorimetric lettering and symbol output that can identify multiple disease markers at extreme sensitivities, and are readable without the need for specialised equipment or a power supply.

9721-4, Session 1

**Extremely sensitive dual imaging system in solid phantoms**

Eran Barnoy, Dror Fixler, Rachela Popovtzer, Tsviya Nayhox, Bar-Ilan Univ. (Israel); Krishanu Ray, Univ. of Maryland School of Medicine (United States)

In our talk we will describe promising results from the combination of fluorescent lifetime imaging microscopy (FLIM) and diffusion reflection (DR) medical imaging techniques. Three different geometries of gold nanoparticles (GNPs) were prepared: spheres of 20nm diameter, rods (GNRs) of aspect ratio (AR) 2.5, and rods of AR 3.3. Each GNP geometry was then conjugated using PEG linkers estimated to be 10nm in length
Plasmonic nanostructure-based sub-diffraction-limited fluorescence microscopy for imaging of gliding biomolecules

Wonju Lee, Youngjin Oh, Yongseoi Univ. (Korea, Republic of); Kyujung Kim, Pusan National Univ. (Korea, Republic of); Yoshiaki Kinoshita, Nagisa Mikami, Takayuki Nishizaka, Gakushuin Univ. (Japan); Donghun Kim, Yongseoi Univ. (Korea, Republic of)

Localization of surface plasmon waves corresponds to constrained electron oscillations near a nanostructure including metallic nanoparticles and subwavelength patterns that are fabricated in a metal film. In recent years, plasmonic localization has attracted tremendous interests in biosensing and bioimaging studies. In this presentation, we explore the feasibility of plasmonic nanostructure-based sub-diffraction-limited nanoscopy for biomolecular imaging. Subwavelength nanoholes were periodically fabricated in a gold film using electron beam lithography. Localized distribution of electromagnetic fields near the nanohole surface was changed by an incident condition of light, and the optimum geometry of a nanohole array such as hole size and array period was determined numerically based on rigorous coupled wave analysis. For experimental proof of concept, a sub-diffraction-limited total internal reflection fluorescence (TIRF) microscope was set up for imaging microtubules gliding on nanohole arrays. Fluorescence emission signals of microtubules were periodically sampled on each nanoscale localized spot, which was created at the side of a hole ridge under TIR with an incidence angle of 70°. We have successfully reconstructed microtubular images with a 70-80 nm effective resolution in the lateral direction. With graded plasmonic nanohole arrays, axial distribution of intracellular molecules was also investigated at sub-diffraction-limited resolution. On the other hand, extraordinary light transmission (EOT) at normal incidence through a nanohole fabricated in a 50-nm-thick was also used to elucidate gliding characteristics of bacteria, which were extracted with the axial resolution down to 50 nm. We finally analyzed axial 3D motions of bacteria on nanohole arrays by mapping to EOT based fields.

Surface plasmon enhanced cell microscopy with blocked random spatial activation

Taehwang Son, Youngjin Oh, Wonju Lee, Heejin Yang, Donghyun Kim, Yongseoi Univ. (Korea, Republic of)

We present surface plasmon enhanced fluorescence microscopy with random spatial sampling using patterned block of silver nanoislands. For proof-of-concept, a 2-nm chromium adhesion layer and a 10-nm silver film were first evaporated on a glass substrate followed by lift-off process of an additional 10-nm silver block defined lithographically. Nanoislands on a 12.2 μm x 12.2 μm block were produced by temperature annealing. Rigorous coupled wave analysis was performed to confirm near-field localization on nanoislands and image reconstruction. For the design of nanoislands, a SEM image of patterned nanoislands was transferred to a binary image. By analyzing random near-field distribution, average size of localized spot was found to be ~135 nm. Raw images were acquired by a total internal reflection fluorescence microscope with a 488-nm diode laser and NA 1.49 objective lens. J774 cell, mouse macrophage cell-line, was cultured on nanoislands, and cell viability was tested using cell counting kit-8 assay to confirm biocompatibility on silver nanoislands. Random localized near-field
In this regard, live force measurements were performed ex-vivo during the interaction of Xylella fastidiosa bacterial cells with InP nanowire arrays. The influence of nanowire array topography and surface chemistry on the response and motion of bacterial cells was studied in detail. The nanowire arrays were also functionalized with different cell adhesive promoters, such as amine and XadA1, an afimbrial protein of X.fastidiosa. By employing the well-defined InP nanowire arrays platform, and single cell confocal imaging system, we were able to trace the bacterial growth pattern, and show that their initial attachment locations are strongly influenced by the surface chemistry and nanoscale surface topography. In addition, we measure the cellular forces down to few nanonewton range using these nanowire arrays. In case of nanowire functionalized with XadA1, the force exerted by vertically and horizontally attached single bacteria on the nanowire is in average 14% and 26% higher than for the pristine array, respectively. These results provide an excellent basis for live-cell force measurements as well as unravel the range of forces involved during the early stages of bacterial adhesion and biofilm formation.

9721-9, Session 1
An optical sensing approach for the noninvasive transdermal monitoring of cortisol
Yongssoon Hwang, Niraj K. Gupta, Yagya R. Ojha, Brent D. Cameron, The Univ. of Toledo (United States)

Cortisol, a biomarker of stress, has recently been shown to have potential in evaluating the physiological state of individuals diagnosed with stress-related conditions including chronic fatigue syndrome. Noninvasive techniques to extract biomarkers from the body are a topic of considerable interest. One such technique to achieve this is known as reverse iontophoresis (RI) which is capable of extracting biomolecules through the skin. Unfortunately, however, the extracted levels are often considerably lower in concentration than those found in blood, thereby requiring a very sensitive analytical method with a low limit of detection. A promising sensing approach, which is well suited to handle such samples, is Surface Plasmon Resonance (SPR) spectroscopy. When coupled with aptamer modified surfaces, such sensors can achieve both selectivity and the required sensitivity. In this study, fabrication and characterization of a RI-based SPR biosensor for the measurement of cortisol has been developed. The optical mount and diffusion cell were both fabricated through the use of 3D printing techniques. The SPR sensor was configured to employ a prism coupler-based arrangement with a laser generation module and CCD line sensor. Cortisol-specific DNA aptamers were immobilized onto a gold surface to achieve the necessary selectivity. For demonstration purposes, cortisol was extracted by the RI system using a skin phantom flow system capable of generating time dependent concentration profiles. The captured sample was then transported using a micro-fluidic platform from the RI collection site to the SPR sensor for real-time monitoring. Analysis and system control was accomplished within a developed LabVIEW program.

9721-10, Session 1
A force sensor using nanowire arrays to understand bacterial cell motility and biofilm formation
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Understanding the cellular signaling and function at the nano-bio interface can pave the way towards developing next-generation smart diagnostic tools. From this perspective, limited reports detail so far the cellular and subcellular forces exerted by bacterial cells during the interaction with abiotic materials. Nanowire arrays with high aspect ratio have been used to detect such small forces.

In this regard, live force measurements were performed ex-vivo during the interaction of Xylella fastidiosa bacterial cells with InP nanowire arrays. The influence of nanowire array topography and surface chemistry on the response and motion of bacterial cells was studied in detail. The nanowire arrays were also functionalized with different cell adhesive promoters, such as amine and XadA1, an afimbrial protein of X.fastidiosa. By employing the well-defined InP nanowire arrays platform, and single cell confocal imaging system, we were able to trace the bacterial growth pattern, and show that their initial attachment locations are strongly influenced by the surface chemistry and nanoscale surface topography. In addition, we measure the cellular forces down to few nanonewton range using these nanowire arrays. In case of nanowire functionalized with XadA1, the force exerted by vertically and horizontally attached single bacteria on the nanowire is in average 14% and 26% higher than for the pristine array, respectively. These results provide an excellent basis for live-cell force measurements as well as unravel the range of forces involved during the early stages of bacterial adhesion and biofilm formation.
9721-31, Session 2

Optoelectronic investigation of nanodiamond interactions with human blood

Mateusz Ficek, Maciej S. Wróbel, Gdansk Univ. of Technology (Poland); Michał Wasowicz, Warsaw Univ. of Life Sciences (Poland); Małgorzata Jedrzejewska-Szczerska, Gdansk Univ. of Technology (Poland)

Nanodiamonds are a relatively new class of carbon nanomaterials. Unique properties of nanodiamonds, including their mechanical and optical characteristics, as well as modifiable surfaces, make them an interesting novel material. They are generally used in biomedical applications, such as biomedical imaging and drug delivery. However, toxicity of carbon nanomaterials varies with their purity, size and surface functional groups. Recent studies suggest that nanodiamonds, in general, are more biocompatible than other carbon materials. Therefore, there is a need to investigate their compatibility with biological materials.

We present the optoelectronic investigation of the interaction of nanodiamond biomarkers with whole human blood. In vitro interactions of human blood and its components with different nanodiamond biomarkers have been examined. Plasmo-chemical modifications of detonation nanodiamond particles give the possibility for controlling their surface for biomedical applications. Optical investigations reveal the biological activity of nanodiamonds in blood dependent on its surface properties. We compare different types of nanodiamonds: commercial non-modified detonation nanodiamonds (grain sizes from 2 to 5 nm), nanodiamonds modified by MW PACVD method in rotary reactor chamber, and chemically modified nanodiamonds. The absorption spectra were measured for whole blood, as well as its separated components: plasma, and washed erythrocytes. Further studies with optical microscopy provide insight into red blood cells morphology and its changes upon interaction with nanodiamonds. The results indicate haemocompatibility of non-modified detonation nanodiamond and modified nanodiamond, and the presence of haemolysis in commercial detonation nanodiamonds.

9721-30, Session 2

Geminal cross-coupling of 1,1-dibromoolefins facilitating multiple topological π-conjugated tetraarylenethenes for optical sensing

Ming-Qiang Zhu, Tao Chen, Ze-Qiang Chen, Huazhong Univ. of Science and Technology (China)

The cross-coupling reactions have been used in C-C bond formation which can be used extensively in optoelectronic materials for organic light emitting diode (OLED), organic photovoltaics and chemical biosensing. Here, we report twofold geminal C-C bond formation at 1,1-dibromoolefins via cross-coupling reactions of aromatic boronic esters over Pd catalysts for multiple topological configurations of π-conjugated molecules. We employ a series of reactions from a precursor toolbox to produce π-conjugated macrocycles, conjugated dendrimers, 1-dimensional linear conjugated polymers, 2-dimensional conjugated microporous polymers (CMPs) and crosslinking conjugated polymer nanoparticles (CCPNs). The π-conjugated macrocycles, dendrimers and 1-D polymers show characteristic aggregation-induced emission properties. 2-D conjugated microporous polymers possess unique porosity of 2-3 nm. This universal strategy towards definite topological configurations of π-conjugated molecules enables efficient coupling of aryl bromides with various coupling partners under mild conditions affording multiple topological conjugated systems with abundant optical and optoelectronic interest.

9721-29, Session 2

Gold nanoparticles based imaging technique and drug delivery for the detection and treatment of atherosclerotic vascular disease

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Atherosclerosis (AS) and its consequences are being a major cause of premature morbidity and death. A large number of biomarkers that relate to lipids, inflammation, immunity, thrombosis and homeostasis, have been regarded as strong predictors of AS lesion progression and their potential as targets for therapy is being investigated. Yet, despite the rapid progression in AS imaging techniques, the identification of inflamed “active” lesions within the coronary circulation remains elusive and the prevention of AS and treatment of its complications are considered to be a clinical challenge.

In our talk we aim to present a new, simple and non-invasive method to detect, locate, and to treat AS at its very early stages. We use gold nanoparticles (GNPs) combined with the diffusion reflection (DR) method to demonstrate the detection of vulnerable AS plaques. Our method is based on the fact that macrophages are a major component in the vulnerable plaque and are able to uptake metal nanoparticles that can be discovered by the DR system. In addition, it is well known that high density lipoprotein (HDL) reduces AS by inhibiting pro-inflammatory factors like lipoprotein-associated phospholipase A2 (Lp-PLA2). Thus, the specific treatment of AS is presented, as the GNPs served as drug carriers of HDL. Early and accurate non-invasive detection of AS plaques by DR method and GNPs may allow serially monitoring the rate of disease progression and thus tailoring therapeutic measures accordingly.
and thrombotic events when undergoing invasive diagnosis like coronary angiography. Therefore, approaches for non-invasive detection and classification of vulnerable plaques in vivo are needed. Whereas classical approaches fail to differentiate plaque types, a new biophotonic method (combining diffuse reflectance (DR) with flow (FCM) or image cytometry (IC)) to analyze gold nanoparticle (GNP) loading of plaques could overcome this limitation. Gold nanorods (GNR; (I) 40x18nm, (II) 65x25nm, (III) 52x13nm in size, absorption peak 630nm) and nanospheres (GNS, average diameter 18.5nm, absorption peak 520nm) were used. Human monocytes were isolated from buffy coat, differentiated into macrophages for 6 days and loaded with GNR or GNS for 24h. GNP loading was determined by FCM, IC and hyperspectral microscopy. Macrophages within tissue-like phantoms were analyzed by DR. After GNR labelling FCM light-scatter increased to 3.7 fold and DR average slope changed from 0.196 (unloaded) to 0.827 (GNR loaded). GNRII had a similar DR slope as control phantoms, indicating that macrophages take up less GNRIII than GNRI or II. IC and microscopy showed that GNP uptake was heterogeneous.

Combining FCM and DR provide a novel, sensitive, non-invasive method to identify atherosclerotic plaques, aimed to develop a tool for in vivo tracking. Further experiments will show if macrophage subtypes (M1 or M2) take up the particles differently and may thereby serve to distinguish stable from vulnerable plaques.

9721-12, Session 3

Plasma dispersion effect assisted nanoscopy based on tuning of absorption and scattering resonances of nanoparticles (Invited Paper)

Zeev Zalevsky, Yossef Danan, Tali Ilovitch, Bar-Ilan Univ. (Israel); Danping Liu, Chongqing Univ. (China); Hadar Pinhas, Moshe Sinvani, Yehonatan Ramon, Jonathan Azougi, Bar-Ilan Univ. (Israel); Alexandre Douplik, Ryerson Univ. (Canada)

Utilizing the localized surface plasmon resonance (LSPR) effect of metallic nanoparticles enables their usage as contrast agents in a variety of applications for medical diagnostics and treatment. Those applications can use both the very strong absorption and scattering properties of the metallic nanoparticle due to their LSPR effects. There are certain applications where domination of the scattering over absorption or vice versa would be an advantage. However, the scattering and absorption resonance peaks have practically the same spectral location for solid noble metal nanoparticles at a certain domination of one over the other.

In this paper we present gold nanoparticles coated with silicon that switch the order between the scattering and the absorption magnitude at the resonance peak and tune the plasmon resonance over the spectrum. This is obtained by modifying the refractive index of the silicon coating of the nanoparticle by illuminating it with a pumping light due to the plasma dispersion effect in silicon. The usage of the above mentioned tunability is applied to create an improved nanoscope. In this paper we report how changing the diffraction limited point spread function through the utilization of plasma dispersion effect of the above mentioned silicon coated nanoparticles allows imaging of sub wavelength resolution. The plasma dispersion effect can increase the absorption coefficient of the silicon, when illuminated with a focused laser beam and as explained above it can also tune the absorption versus scattering properties of the nanoparticle. Due to the Gaussian nature of the laser illumination which has higher intensity at its peak, the absorbance change is more significant at the center of the illumination. As a consequence, the reflected light has a sub wavelength dip that overlaps with the location of the illumination peak. This dip has higher spatial frequency than ordinary Gaussian, which enables to achieve sub wavelength super resolution results. By tuning the absorption versus the scattering properties the proposed concept can be a modified and a generalized mix between STED and PALM microscopes based upon plasma dispersion effect in silicon.

9721-13, Session 3

Fundamental limits of super-resolution microscopy by dielectric microspheres and microfibers

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In recent years, optical super-resolution by microspheres and microfibers emerged as a new paradigm in nanoscale label-free and fluoroscent imaging. However, the mechanisms of such imaging are still not completely understood and the resolution values are debated. In this work, the fundamental limits of super-resolution imaging by high index barium-titanate microspheres and silicon microspheres are studied using nanoplasmonic arrays made from Au and Al [1,2]. A rigorous resolution analysis is developed based on the object’s convolution with the point-spread function that has width well below the conventional (-λ/2) diffraction limit, where λ is the illumination wavelength [2]. A resolution of -λ/6-λ/7 is demonstrated for imaging nanoplasmonic arrays by microspheres. Similar resolution was demonstrated for microfibers in the direction perpendicular to the fiber axis with hundreds of times larger field-of-view in comparison to microspheres. Using numerical solution of Maxwell’s equations, it is shown that extraordinary close point objects can be resolved in the far field, if they oscillate out of phase. Possible super-resolution using resonant excitation of Whispering gallery modes is also studied. These structures can be used as substrates with engineered nanoplasmonic properties for biomedical imaging applications.


9721-14, Session 3

Broadband energy-entangled photon for high resolution temporal sensing

André Stefanov, Stefan Lerch, Manuel Unternährer, Univ. Bern (Switzerland); Jos Kohn, Univ. Bern (Switzerland) and Univ. de Fribourg (Switzerland)

Broadband energy-time entangled photon pairs are produced by pumping a non-linear crystal with a cw laser. Because of their quantum nature, they exhibit at the same time narrowband and short time features. Indeed the sum energy of both photons is equal to the well defined energy of the pump photon, whereas the correlation time between the two photons is of the order of few tens of femtoseconds. Those properties can be used for measurements beyond the capabilities of classical devices. Here we show how to make use of those features to study the temporal properties of photons through various media. The propagation of the entangled two-photon quantum states is described by a temporal wavefunction which is comparable for certain aspects to the one of coherent ultrashort laser pulses. However, because this light is in a continuous way regime, femtosecond timing can be performed without relying on high intensities which could destroy the investigated sample, like with short pulses.
As application, we show a proof of principle experiment where ultrafast optical coincidences of the photon pairs allow selecting only the ballistic photons. Imaging through a scattering medium can therefore be performed. Using techniques from the ultrafast optics, we are able to manipulate the two-photon wave function with the help of a pulse shaper, which combines dispersive elements and a spatial light modulator. In that way, the temporal shape of the two-photon states before and after being transmitted through a sample can be reconstructed, leading to the dispersion properties of the sample.

9721-15, Session 4
Water nanodroplets on micro/nano-arrays: visualization by AFM and simulation
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No Abstract Available

9721-16, Session 4
Near infrared hyperspectral microscopy of carbon nanotube photoluminescence enables 17-color imaging
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The intrinsic near-infrared photoluminescence (fluorescence) of single-walled carbon nanotubes exhibits unique photostability, narrow bandwidth, penetration through biological media, environmental sensitivity, and both chromatic variety and range. Biomedical applications exploiting this large family of fluorophores will require the spectral and spatial resolution of individual (n,m) nanotube species’ fluorescence and its modulation within live cells and tissues, which is not possible with current microscopy methods. We present a wide-field hyperspectral approach to spatially delineate and spectroscopically measure single nanotube fluorescence in living systems. This approach resolved up to 17 distinct (n,m) species (chiralities) with single nanotube spatial resolution in live mammalian cells, murine tissues ex vivo, and zebrafish endothelium in vivo. We anticipate that this approach will facilitate multiplexed nanotube imaging in biomedical applications while enabling deep-tissue optical penetration, exceptional photostability, and single-molecule resolution in vivo.

9721-17, Session 4
Cross slit-grooves grid structure for surface plasmon resonant sensor
Joo ho Lee, Bikash Nakarmi, Bong Ho Kim, Wonjae Jang, Yong Hyub Won, KAIST (Korea, Republic of)
Surface plasmon resonant (SPR) phenomenon is widely researched for various purposes, among which biomedical sensing is getting more attentions as they are suitable for surface functionalization acting as a bio recognition element to detect different biological infections. The common method of surface resonant is attenuated total reflection method but it is not suitable to nano-scale device. Another method which is widely used for SPR is nanostructures in thin metal. Various structures such as slit only, slit+groove and slit-multiple groove are used for generation of SPR and obtaining the optimum optical transmission through the structure. The number and position of slits and grooves affect transmittance through the structure. In this paper we propose a new structure of cross slit-grooves structure, which includes slit-groove structure in grid form. The slit-grooves structures are arranged in such a way that it forms symmetrical structure in two dimension with slit and groove and hence the transmission with cross slit-grooves structure increases significantly. The cross slit-grooves structure takes the advantage of symmetrical slit and groove by using both dimensional structure for generating SPR which increases the transmission through the structure. A comparison of proposed slit-grooves grid structure with slit only, slit groove structure is carried out to show the increase in transmission through the cross slit-grooves grid structure. Plane wavelength of 400 nm to 900 nm is used for the analysis of transmission through the Ag slit-grooves grid structures with glass substrate. We also measure the change in transmission with change in refractive index, which can be helpful for measuring different chemical analytes, and hence can be used for different chemical and biosensors applications.

9721-18, Session 4
Detection of organic nanoparticles within tissues using optical iterative method
Inbar Yariv, Dror Fixler, Rachel Lubart, Hamootal Duadi, Anat Lipovsky, Bar-Ilan Univ. (Israel)
In recent years nanoparticles (NPs) have become very attractive due to their increased biological activity and penetration depth into human tissues. However, determining their penetration depth remained a challenge. Our research suggests an optical study which promotes the determination of organic NPs (ONPs) penetration depth into tissues. A novel optical technique, that detects ONPs within tissues by extracting their reduced scattering coefficient (µs'), was developed. The suggested optical technique, which examines the light transmission through or reflection from tissues, is based on iterative Gerchberg-Saxton (G-S) algorithm. It uses the multiple G-S algorithm in order to reconstruct the light phase created by the tissue. The ONPs within the tissue create an additional scattering component which is observed by the changes of the light phase that can be measured by the standard deviation (STD). The light phase STD together with the tissue thickness provides estimation for µs' and the ONPs penetration depth.
A simulation that calculates the STD for different µs', different tissue thickness and different penetration depths was developed. The results of the simulation indicate a linear ratio between the STD and the scattering components. A linear ratio was also observed in the experiments of tissue-like phantoms and chicken skin with and without different types of NPs. The NPs presence within the tissue was observed by the change in STD which was up to 40% when the NPs were added. The results indicate that our novel technique succeeded to detect NPs within tissues and can provide estimation for their penetration depth.

9721-19, Session 4
New confocal microscopy hyperspectral imager for NIR-emitting bioprobes: high spectral resolution for a wide spectral range
Stéphane Marcet, Photon etc. Inc. (Canada); Antonio Benayas, Marta Quinterina, Institut National de la Recherche Scientifique (Canada); Francesca Mangiarini, Marc Verhaegen, Photon etc. Inc. (Canada); Fiorenzo Vetrone, Institut National de la Recherche Scientifique (Canada); Sébastien Blais-Ouellette, Photon etc. Inc.
Functional nanoscale materials are being extensively investigated for applications in biology and medicine and are ready to make significant contributions in the realization of exciting advancements in diverse areas of diagnostics and therapeutics. Aiming for more accurate, efficient, non-invasive and fast diagnostic tools, the use of near-infrared (NIR) light in the range of the 1st and 2nd biological window (NIR-I: 0.70-0.85 μm; NIR-II: 1.00-1.35 μm) provides deeper penetration depth into biological tissue, better image contrast, reduced phototoxicity and photobleaching. Consequently, NIR-based bioimaging became a quickly emerging field and manifold new NIR-emitting bioprobes have been reported. Since commercially available microscopes are not optimized for this kind of NPs, a new microscopy hyperspectral confocal imager has been developed to cover a broad spectral range (400 to 1700 nm) with high spectral resolution. The smallest spectral variation can be easily monitored thanks to the high spectral resolution (as low as 0.2 nm). This is possible thanks to a combination of an EMCCD and an InGaAs camera with a high resolution spectrometer. An extended number of NPs can be excited with a Ti:Sapphire laser, which provides tunable illumination within 690-1040 nm. Cells and tissues can be mapped in less than 100 ms, allowing in-vivo imaging. As a proof of concept, here we present the preliminary results of the spatial distribution of the fluorescence signal intensity from lanthanide doped nanoparticles incorporated into a system of biological interest. The temperature sub-mm gradient – analyzing the spectral features so gathered through an all-optical route is also thoroughly discussed.

Non-radiative excitation fluorescence microscopy

Lina Riachy, Cyrille Vézy, Rodolphe Jaffiol, Univ. de Technologie Troyes (France)

Non-radiative Excitation Fluorescence Microscopy (NEFM) constitutes a new way to observe biological samples beyond the diffraction limit [1]. Non-radiative excitation of the samples is achieved by coating the substrate with the donor species, such as quantum dots (QDs). The dyes will not be excited directly by the laser source, as in common microscopy, but through a non-radiative energy transfer. To prevent dewetting of the donor film, we have recently implemented a silanization process to covalently bond the QDs on the substrate. An homogenous monolayer of QDs is then created on one side of the substrate. Atomic force microscopy and ellipsometry were used to characterize the QD layer. We demonstrate the potential of our method through the study of phospholipid membranes, such as Giant Unilamellar Vesicles (GUVs) labeled with DiD as acceptor, in interaction with different functionalized surfaces (Poly-L-Lysin, biotin-streptavidin). In the presence of GUVs on a QDs monolayer, we observed together a quenching of the QDs emission and emission of the DiD located in the membrane, which clearly indicate that non-radiative energy transfer from QDs to DiD occurs. Further experiments were also conducted to investigate steric and undulation forces which appear during GUV adhesion. Based on this study, our functionalization technique is also used to observe other biological samples such as adhesion of living cells.

Non-contact optical sensor for detection of glucose concentration using a magneto-optic effect

Nisan Ozana, Yevgeny Beiderman, Bar-Ilan Univ. (Israel); Arun Anand, The Maharaja Sayajirao Univ. of Baroda (India); Baharam Javidi, Univ. of Connecticut (United States); Javier García-Monreal, Univ. de València (Spain); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

This paper presents the development of a non-contact method for measurement of glucose concentration in blood stream. The final device aims to contain a single wristwatch-style device containing an AC electromagnet generated by a solenoid, a laser and a camera. This measurement is based on two effects. The first is the extraction and separation of remote vibration sources (caused due to the pulsation of the blood stream) and the second is the effect on the polarization of the wavefront of light by the glucose exposed to magnetic fields (applied at the frequency of the solenoid which differs from the blood pulsation frequency). The technique is based on tracking of temporal changes of reflected secondary speckle patterns produced from the skin near the wrist when being illuminated by a laser beam. Change in skin’s temporal vibration profile together with the change in the magnetic medium that is generated by time varied glucose concentration cause those temporal variations. When a glucose substance was inserted into the vascular stream, the AC magnetic field was found to have a lock-in amplification role which increased the observability of the relatively small magneto-optic effect. Experimental results to support the proposed concept are presented.

A novel method for sensing metastatic cells in the CSF of pediatric population with medulloblastoma by frequency domain FLIM system

Gilad Yahav, Dror Fixler, Sivan Gershonov, Helen Toledano, Shalom Michowitz, Nitza Goldenberg-Cohen, Bar-Ilan Univ. (Israel)

Fluorescence lifetime (FLT) is considered more advanced sensing method than the classical fluorescence intensity (FI) as it is not exposed to many of its artifacts. Furthermore FLT provides a means of probing changes in the local fluorophore’s environment such as viscosity and pH. In frequency domain FLIM the FLT is extracted from the amplitude attenuation and the phase shift between the FI emission and the exciting light source. This leads FLT measurements to provide a means of sensing changes in the local fluorophore physical, chemical and biological environment. Cancerous cells are known for changing several environmental factors include pH and the viscosity. As a result they can theoretically be sensed by the FLIM system.

In our talk we will present for the first time the variations of the FLT of DAPI in medulloblastoma (MB) patient’s tissues. The cells were extracted from tumor and cerebrospinal fluids (CSF) of children diagnosed with MB following nuclear staining of DAPI via FD-FLIM technology. The FLT of cells from the original tumors and the metastatic cells was greatly extended (median 5.73ns, 6.47ns respectively; p-value=8.231*10-9) relate to the normal/medium FLT measured in inflammatory samples from non-oncology pediatric patients who served as controls (median 2.69ns; p-value=2.2*10-16). In short FLT value was measured in samples from children post treatment – either chemotherapy or craniospinal radiation (median 1.6ns; p-value<2.2*10-16). These findings may pave the way for better detection of the tumors and metastatic cells, and may guide personally tailored treatment, improve outcome and increase survival as well as for others biomedical applications.

Seeing the unseen with localized optical contrast

Swathi Suran, Krishna Bharadwaj, Srinivasan Raghavan, Manoj M. Varma, Indian Institute of Science (India)

Optical wide-field imaging of sub-diffraction limit nanostructures is of interest in a wide array of applications. In applications where the nanostructures to be visualized are well isolated, a high enough optical contrast is sufficient to detect these. Here we demonstrate a technique to...
visualize nanoscale features, such as grain boundaries in Chemical Vapor Deposited (CVD) single layer graphene (CVD-SLG), which are just a few atom length defects, using regular bright field optical microscopy. This remarkably low lateral length scale was imaged using of a special thin film structure consisting of a water-soluble thin film layer deposited on a metal substrate, which produces a strong color change as a function of the film thickness. Small local water transport differences in the graphene layer result in thickness variation of the underlying thin film due to its solubility in water and produces color contrast readily observable under a normal bright-field optical microscope with the naked eye. The same technique also permits the direct optical visualization of grain boundaries in graphene and single metal or dielectric nano-particles down to 40 nm. By using super-resolution image processing algorithms we may be able to detect structure even smaller in size than currently achieved. We believe that this technique will be useful in applications ranging from single molecule detection to nano-scale solute transport studies in 2D materials.

9721-24, Session 4

Temporally flickering nanoparticles for compound cellular imaging and super resolution

Tali Ilovitsh, Yosses Danan, Rinat Meir, Bar-Ilan Univ. (Israel); Amihai Meiri, The Univ. of Utah (United States); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

This work presents a novel method that enables the simultaneous superresolved imaging of multiple types of gold nanoparticles (GNPs) that label targets of interest within biological samples. The method utilizes a lock-in technique at which the imaging of the sample is done using a number of time-modulated modulated laser beams that match the number of these types of GNPs that label a given sample, and resulting in the excitation of the temporal flickering of the scattered light at known temporal frequencies. The final image where the GNPs are spatially separated is obtained using post processing where the proper spectral components corresponding to the different modulation frequencies are extracted.

The proposed method enables the detection of overlapping types of GNPs, at significantly sub-diffraction distances, making it attractive for super resolving localization microscopy techniques. In addition, by targeting each type of GNPs to different areas within the sample, the cite-specified areas can be simultaneously imaged. Furthermore, the spatial separation of the GNPs can be done even at poor signal to noise (SNR) conditions, where the inspected signal is indistinguishable in the given noisy environment.

We believe that the proposed concept can be a revolutionizing technique extremely applicable to the field of biomedical imaging which can have a crucial future role in understanding cellular trafficking pathways, identifying receptor expression and in facilitating the understanding of cellular signaling pathways. All this pave the way to be able to design effective therapeutic procedures.

9721-25, Session 4

Optimization of imaging parameters for high sensitivity detection of skin cancer at the THz

Michael Ney, Ibrahim Abdulhalim, Ben-Gurion Univ. of the Negev (Israel)

Skin cancer detection at its’ early stages has been the focus of a large number of experimental and theoretical studies during the past decades. Throughout these studies, Monte-Carlo (MC) simulations have demonstrated high reliability in predicting the spectral and spatial behavior of the back-scattered electromagnetic field from the tissue under examination. Among these experimental and theoretical studies, two prominent approaches presenting high potential and applicability are sensing at the THz wavelengths region, presenting low scattering and long penetration lengths, and polarimetric techniques for pathological tissue differentiation presenting higher sensitivity and contrast than reflectometric techniques.

A comprehensive MC simulation of radiative transfer in a complex skin tissue model including simultaneously skin’s stratified structure, tissue material optical dispersion modeling, surface roughness, scatterers, and substructure organelles has been developed, combining both THz imaging with Muller matrix differential analysis, and is presented and utilized for the determination of the optimal imaging configuration and parameters for highest detection sensitivity. Sensitivity enhancement by the introduction of Parylene-C coated InN nano-particles into the skin tissue is also demonstrated. Current study is still ongoing and being further extended to improved modelling of the skin structure and the EM field-skin interaction.

9721-26, Session 4

Characterizing single molecule dynamics on surfaces

Siheng He, Megan Armstrong, Corina Curschellas, Henry Hess, Columbia Univ. (United States)

Directed transport of molecules via surface chemical potential gradients can accelerate analyte collection on nanoscale sensor surfaces by several orders of magnitude (Nano Letters, vol. 15, p. 3341). Single molecule imaging of the dynamics and spatial distributions of molecules on the surface yields insights into the characteristic of directed transport behavior. Image processing software such as FIESTA (Biophysical Journal, vol. 100, p. 2820) is critical to advance our analysis of nanoscale imaging. Here we describe a new software package called Fluorescent Single Molecule Imaging Analysis (FSMIA), which is specifically designed for single molecule imaging. The software can correct for imaging imperfections such as non-uniform illumination and noisy single pixels. Fitting Gaussian models to the intensity profile of nanoscale objects determines the localization with ~20 nm precision. Moreover, automated single molecule tracking across image stacks assembles molecular trajectories for dynamics analysis. We used FSMIA to characterize the dynamics of protein molecules and single-stranded DNA adsorbed to glass substrates. As a result, we identified several distinct molecular subpopulations and calculated their associated biophysical parameters at the interface. The interfacial dynamics will inform the design of non-fouling surfaces and biosensor surfaces.
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9722-1, Session 1

Luminescent rare earth vanadate nanoparticles doped with Eu3+ and Bi3+ for sensing and imaging applications
Alberto Escudero Belmonte, Carolina Carrillo-Carrión, Mikhail Zyuzin, Raimo Hartmann, Sumaira Ashraf, Wolfgang J. Parak, Philippus-Philipps Univ. Marburg (Germany)

Nanoparticles are attracting interest in nanomedicine not only due to their potential medical applications, ranging from optical biolabels and contrast agents for magnetic resonance imaging to carriers for drug and gene delivery for disease therapy [1]. RE-based nanophosphors exhibit important advantages compared with the other available luminescent materials such as quantum dots and nanostructures functionalized with organic dyes, due to their lower toxicities, photostabilities, high thermal and chemical stabilities, high luminescence quantum yields, and sharp emission bands [2].

Yttrium orthovanadate nanoparticles doped with Eu3+ and Bi3+, functionalised with poly acryl acid (PAA), and excitable by near-ultraviolet light have been synthesised by homogeneous precipitation at 120 °C from solutions of rare earth precursors (yttrium acetylacetonate and europium nitrate), bismuth nitrate, sodium orthovanadate, and PAA, in an ethylene glycol/water mixture. Quasispheres of a mean size of 90 (15) nm were obtained. The as synthesised nanoparticles were already functionalised with PAA, and more layers of polyelectrolytes such as poly allylamine (PAH) and PAA could be further added by using a Layer-by-Layer (LbL) approach. The nanoparticles showed the typical red luminescence of Eu3+, which can be excited with near-UV light through an energy transfer from the vanadate anion. The presence of Bi3+ shifts the maximum of the broad excitation band from 280 nm to 330 nm. This excitation path is much more efficient than the direct excitation of the Eu3+ electronic levels, and results in a much higher luminescence. The functionalised nanophosphors showed negligible toxicity for cells and a very high colloidal stability in different physiological media buffers. The nanoparticles can be uptaken by HeLa cells, and are eventually located in the lysosomes after being internalized. Finally, the functionalization with PAA and PAH provides -COOH and -NH2 anchors for adding functional ligands of biomedical interest that can be used for sensing applications.

References

9722-2, Session 1

Design of a multi-coordinating polymer as a platform for functionalizing metal, metal oxide and semiconductor nanocrystals
 Wentao Wang, Anshika Kapur, Florida State Univ. (United States); Xin Ji, Florida State Univ. (United States) and Ocean Nanotech, LLC (United States); Hedi Mattoussi, Florida State Univ. (United States)

Inorganic nanomaterials such as those made of semiconductors, metals and metal oxides possess unique photo-physical properties that can be tuned via size and/or composition. For instance, gold nanorods exhibit tunable longitudinal surface plasmon resonance. Similarly, semiconductor quantum dots (QDs) exhibit narrow emission that is tunable over a wide range of the visible spectrum, combined with high quantum yields. These unique features have made them greatly appealing for use as in vivo and in vitro probes in a variety of biomedical applications.

We introduce a new set of polymers as multifunctional, metal-coordinating ligands adapted to surface functionalize QDs, iron oxide nanoparticles and gold nanoparticles/nanorods alike. The ligand design relies on the introduction of several anchoring groups, hydrophilic moieties and reactive functionalities into a polymer chain, via one-step nucleophilic addition reaction. In particular, this synthetic scheme allows the insertion of target biomolecules during the ligand synthesis. This surface functionalization route yields nanocrystals that exhibit long-term colloidal stability over a broad range of biological conditions, such as pH changes and when mixed with growth media, while preventing corona formation. When zwitterion groups are used as hydrophilic blocks, this provides compact nanocrystals that are compatible with conjugation to proteins via metal-polylhistidine self-assembly. We show that QDs ligated with these polymers can engage in energy and charge transfer interactions. Furthermore, nanocrystals coated with folic acid-modified polymers could promote the delivery of nanoparticle-conjugates into cancer cells via folate receptor-mediated endocytosis.

9722-3, Session 1

Colloidal core-seeded semiconductor nanorods as fluorescent labels for in-vitro diagnostics (Invited Paper)
Yin Thai Chan, National Univ. of Singapore (Singapore)

Colloidal semiconductor nanocrystals are ideal fluorophores for clinical diagnostics, therapeutics, and highly sensitive biochip applications due to their high photostability, size-tunable color of emission and flexible surface chemistry. The relatively recent development of core-seeded semiconductor nanorods showed that the presence of a rod-like shell can confer even more advantageous physicochemical properties than their spherical counterparts, such as large multi-photon absorption cross-sections and facet-specific chemistry that can be exploited to deposit secondary nanoparticles. It may be envisaged that these highly fluorescent nanorods can be integrated with large scale integrated (LSI) microfluidic systems that allow miniaturization and integration of multiple biochemical processes in a single device at the nanoliter scale, resulting in a highly sensitive and automated detection platform. In this talk, I will describe a LSI microfluidic device that integrates RNA extraction, reverse transcription to cDNA, amplification and target pull-down to detect histidine decarboxylase (HDC) gene directly from human white blood cells samples. When the biotinylated colloidal semiconductor nanorods (NRs) were used as the fluorescent readout, the detection limit was found to be 0.4 ng of total RNA, which was much lower than that obtained using spherical quantum dots (QDs) or organic dyes. This was attributed to the large action cross-section of NRs and their high probability of target capture in a pull-down detection scheme. The combination of large scale integrated microfluidics with highly fluorescent semiconductor NRs may find widespread utility in point-of-care devices and multi-target diagnostics.
Formation of upconversion nanoparticles of Ce:YAG, Eu:KYW and 18%Yb:1%Er:NaYF4 by ultra-short pulse laser ablation in water (Invited Paper)
Laura Gemini, Marie Caroline Hernandez, Rainer Kling, ALPhA NOV (France)

Laser ablation in liquid (LAL) is nowadays gaining popularity as innovative, reliable and efficient technique to produce high-purity nanoparticles (NPs) of many inorganic and organic materials. In this contest, attention has been focused in the past decade on luminescent up-conversion NPs (UCNPs) which, being characterized by sharp emission bands in UV-visible range upon NIR irradiation, are in fact of great interest in many biological and biomedical applications. Moreover, with respect to organic dyes NPs and Quantum dots, UCNPs show less toxicity, increased chemical stability, long-lifetime decays and lack of bleaching. Our research focuses on generation of UCNPs of rare earth lanthanide-doped crystalline materials, namely Ce:YAG, Eu:KYW and 18%Yb:1%Er:NaYF4, by LAL in water. It is well known that optical properties of NPs strongly depend on their features, as for instance size and shape, which in turn are mainly defined by laser ablation parameters. Therefore, two different laser sources are used for the ablation processes in order to find the set of laser parameter, i.e. laser wavelength, pulse duration and laser fluence, for which the luminescence of UCNPs is optimized:

- Amplitude Tangerine system: 350 fs pulse duration, 1030 & 515 nm (SH) wavelengths
- TemPhotronics PicoSpark system: 450 ps pulse duration, 532 nm wavelength

UCNPs are then characterized by spectrophotometer analyses to define emission range and intensity under NIR light and by transmission electron microscopy (TEM) to determine their size and shape. Luminescence UCNPs lifetimes are measured coupling the emission spectra with a photomultiplier as a detector.

Hollow metal nanostructures for enhanced plasmonics (Invited Paper)
Aziz Genç, Institut de Ciencia de Materials de Barcelona (Spain); Javier Patarroyo, Institut Català de Nanociència i Nanotecnologia (ICN2) (Spain); Jordi Sancho-Parramon, Institut Ruder Boškovic (Croatia); Martial Duchamp, Forschungszentrum Jülich GmbH (Germany); Edgar Gonzalez, Instituto Geofísico, Pontificia Univ. Javeriana Bogotá (Colombia); Neus G. Bastus, Institut Català de Nanociència i Nanotecnologia (ICN2) (Spain); Lothar Houben, Rafal Dunin-Borkowski, Forschungszentrum Jülich GmbH (Germany); Victor F. Puntes, Institut Català de Nanociència i Nanotecnologia (ICN2) (Spain) and Institució Catalana de Recerca i Estudis Avançats (Spain) and Vall d’Hebron Institut de Recerca (Spain); Jordi Arbiol, Institut de Ciencia de Materials de Barcelona (Spain) and Institució Catalana de Recerca i Estudis Avançats (Spain)

Complex metal nanoparticles offer a great playground for plasmonic nanoeengineering, where it is possible to cover plasmon resonances from ultraviolet to near infrared by modifying the morphologies from solid nanocubes to nanoframes, multiwalled hollow nanoboxes or even nanotubes with hybrid (alternating solid and hollow) structures. We experimentally show that structural modifications, i.e. void size and final morphology, are the dominant determinants for the final plasmonic properties, while compositional variations allow us to get a fine tuning. EELS mappings of localized surface plasmon resonances (LSPRs) reveal an enhanced plasmon field inside the voids of hollow AuAg nanostructures along with a more homogeneous distributions of the plasmon fields around the nanostructures.

With the present methodology and the appropriate samples we are able to compare the effects of hybridization at the nanoscale in hollow nanostructures. Boundary element method (BEM) simulations also reveal the effects of structural nanoengineering on plasmonic properties of hollow metal nanostructures. Possibility of tuning the LSPR properties of hollow metal nanostructures in a wide range of energy by modifying the void size/shell thickness is shown by BEM simulations, which reveals that void size is the dominant factor for tuning the LSPRs. As a proof of concept for enhanced plasmonic properties, we show effective label free sensing of bovine serum albumin (BSA) with some of our hollow nanostructures. In addition, the different plasmonic modes observed have also been studied and mapped in 3D.

Growth of fluorescence-tunable gold clusters using photo-chemically activated ligands (Invited Paper)
Dinesh Mishra, Fadi Aldeek, Goutam Palui, Hedi Mattoussi, Florida State Univ. (United States)

Interest in developing fluorescent metal clusters made of gold has substantially grown in the past decade. Such interest is motivated by a desire to understand their unique structures and photophysical properties, supplemental by the potential use in an array of applications including biological imaging. We introduce a one-step aqueous reduction of gold precursors in the presence of a cationic acid appended with a poly(ethylene glycol) short chain (LA-PEG) gold clusters with emission that can be tuned from the blue, yellow to the red regions of the visible spectrum. The synthesis does not rely on the usual borohydride reduction, but rather starts with “photochemically-transformed” LA-PEG mixed with AuCl3.3H2O under alkaline conditions (pH 11). Photochemical transformation of the dithiolane group in LA-PEG is achieved using UV-irradiation.

We find that the emission of the prepared clusters is influenced by the time of the growth reaction and the terminal functional group of the ligand used. For instance we find that heating provides red- or blue-emitting clusters, depending on the nature of the terminal functional group in the ligand; LA-PEG-OH3 promotes the formation of red-emitting clusters while amine-terminated LA-PEG promotes the growth of blue-emitting materials. The clusters have been characterized using optical absorption, fluorescence, and DOSY-NMR spectroscopy. The emission location correlates with cluster size as extracted from DOSY-NMR data. The combination of 1 nm or smaller size nanoclusters with tunable fluorescence and high colloidal stability make these materials greatly promising for use in biological applications.

A facile method to prepare NaMnF3:Yb,Er/Tm upconversion nanoparticles with single band
Xiao Peng, Shuai Ye, Yuliang Tian, Jun Song, Guangsheng Wang, Maozhen Xiong, Dong Wang, Hanben Niu, Junle Qu, Shenzhen Univ. (China)

Here, we reported a novel and facile thermal decomposed method to prepare NaMnF3 :Yb,Er/Tm upconversion nanoparticles (UCNPs). In this method, Rare earth acetate and manganese(II) 2,4-pentanedionato were used as raw material. The as-synthesized NaMnF3 :Yb,Er/Tm nanoparticles were monodispersed, uniform and their sizes were both smaller than 10 nm. The NaMnF3 :Yb,Er and NaMnF3 :Yb,Tm nanoparticles radiated intense pure
red emission and near-infrared emission under the excitation of 980 nm laser. Then, the core/shell structured NaMnF3:Yb,Er/Tm@NaMnF3 nanoparticles were prepared to enhance the UC emission effectively.

9722-8, Session 1

**Simple method for the quantification of PEG ligands and guidelines to the spectroscopic characterization of upconversion nanoparticles (Invited Paper)**

Ute Resch-Genger, Marko Moser, Marco Kraft, Thomas Behnke, Jana Falkenhagen, Martin Kaiser, Bundesanstalt für Materialforschung und -prüfung (Germany); Verena Muhr, Institut für Analytische Chemie-und Biosensorik (Germany); Thomas Hirsch, Univ. Regensburg (Germany)

The surface modification of nanometer- and micrometer-sized particles with polyethylene glycol (PEG) ligands of varying length is a very common strategy to tune their hydrophilicity and biocompatibility, minimize unspecific interactions, improve biofunctionalization efficiencies, and enhance blood circulation times [1]. Nevertheless, simple methods for the quantification of PEG ligands are rare. This is similarly true for the spectroscopic characterization of lanthanide-doped upconverting nanoparticles (UCNPs), novel near infrared (NIR)-excitable nonlinear fluorescence reporters for bioanalysis and theranostics, providing background-free multiple narrow emission bands in the visible and NIR, excellent photostability, and long luminescence lifetimes [2,3]. We present here a new and versatile concept for the quantification of PEG ligands on different types of nanomaterials and determination of PEG coupling efficiencies [4], using a new concept based upon the photometric Elman’s test. Subsequently, we reveal straightforward spectroscopic screening methods for the fast identification of nonradiative deactivation pathways in UCNPs, developed based upon the systematic spectroscopic study of UCNPs of varying size and surface chemistry in different microenvironments [5,6].

9722-9, Session 2

**Controlled assembly of biocompatible metallic nanoaggregates using a small molecule crosslinker (Invited Paper)**

Desiree Van Haute, Alice Liu, Jacob M. Berlin, City of Hope Beckman Research Institute (United States)

The controlled assembly of biocompatible nanoparticle aggregates using small molecule crosslinker has been a long standing challenge, likely owing to difficulties in controlling rates of initiation, propagation and termination. Here we demonstrate that adjusting the concentration of the starting nanoparticles or the crosslinker allows for the preparation of relatively homogeneous aggregates from metallic nanoparticles of varied composition and size, presumably by controlling the rates of initiation and propagation. Capturing reactive thiol groups on the formed aggregates with PEG-maleimide provides a termination step and renders the aggregates stable and biocompatible. The size of the aggregates can be systematically adjusted. The aggregates are biocompatible and show no toxicity when incubated with cells. Finally, the aggregates are highly stable and appear unchanged after uptake by cells. It is expected that this straightforward and inexpensive assembly of highly stable nanoparticle aggregates will expand the biological applications of this class of materials. Furthermore, this method for preparing aggregates is highly modular as the crosslinker, the building block nanoparticles and the exterior coating can all be independently varied and the use of alternative crosslinkers and capping agents will enable applications in diverse material applications.

9722-10, Session 2

**Preparation of cellular vehicles for delivery of gold nanorods to tumors**

Sara Lai, Sonia Centi, Istituto di Fisica Applicata “Nello Carrara” (Italy); Claudia Borri, Univ. degli Studi di Firenze (Italy); Francesca Tatini, Istituto di Fisica Applicata “Nello Carrara” (Italy); Marisa Benagiano, Chiara Della Bella, Alessia Grassi, Stefano Colagrande, Mario M. D’Eliaos, Univ. degli Studi di Firenze (Italy); Fulvio Ratto, Roberto Pini, Istituto di Fisica Applicata “Nello Carrara” (Italy)

Over recent years, gold nanorods (GNRs) have emerged as a promising material in biomedical optics and have been proposed as contrast agents for the photothermal therapy and the photoacoustic imaging of tumors. A pioneering approach to target tumors is the use of cellular vehicles, i.e. cells of the immune system that exhibit an innate tropism to tumors and that can serve as Trojan horses. This strategy relies on cell types, such as tumor-associated macrophages or T cells, that are recruited by or naturally traffic to the microenvironment of tumors and that can be isolated from a patient and loaded with plasmonic particles in vitro. In this work, GNRs were synthesized and designed to combine high optical and photo-stability and the ability to accumulate into cells of the immune system. Particles were silanized, PEGylated and conjugated with cationic moieties. Different cationic compounds were tested and the cell viability and uptake of the particles were studied on complementary cell types. The cytotoxicity test was based on a colorimetric WST-8 assay while the intracellular amount of gold and the optical absorbance of the cells were quantified by spectrophotometry. Moreover, we investigated the effect of GNRs on the cell migration and the production of cytokines in the presence of pro-inflammatory stimuli, which provide a functional overview on the feasibility of this approach to target tumors.

9722-11, Session 2

**Supramolecular nanocarriers with photoresponsive cargo (Invited Paper)**

Yang Zhang, Sicheng Tang, Ek Raj Thapaliya, Francisco Raymo, Univ. of Miami (United States)

Nanoparticles with photoresponsive character can be assembled from amphiphilic macromolecular components and hydrophobic chromophores. In aqueous solutions, the hydrophobic domains of these species associate to produce spontaneously nanosized hosts with multiple photoresponsive guests in their interior. The modularity of this supramolecular approach to nanostructured assemblies permits the co-encapsulation of distinct subsets of guests within the very same host. In turn, the entrapped guests can be designed to interact upon light excitation and exchange electrons, energy or protons. Such photoinduced processes permit the engineering of properties into these supramolecular constructs that would otherwise be impossible to replicate with the separate components. In alternative, noninteracting guests with distinct functions can be entrapped in these supramolecular containers to ensure multifunctional character. In fact, biocompatible luminescent probes with unique photochemical and photophysical signatures have already emerged from these fascinating investigations. Thus, polymer nanocarriers can become invaluable supramolecular scaffolds for the realization of multifunctional and photoresponsive tools for a diversity of biomedical applications.

9722-12, Session 2

**Dielectric platforms for surface enhanced spectroscopies (Invited Paper)**

Stefan A. Maier, Imperial College London (United Kingdom)
Plasmonic nanostructures serve as the main backbone of surface enhanced sensing methodologies, yet the associated optical losses lead to localized heating as well as quenching of molecules, complicating their use for enhancement of fluorescent emission. Additionally, conventional plasmonic materials are limited to operation in the visible part of the spectrum. We will elucidate how nanostructures consisting of conventional and polar dielectrics can be employed as a highly promising alternative platform. Dielectric nanostructures can sustain scattering resonances due to both electric and magnetic Mie modes. We have recently predicted high enhanced local electromagnetic field hot spots in dielectric nanoantenna dimers, with the hallmark of spot sizes comparable to those achievable with plasmonic antennas, but with lower optical losses. Here, we will present first experimental evidence for both fluorescence and Raman enhancement in dielectric nanoantennas, including a direct determination of localized heating, and compare to conventional Au dimer antennas. The second part of the talk will focus on the mid-infrared regime of the electromagnetic spectrum, outlining possibilities for surface enhanced infrared absorption spectroscopy based on polar and hyperbolic dielectrics.

9722-13, Session 2

Biomedical applications of magneto-plasmonic nanoclusters (Invited Paper)

Konstantin V. Sokolov, Chun-Hsien Wu, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); Jason Cook, The Univ. of Texas at Austin (United States); Tomasz Zal, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); Stanislav Y. Emelianov, The Univ. of Texas at Austin (United States)

Perhaps one of the most intriguing aspects of nanotechnology is the ability to create multimodal and multifunctional nanostructures that can open new venues in solving challenging biomedical problems. Here, we present multimodal magneto-plasmonic nanoparticles (MPNs) with a strong red-NIR absorbance, superparamagnetic properties, and a high magnetic moment in an external magnetic field. Our design is based on self-assembly of 6 nm primary particles which consist of 5 nm diameter iron-oxide cores coated with a very thin ca. 0.5 nm gold shell. The assembly results in spherical highly uniform MPNs. We developed antibody targeted MPNs to address two highly challenging applications: (i) development of real-time assays for capture, enumeration and characterization of circulating tumor cells (CTCs), and (ii) enhancement of adoptive cell immunotherapy (ACT). Our results showed that MPNs can be used for simultaneous magnetic capture and photoacoustic (PA) detection of cancer cells in whole blood with no laborious processing steps. Furthermore, we demonstrated that MPNs conjugated with anti-CD8 antibodies, which are specific for cytotoxic T cells used in ACT, label CDB8+ T cells with high specificity ex vivo and in vivo. Labeled T cells can be easily manipulated by a small magnet in suspension and under flow conditions. In addition, MPNs generate high contrast in MRI and PA imaging with the potential to detect just few cells per imaging voxel. These results show that immunotargeted MPNs can be explored for simultaneous visualization and magnetic guidance of T cell subsets in vivo for cancer treatment.

9722-15, Session 2

Energy transfer study between luminescent gold nanocluster and fluorescent donors (Invited Paper)

Eunkeu Oh, Alan L. Huston, Andrew Shabaev, Alexander L. Efros, Marc Currie, Kimihiro Susumu, Konrad M. Bussmann, Ramasis Goswami, Fredrik K. Fatemi, Igor L. Medintz, U.S. Naval Research Lab. (United States)

We characterize energy transfer between luminescent 1.5 nm diameter gold nanocrystal (AuNC) acceptors and three structurally/functionally-distinct classes of emissive donors including organic dyes, metal chelates and semiconductor quantum dots (QDs). Energy transfer efficiencies within the donor-AuNC assemblies were evaluated with steady-state and time-resolved measurements. Donor quenching was observed for every donor-acceptor pair although AuNC sensitization was only observed from metal-chelates and QDs. Results were analyzed with Förster’s dipole-dipole coupling model (FRET) and dipole-metal damping models including nanosurface energy transfer (NSET) and nanovolume energy transfer (NVET). FRET dramatically underestimated energy transfer efficiencies while the damping models provided qualitatively better fits to the data although neither fully reproduces the experimental data. Analysis suggests that organic dye donor quenching without corresponding AuNC sensitization results from enhanced intersystem crossing between dye singlet and triplet states driven by AuNC magnetic dipoles. We further consider factors that account for the unique electronic properties of the ultra-small luminescent AuNCs including the high excited state densities, rapid dephasing time and strong electron confinement as well as paramagnetic properties. Overall, the results provide insight into requirements necessary for realizing applications based on AuNC acceptor sensitization.

9722-45, Session 2

Topical drug delivery by nanocarriers probed by spectromicroscopy (Invited Paper)

Kanji Yamamoto, Andre Klossek, Robert Schulz, Freie Univ. Berlin (Germany); Takuji Ohigashi, Institute for Molecular Science (Japan); Markus Weigand, Max-Planck-Institut für Intelligente Systeme (Germany); Roman Flesch, Freie Univ. Berlin (Germany); Sebastian Ahlberg, Fiorenza Rancan, Annika Vogt, Ulrike Blume-Peytavi, Petra Schrade, Sebastian Bachmann, Charité Universitätsmedizin Berlin (Germany); Rainer Haag, Emanuel Fleige, Sarah Hedtrich, Monika Schaefer-Korting, Freie Univ. Berlin (Germany); Nobuhiro Kosugi, Institute for Molecular Science (Japan); Ulrike Alexiev, Roland Netz, Eckart Ruehl, Freie Univ. Berlin (Germany)

Recent progress on nanocarrier-enhanced topical drug delivery processes in skin and cells is reported. Ex vivo human skin samples are exposed to formulations such as HEC gel, containing either neat or nanocarrier-bound anti-inflammatory drugs for variable time periods. The penetration of drugs is followed by label-free approaches, primarily X-ray microscopy. This yields absolute concentration profiles as a function of penetration depth. Significant differences in drug distribution in the stratum corneum, the viable epidermis, and the dermis are deduced from these studies, allowing us to determine the role of core-multishell nanocarriers for drug penetration processes. Complementary work on tape-stripped skin is reported. These results indicate that drug penetration is significantly enhanced, if the stratum corneum, the top horny layer of skin is partially removed or damaged. The experimental results are modeled by the diffusion of drugs using Fokker-Planck equation. This provides evidence for a diffusion and free energy barrier that is located near the interface between the stratum corneum and the viable epidermis. Complementary studies using stimulated Raman microscopy, as another label-free detection technique, as well as results from fluorescence microscopy, making use of dyes and dye-labeled drug nanocarriers are reported, as well. A comparison between different spectromicroscopy techniques relying either on labels or label-free approaches will be made.
Nanoparticles in sensing (Invited Paper)
Amelie Heuer-Jungemann, Antonios G. Kanaras, Univ. of Southampton (United Kingdom)

Nanoparticles are of importance in biomedical applications due to their intrinsic properties. While the ligand coating of the nanoparticle surface is critical for colloidal robustness, targeting and sensing the chemical composition of the inner core is important in defining the optical, electrical and magnetic properties of the probe. As the chemical synthesis of nanoparticles advances during the years, functional nanoparticles become more complex performing multitasking roles. In this presentation we will discuss the current work of the group in the development of sophisticated nanoparticles for bio-sensing and drug delivery in simple and more complex environments.

Mechanogenetic nanoprobes to interrogate spatial, temporal, and mechanical responses of cells
Daeha Seo, Univ. of California, San Francisco (United States); Ji-wook Kim, Yonsei Univ. (Korea, Republic of); Kade Southard, Justin Farlow, Hyun Jung Lee, Thomas Haas, Univ. of California, San Francisco (United States); Jinwoo Cheon, Yonsei Univ. (Korea, Republic of); A. Paul Alivisatos, Univ. of California (United States) and Lawrence Berkeley National Lab. (United States); Zev Gartner, Young-wook Jun, Univ. of California, San Francisco (United States)

The capacity to manipulate the cellular activities using light-sensitive optogenetic modules in both space and time provides an unprecedented means of interrogating cellular information processing at the systems level. However, analogous manipulation of mechanosensitive proteins has largely eluded control so far despite their importance in various physiological, developmental, and pathological processes. In this talk, we present design and synthesis of new colloidal magneto-plasmonic nanoparticle probes, comprised of a Zn-doped magnetite core, a silica spacer, a Au shell, and a monovalently attached oligonucleotide, where the magnetic core, the plasmonic shell, the oligonucleotide serve as the force generating, the single-molecule imaging, and modular functionalization components, respectively. We demonstrate their multifunctional capabilities of imaging, localizing, and mechanically loading targeted proteins in two relevant states), offering the opportunity to investigate primary tumor as well as metastasis. The existing detection strategies encounter various issues such as false negative and low specificity. Therefore, we propose a distinctive detection approach based on the metabolic differences between cancer and healthy cells by using the metabolic indicator. To maximize the difference between cancer and healthy cells, incubation time and oxygen concentration were optimized. Importantly, under the optimized condition, the cancer cell can be clearly identified from the surrounded healthy cells based on the greater brightness in the fluorescence image of the indicator. Implementation of the assay within the microfluidic chips (mixing, purification and detection chips) allows automating the whole procedure, being feasible for in-situ measurements, thus evolving in portable devices. The obtained results using these microsystems are in concordance with those obtained for cells fixed to the tissue culture plate, which also corroborates the robustness of the approach. The demonstrated experimental evidences provide a solid support for our proposed approach which specifically paves the way for the application in liquid biopsy.

Quantum dot based enzyme activity sensors present deviations from Michaelis-Menten kinetic model (Invited Paper)
Sebastián A. Díaz, Carl W. Brown III, U.S. Naval Research Lab. (United States); Anthony P Malanoski, US Naval Research Lab (United States); Eunkeu Oh, Kimihiro Susumuu, Igor L. Medintz, U.S. Naval Research Lab (United States)

Nanosensors employing quantum dots (QDs) and enzyme substrates with fluorescent moieties offer tremendous promise for disease surveillance/diagnostics and as high-throughput co-factor assays. Advantages of QDs over other nanoscaffolds include their small size and inherent photophysical properties such as size tunable fluorescence, ease in attaching functional moieties, and resistance to photobleaching. These properties make QDs excellent Förster Resonance Energy Transfer (FRET) donors; well-suited for rapid, optical measurement applications.

We report enzyme sensors designed with a single FRET donor, the QD donor acting as a scaffold to multiple substrates or acceptors. The QD-Sensor follows the actual activity of the enzyme, as compared to the most common methodologies that quantify the enzyme amount or its mRNA precursor. As the sensor reports on the enzyme activity in real-time we can actively follow the kinetics of the enzyme. Though classic Michaelis-Menten (MM) parameters can be obtained to describe the activity. In the course of these experiments deviations, both decreasing and increasing the kinetics, from the common MM model were observed upon close examinations.

From these observations additional experiments and modeling were undertaken to understand the varying mechanisms. Different enzymes can present different deviations depending on the construction of the sensor, e.g. trypsin appears to present a positive hopping mechanism while collagenase demonstrates a QD caused reversible inhibition.
Colorimetric monitoring of nanometer distance changes in DNA-templated plasmon rulers

Laurent Lermusiaux, Sebastien Bidault, Institut Langevin (France)

The nanometer-scale sensitivity of plasmon coupling allows the translation of minute morphological changes in nanostructures into macroscopic optical signals. In particular, single nanoscale scattering spectroscopy provides a direct estimation of interparticle distances in gold nanoparticle (AuNP) dimers linked by a short DNA double-strand [M. P. Busson et al, Nano Lett. 11, 5060 (2011)].

We demonstrate here that this spectroscopic information can be inferred from simple widefield measurements on a calibrated color camera [L. Lermusiaux et al, ACS Nano 9, 978 (2015)]. This allows us to analyze the influence of electrostatic and steric interparticle interactions on the morphology of DNA-templated AuNP groupings. Furthermore, polarization-resolved measurements on a color CCD provide a parallel imaging of AuNP dimer orientations.

We apply this spectroscopic characterization to identify dimers featuring two different conformations of the same DNA template. In practice, the biomolecular scaffold contains a hairpin-loop that opens after hybridization to a specific DNA sequence and increases the interparticle distance [L. Lermusiaux et al, ACS Nano 6, 10992 (2012)]. These results open exciting perspectives for the parallel sensing of single specific DNA strands using plasmon rulers. We discuss the limits of this approach in terms of the physicochemical stability and reactivity of these nanostructures and demonstrate the importance of engineering the AuNP surface chemistry, in particular using amphiphilic ligands [L. Lermusiaux & S. Bidault, Small (2015), in press].

Zwitterionic quantum dots and amphiphilic polymer quantum dot composites: spray-and-wash multiplex colon cancer diagnosis and platform for cellular labeling and ratiometric oxygen sensing (Invited Paper)

Sunghee Kim, Youngrong Park, Pohang Univ. of Science and Technology (Korea, Republic of); Yeon-Mi Ryu, Asan Medical Ctr. (Korea, Republic of); Joonhyuck Park, Pohang Univ. of Science and Technology (Korea, Republic of); Sang Mun Bae, Jaeil Kim, Eun-Ju Do, Sang-Yeob Kim, Jun Ki Kim, Asan Medical Ctr. (Korea, Republic of); Euiheon Chung, Gwangju Institute of Science and Technology (Korea, Republic of); G-One Ahn, Ki Hean Kim, Pohang Univ. of Science and Technology (Korea, Republic of); Seung-Jae Myung, Asan Medical Ctr. (Korea, Republic of)

Quantum dots (QDs) were conjugated to matrix metalloproteinases (MMP) 9, MMP 14, or carcinoembryonic antigen (CEA) antibodies (Abs) with zwitterionic surface coating to reduce nonspecific bindings. The Ab-QD probes can diagnose tumors on sectioned mouse tissues, fresh mouse colon stained ex vivo and also in vivo as well as fresh human colon adenoma tissues in 30 min and can be imaged with a depth of 100 μm. The probes successfully detected not only cancers that are readily discernible by bare eyes but also hyperplasia and adenoma regions. This multiplexed QD, spray-and-wash, and endoscopy approach provides a significant advantage for detecting small or flat tumors that may be missed by conventional endoscopic examinations and bestows a strategy for the improvement of cancer diagnosis. On the other hand, amphiphilic polyethyleneimine derivatives (amPEIs) were synthesized and used to encapsulate dozens of QDs. The QD-amPEI showed very efficient QD cellular labeling with the labeled cell fluorescence intensity more than 10 times higher than conventional techniques such as Lipofectamine-assisted QD delivery. The QD-amPEI platform has two partitions: positive outer surface and hydrophobic inside pocket. The outer positive surface was further exploited for gene delivery and targeting. Co-delivery of QDs and GFP silencing RNAs and tethering hyaluronic acids for targeting were successfully demonstrated by assembling them to the outer surfaces. The inside hydrophobic compartment was further applied for cohosting oxygen sensing phosphorescence Ru dyes along with QDs. The oxygen probe showed accurate and reversible oxygen sensing capability by the ratiometric photoluminescence signals, which was successfully applied to cellular and spheroid models.


Label-free direct surface-enhanced Raman scattering (SERS) of nucleic acids (Invited Paper)

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Recently, plasmonic-based biosensing has experienced an unprecedented level of attention, with a particular focus on the nucleic acid detection, offering efficient solutions to engineer simple, fast, highly sensitive sensing platforms while overcoming important limitations of PCR and microarray techniques. In the broad field of plasmonics, surface-enhanced Raman scattering (SERS) spectroscopy has arisen as a powerful analytical tool for detection and structural characterization of biomolecules. Today, applications of SERS to nucleic acid analysis largely rely on indirect strategies, which have been demonstrated very pure for sensing purposes but completely dismiss the exquisite structural information provided by the direct acquisition of the biomolecular vibrational fingerprint. Contrarily, direct label-free SERS of nucleic acid shows an outstanding potential in terms of chemical-specific information which however, remained largely unexpressed mainly because of the inherent poor spectral reproducibility and/or limited sensitivity.

To address these limitations, we developed a fast and affordable high-throughput screening direct SERS method for gaining detailed genomic information on nucleic acids (DNA and RNA) and for the characterization and quantitative recognition of DNA interactions with exogenous agents. The simple strategies rely on the electrostatic adhesion of DNA/RNA onto positively-charged silver colloids that promotes the nanoparticle aggregation into stable clusters yielding intense and reproducible SERS spectra at picogram level (i.e. the analysis can be performed without the necessity of amplification steps thus providing realistic direct information of the nucleic acid in its native state). We anticipate this method to gain a vast impact and set of applications in different fields, including medical diagnostics, genomic screening, drug discovery, forensic science and even molecular electronics.

9722-24, Session 4

Standardized toxicological assays for utilization of colloidal nanoparticles in biomedical applications (Invited Paper)

Christoph Rehbock, Univ. Duisburg-Essen (Germany); Ulrike Taylor, Detlef Rath, Friedrich-Loeffler-Institut (Germany); Stephan Barcikowski, Univ. Duisburg-Essen (Germany)

Nowadays exposure to nanomaterials has intensified due to their frequent utilization in biomedical applications. Consequently, risk assessment of nanoparticles is an issue of paramount global significance. Even though countless studies have been published addressing nanotoxicology, the outcome remains diverse. This is predominantly attributed to the lack of proper standards for nanoparticles.

In this work, strategies for the standardization of nanotoxicological assays are presented. Thereto, it is important to always adapt the used nanoparticles to the addressed exposure scenario. Particularly the impact of surface ligands is a critical issue, which may be assessed when ligand-free standards e.g. from laser ablation in liquids are used. In addition proper dosing is critical. In contrast to commonly applied mass doses, surface dosing in reference to the relevant biological entity should be used. Furthermore, the choice of an appropriate biological system warrants special attention. In this context, approval of functional toxicity could be more relevant requiring detection of more subtle adverse effects, especially where reproductive biology with its impact on offspring is involved.

References

9722-25, Session 4

What happens to nanoparticles once they are incorporated by cells in vitro and in vivo?

Wolfgang J. Parak, Philipps-Univ. Marburg (Germany) and CIC Biomagque (Spain)

What happens to inorganic nanoparticles (NPs), such as plasmonic gold or silver, superparamagnetic iron oxide, or fluorescent quantum dot NPs, after they have been administrated to an animal or a human being? The review discusses the integrity, biodistribution, and fate of NPs after in vivo administration. First the hybrid nature of the NPs is described, by conceptually dividing them into the inorganic NP core, an engineered surface coating around the core which comprises the ligand shell and optionally also bioconjugation, and into the corona of adsorbed biological molecules. It is shown that in vivo all of these three compounds may degrade individually and that each of them can drastically modify the life-cycle and biodistribution of the whole hetero-structure. The NPs thus may be disintegrated into different parts, of which biodistribution and fate would need to be analyzed individually. Multiple labelling and quantification strategies for such purpose will be discussed. All reviewed data indicate that in vivo NPs no longer should be considered as homogeneous entity, but should be seen as inorganic/organic/biological nano-hybrids with complex and intricately linked degradation pathways.

9722-26, Session 4

The influence of cell penetrating peptide branching on cellular uptake of QDs

Joyce Breger, James Delechanty, Kimihito Susumu, George Anderson, U.S. Naval Research Lab. (United States); Markus Muttenhaler, Philip Dawson, The Scripps Research Institute (United States); Igor L. Medintz, U.S. Naval Research Lab. (United States)

To overcome the liabilities associated with traditional, systemic drug delivery, recent efforts have focused on developing nanoparticles as carriers or vectors for targeted drug delivery systems. These systems employ nanoparticle platforms to assemble complex theranostic structures which have poorly understood cellular interactions. Semiconductor quantum dots (QDs) can be incredibly valuable as a platform for understanding the intricacies of nanoparticle cellular uptake and intracellular fate. Because a number of different functional moieties, such as targeting moieties and drugs, must be attached to the nanoparticle surface for efficient drug delivery and monitoring, the number of internalization peptides necessary for efficient uptake should be minimized. Therefore, we have designed a range of polyarginine (Arg9) peptides which are repeatedly displayed in branches (n = 1 to 16 repeats) when attached to the backbone of a dendritic metal affinity coordination to QDs at display ratios ranging from 0.5 up to 0.5 peptides per QD. The efficiency and amount of QD uptake was quantified over time (up to 24 hrs). Increasing the number of polyarginine branches per peptide correlated with a higher degree of cellular uptake which was most noticeable at the lowest ratio of peptide (R= 0.5) and shortest time point tested (30 min). By increasing the polyarginine branching in the internalization peptide and thus utilizing less peptide per QD, more cargo delivery space becomes available on the nanoparticle surface for other
functional moieties such as sensors or drugs while still achieving efficient cellular uptake.

9722-27, Session 4

Imaging cellular membrane potential through ionization of quantum dots (Invited Paper)


Recent interest in quantum dots (QDs) stems from the plethora of potential applications that arises from their tunable absorption and emission profiles, high absorption cross sections, resistance to photobleaching, functionalizable surfaces, and physical robustness. The emergent use of QDs in biological imaging exploits these and other intrinsic properties. For example, quantum confined Stark effect (QCSE), which describes changes in the photoluminescence (PL) of QDs driven by the application of an electric field, provides an inherent means of detecting changes in electric fields by monitoring QD emission and thus points to a ready mean of imaging membrane potential (and action potentials) in electrically active cells. Here we examine the changing PL of various QDs subjected to electric fields comparable to those found across a cellular membrane. By pairing static and time-resolved PL measurements, we attempt to understand the mechanism driving electric-field-induced PL quenching and ultimately conclude that ionization plays a substantial role in initiating PL changes in systems where QCSE has traditionally been credited. Expanding on these findings, we explore the rapidity of response of the QD PL to applied electric fields and demonstrate changes amply able to capture the millisecond timescale of cellular action potentials.

9722-29, Session 4

Size dependent gold nanoparticle interaction at nano-micro interface using both monolayer and multilayer (tissue-like) cell models (Invited Paper)

Devika B. Chithrani, Ryerson Univ. (Canada)

Gold nanoparticles (GNPs) are emerging as a novel tool to improve existing cancer therapeutics. GNPs are being used as radiation dose enhancers in radiation therapy as well as an anticancer drug carrier in chemotherapy. However, the success of GNP-based therapeutics depends on their ability to penetrate in tumor tissue. GNPs of 20 nm and 50 nm diameters were used to elucidate the effects of size on the GNP interaction with tumor cells at monolayer and multilayer level. At monolayer cell level, smaller NPs had a lower uptake compared to larger NPs at monolayer cell level. However, the order was reversed at tissue-like multilayer level. The smaller NPs penetrated better as compared to larger NPs in tumor tissue. This study showed that smaller NPs are better for future therapeutics since they can penetrate better in tumor tissue once leave the leaky blood vessels. In this work, tissue-like multilayer cellular structures (MLCs) were grown to model the post-vascular tumor environment. The MLCs exhibited a much more extensive extracellular matrix (ECM) than monolayer cell cultures. The MLC model can be used to optimize nano-micro interface at tissue level before moving into animal models. This would accelerate the use of NPs in future cancer therapeutics.

9722-30, Session 4

Photo thermal efficacy of green light emitting diode and gold nano spheres for malignancy

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Photoirradiation effect of 30nm Gold nanoparticle(GNPs) in combination with light emitting diode(LED) found to be remarkable and this work concentrates on optimizing concentration of GNP and light source in malignant and normal cells lines. The biochemical mechanism behind the cell lethality were studied which is varied based on Fluence, Nature of Light source, Concentration of nanoparticles and Post irradiation effects. 30nm GNPs and LED with power 30mW were used against Vero and HeLa cell lines followed by MTT assay. We have compared the cytotoxicity, phototoxicity, post irradiation effect for a period of 24 and 48 hrs with varying concentration. By photo irradiation, irradiated spot showed morphological disturbance but the cell monolayer was undisturbed in the non-irradiated area. Floating cells were not observed which confirms that there is no necrotic effect. After 24 hrs, the irradiated spot which showed slight disturbance at 2 hrs time interval has now developed clear apoptotic bodies. On closer examination, the growth of the cells in non-irradiated area can be observed. Thus the treatment is completely localized. The LED (530 nm) light source of 30 mW power irradiated for 2 minutes induces apoptosis and the same effect is replicated with the laser of the same wavelength of 7 mW and irradiated for 5 minutes. This effect may be due to hyperthermia which is known to induce apoptotic cell death in many tissues and has been shown to increase local control and overall survival.

9722-31, Session 4

Lanthanum fluoride nanoparticles for radiosensitization of tumors (Invited Paper)

Jay L. Nadeau, California Institute of Technology (United States) and McGill Univ. (Canada); Daniel Cooper, Devesh Bekah, McGill Univ. (Canada); Ido Badash, Collin T. Mayemura, Colin Hill, Stephen E. Bradforth, The Univ. of Southern California (United States)

Dense inorganic nanoparticles have recently been identified as promising radiosensitizers. In addition to dose enhancement through increased attenuation of ionizing radiation relative to biological tissue, scintillating nanoparticles can transfer energy to coupled photosensitizers to amplify production of reactive oxygen species, as well as provide UV-visible emission for optical imaging. Lanthanum fluoride is a transparent material that is easily prepared as nanocrystals, and which can provide radioluminescence at a number of wavelengths through simple substitution of lanthanum ions with other luminescent lanthanides. We have prepared lanthanum fluoride nanoparticles doped with cerium, terbium, or both, that have good spectral overlap with chlorin e6 or rose bengal photosensitizer molecules. We have also developed a strategy for stable conjugation of the photosensitizers to the nanoparticle surface, allowing for high energy transfer efficiencies on a per molecule basis. Additionally, we have succeeded in making our conjugates colloially stable under physiological conditions. Here we present our latest results, using nanoparticles and nanoparticle-photosensitizer conjugates to demonstrate radiation dose enhancement in two melanoma cell lines in vitro: B16, a murine melanoma, and MeWo, a human melanoma. The effects of nanoparticle treatment prior to 250 kVp x-ray irradiation were investigated through clonogenic survival assays, cell cycle analysis, 5H2AX and comet assays. Using a custom apparatus, we have also observed scintillation of the nanoparticles and conjugates under the same conditions that the cell samples are irradiated.
9722-32, Session 4

Attenuating the neurotoxic aggregation of the Alzheimer’s peptide α? with functionalized gold nanoparticles

Christoph Rehbock, Carmen Streich, Laura Akkari, Thomas Schrader, Stephan Barcikowski, Univ. Duisburg-Essen (Germany)

Neurodegenerative disorders like Alzheimer’s disease affect an ever increasing number of people. Many of these diseases involve protein misfolding. In our study, gold nanoparticles are employed as organizational platform and transport vehicle for ligands [1], specifically targeting the A? peptide associated with the development of Alzheimer’s disease. Pulsed laser ablation in liquid was chosen as nanoparticle fabrication method because completely ligand-free nanoparticles can be obtained [2], featuring a high purity and surface accessibility for subsequent ligand functionalization. Conjugation efficiency and conjugate stability were analyzed as function of ligand-to-nanoparticle ratio. Thereby different ligand coverages on the nanoparticle surface could be established and regimes of high colloidal stability were identified for further tests. [3] The interaction of nanoparticle-ligand conjugates with the Alzheimer peptide A? was characterized via dynamic light scattering and zeta potential measurements. Even though A? binds to both, ligand-free and ligand-functionalized nanoparticles only functionalized nanoparticles seem to inhibit A? aggregation. Interestingly, ligands immobilized on the nanoparticle surface show increased efficacy compared to free ligands at the same dose. This may be due to high local ligand concentrations at the particle surface and an increased avidity towards A? via synergistic effects.

References:

9722-49, Session PSun

Design of Raman active nanoparticles for SERS-based detection

Javier T. Garza, Gerard L. Cote, Texas A&M Univ. (United States)

Timely detection of cardiac biomarkers is needed to diagnose acute myocardial infarction, implement the appropriate early treatment, and significantly reduce the chance of mortality. Ideally, for maximizing patient impact, a point of care device needs to be designed that is fast, sensitive, reliable, and small enough to be used in the ambulance and emergency department. Surface enhanced Raman spectroscopy (SERS) is a sensitive optical technique that can potentially be used to quantify the cardiac biomarkers of interest. In this work, silver nanoparticles were functionalized with a Raman reporter molecule and human cardiac Troponin I as an essential component of a competitive binding assay. Aggregated nanoparticles with the Raman reporter molecules were encapsulated in a silica shell to form SERS hotspots. Besides having a specific Raman spectra and binding affinity to cardiac Troponin I antibodies, the nanoparticles were designed to exhibit stability by using silica and polyethylene glycol (PEG) as part of the bioconjugation strategy. The specific narrow peaks from the Raman reporter molecule SERS signal allow for potential multiplexing capabilities as different Raman reporter molecules can be used in functionalized nanoparticles with different cardiac biomarkers. The SERS peak intensities of different concentrations of functionalized nanoparticles were measured to determine the minimum and maximum amount of nanoparticles that can be detected.

9722-50, Session PSun

Measuring pair-wise molecular interactions in a complex mixture

Krishnendu Chakraborty, Manoj M. Varma, Murugesan Venkatapathi, Indian Institute of Science (India)

Complex biological samples such as serum contain thousands of proteins and other molecules spanning up to 13 orders of magnitude in concentration. Present measurement techniques do not permit the analysis of all pair-wise interactions between the components of such a complex mixture to a given target molecule. In this work we explore the use of nanoparticle tags which encode the identity of the molecule to obtain the statistical distribution of pair-wise interactions using their Localized Surface Plasmon Resonance (LSPR) signals. The nanoparticle tags are chosen such that the binding between two molecules conjugated to the respective nanoparticle tags can be recognized by the coupling of their LSPR signals. We used a numerical code using the Discrete Dipole Approximation (DDA) to investigate this approach using a reduced system consisting of three nanoparticles (a gold ellipsoid with aspect ratio 2.5 and short axis 16 nm, and two silver ellipsoids with aspect ratios 3 and 2 and short axes 8 nm and 10 nm respectively) and the set of all possible pair-wise interactions between the nanoparticles. Incident light was circularly polarized and all possible particle and dimer orientations were considered. We observed that for this set of particles with close dimensions and similar shapes, the minimum LSPR peak separation was 5 nm (maximum separation was 184 nm) which is readily detected using state of the art instruments permitting the identification of the molecules involved in the interaction.

9722-48, Session PSun

In vitro antitumor efficacy of berberine-solid lipid nanoparticles against human HepG2, Huh7 and EC9706 cancer cell lines

Zhi-ping Wang, Guangdong Pharmaceutical Univ. (China); Hua Fan, Guangdong Hinabiotech Co., Ltd. (China); Ju Jin, Guangdong Pharmaceutical Univ. (China); Yifei Wang, Institute of Biological Medicine, Jinan Univ. (China); Tongsheng Chen, Institute of Laser Life Science, 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 resistance of the advanced hepatocarcinoma to chemotherapy. Berberine (Ber), an isoquinoline derivative alkaloid, has a wide range of pharmacological properties and is considered to have anti-heptocarcinoma effects. However its low oral bioavailability restricts its wide application. In this report, Ber loaded solid lipid nanoparticles (Ber-SLN) was prepared by hot melting and then high pressure homogenization technique. The in vitro anti-heptocarcinoma effects of Ber-SLN relative to efficacy of bulk Ber were evaluated. The particle size and zeta potential of Ber-SLN were 95.2 ± 0.8 nm and 118.63 ± 0.99 mV, respectively. MTT assay showed that Ber-SLN effectively inhibited the proliferation of human HepG2 and Huh7 and SMMC7721 cells, and the corresponding IC50 values were 11.6, 8.3, and 9.1 μg/ml (18.3, 14.1, and 14.7 μg/ml of bulk Ber). These results suggest that the delivery of Ber-SLN is a promising approach for treating tumors.

9722-51, Session PSun

Evaluation of free radical scavenging capacity and antioxidative damage effect of resveratrol-nanostructured lipid carrier

Zhi-ping Wang, Ju Jin, Guangdong Pharmaceutical Univ. (China); Hua Fan, Guangdong Hinabiotech Co., Ltd. (China); Ju Jin, Guangdong Pharmaceutical Univ. (China); Yifei Wang, Institute of Biological Medicine, Jinan Univ. (China); Tongsheng Chen, Institute of Laser Life Science, South China Normal Univ. (China)
Donald A. Fernandes, Ryerson Univ. (Canada); Dennis

In vitro studies of multifunctional perfluorocarbon nanoemulsions for cancer therapy and imaging

Donald A. Fernandes, Ryerson Univ. (Canada); Dennis

Perfluorocarbon nanoemulsions are a unique class of theranostic agents which are able to solubilize a wide variety of therapeutic agents, at the same time have optical properties for tumor detection using various imaging modalities. Perfluorocarbon (PFC) nanoemulsions allow for a range of functions in vivo because of their biological inertness, high stability, and reduced cytotoxicity. Like many other nanoparticles, the nanoemulsions can be designed to have various targeting ligands on their surface to direct them to various biomarkers associated with cancer. It can at the same time carry a variety of extrinsic imaging agents such as chromophores for fluorescence imaging or use its intrinsic physical properties such as its density or ability to vaporize upon expansion for ultrasound and photoacoustic imaging. Its phase transition from a liquid droplet to a microbubble can be accomplished by either directly providing energy using acoustic waves or indirectly through the use of optical absorbers (i.e. gold nanoparticles) which can transfer energy as heat to PFC nanodroplets. This work will study the effect of perfluorocarbon nanoemulsions in breast cancer cell lines (i.e. MCF-7, MDA-MB 231) to determine their efficacy as theranostic agents.

Bio-modified cobalt core/carbon shell nanoparticle for MR/photoacoustic/microwave-induced thermoacoustic triple-modality imaging

Huan Qin, Dong Xu, South China Normal Univ. (China)

Multimodality imaging based on complementary detection principles has broad clinical applications and promises to improve the accuracy of tumor diagnosis. In the present work, we have developed a novel cobalt core/carbon shell (Co/C) based probe for targeting and triple-modality imaging of tumors. The nanostructure consists of ferromagnetic (Co) particles coated with carbon (C) for biocompatibility and optical absorption. The Co/C nanoparticles have been characterized by transmission electron microscopy (TEM) and visible-near infrared spectra. Experiments verify that the designed Co/C nanoparticles can be detected by the magnetic resonance imaging, photoacoustic imaging and microwave-induced thermoacoustic imaging with at least a nanomole sensitivity both in vitro and in vivo. Importantly, when conjugated with a tumor targeted molecular, the Co/C nanoparticles could be targeted to the corresponding tumors, allowing for a noninvasive tumor delineation using all three modalities. The bio-modified Co/C nanoparticles are likely to become a promising candidate for triple-modality imaging of tumor.

Persistent nanophosphors for in vivo optical imaging

Cyrille Richard, Univ. Paris Descartes (France)

Optical imaging has become a dominant visualization method in biomedical research due to its high sensitivity, low cost and its ease of use. However, tissue autofluorescence produces a substantial background signal that severely limits the quality of images. To overcome this limitation, our team pioneered the use of persistent luminescence nanoparticles (PLNP) for optical real-time imaging in small animals. These nanophosphors have the ability to store the excitation light in traps and to emit light from minutes to optical real-time imaging in small animals.2 These nanophosphors have the ability to store the excitation light in traps and to emit light from minutes to
hours after the end of the excitation through a thermal activation in tissues. This property allows the detection of PLNP in tissues with high target to background ratio.

We will report the synthesis of these different nanomaterials and their use for in vivo optical imaging. We will show how the functionalization of the surface allows the targeting of several cancer cell lines.3,4 We will retrace the steps that led us to move from the first generation of nanoparticles, only visible for 1 hour in vivo, to the intermediate material 5 times longer luminescent5 to the actual material, in situ re-excitable allowing monitoring the probe in vivo for several weeks.6 Finally, we will present examples using this technology either for tumour-targeting in vivo, cell tagging to track cells biological fate in vivo,7 as well as to the preparation of a two nanohybrids, one for bimodal imaging8 and the other, a porous nanohybrid, for theranostics applications.9

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9722-34, Session 5

**Single gold@silver nanoprobes for real-time tracing autophagy process at single-cell level (Invited Paper)**

Junjie Zhu, Nanjing Univ. (China)

This work described a multi-modified core-shell gold@silver nanoprobe for real-time monitoring the entire autophagy process at single-cell level. Autophagy is a regulated lysosomal degradation pathway along with the formation of double vacuoles, called autophagosomes. Autophagy is vital for understanding the mechanisms of human pathologies, developing novel drugs and exploring approaches for autophagy controlling. Major challenge for autophagy study lies in real-time monitoring. When cells were under starvation condition, the mitochondrial-mediated oxidative phosphorylation became incomplete and the production of O2•− was upregulated, which induced the autophagy. Thus, one solution might come from real-time detection of in-situ superoxide radicals (O2•−) because it is the main regulator of autophagy. In this work, we report a plasmon resonance scattering (PRS) nanoprobe-based approach for real-time monitoring the intracellular O2•− level. This design was realized by notable PRS spectral shift ?max of nanoprobe when the silver shell was etched by the generated O2•−. Both the experimental and simulated results suggested the wavelength change rate correlated well with O2•− level. This response enabled its application in real-time in-situ quantification of O2•− during autophagy course. More importantly, with the introduction of ‘relay probe’ operation, two types of O2•−-regulating autophagy processes were successfully traced from the beginning to the end, and the possible mechanism was also proposed. According to our results, intracellular O2•− level controlled the autophagy process by mediating the autolysosome generation. Different starvation approaches can induce different autophagy processes, such as diverse steady state time-consuming.

9722-35, Session 5

**Photoluminescent nanodiamonds for bioimaging applications**

Ishan D. Rastogi, Carlo Bradac, Thomas Volz, Macquarie Univ. (Australia); Philipp Reineck, Brant Gibson, RMIT Univ. (Australia); Louise J. Brown, Macquarie Univ. (Australia)

Nanodiamond (ND) particles (<100nm) are biocompatible, chemically stable and have point crystal defects in their core lattice like Nitrogen-Vacancy (NV) centre. Unique material and optical properties like facile surface functionalization, photostable fluorescence, long lifetime and a quantum yield near unity, make nanodiamonds an efficient fluorescent bio-label for molecular tracking and imaging.

However, the challenges in developing this material as a bio-label, deal with the procurement of a monodisperse preparation for smaller nanodiamonds (<20nm) and their reduced brightness. Here we present a purification and processing method for isolating nanodiamonds of various sizes from 4 nm to 100 nm. Comparative analysis of brightness for NDs of different sizes suggests the best suitable size range (~20-30nm) for bioimaging applications. NDs in this size range are small enough to be relevant on biomolecular size scale and large enough to accommodate enough optical centres that are stable and therefore emit a sufficiently bright signal. We also demonstrate functionalization of nanodiamond surface to conjugate to the biological molecules, specifically actin filaments. The specific labelling of the target biomolecule is shown using several independent techniques including confocal microscopy, transmission electron microscopy and fluorescence spectroscopy. These results open up many possibilities for using nanodiamonds as an imaging label in complex biological systems.

9722-36, Session 5

**Facile synthesis of NaYF4:Yb/Ho upconversion nanoparticles for NIR-excited fluorescent bio-imaging**

Xiao Peng, Shuai Ye, Guangsheng Wang, Yuliang Tian, Ming Zhu, Wei Yan, Jun Song, Junle Qu, Shenzhen Univ. (China)

Recently, upconversion nanoparticles (UCNPs) have attracted increasing interests in bio-imaging applications. Here, the Yb/Ho codoped NaYF4 nanoparticles with strong red emission (~650 nm) under the 800 nm excitation were synthesized by using a facile method. Then, the modified NaYF4 core-shell nanoparticles were introduced into cells and the light damage effects under different excitation wavelengths were compared. These results showed that the survival rates of the cells under 800 nm excitation wavelength were much higher than those under 980 nm, indicating that the synthesized UCNPs have great potentials in optical cellular imaging applications with lower light damage for normal tissues.

9722-37, Session 5

**Magnetic nanoparticle hyperthermia dosimetry by biomechanical properties revealed in magnetomotive optical coherence elastography**

Pin-Chieh Huang, Marina Marjanovic, Darold R. Spillman Jr., Boris M. Odintsov, Stephen A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Magnetic nanoparticles (MNPs) have been utilized in magnetic hyperthermia to treat solid tumors. Under an appropriate AC magnetic field, energy can be transferred to the MNPs to heat up the intended tissue target while sparing non-targeted healthy tissue. However, a sensitive monitoring technique for the dose of MNP thermal therapy is desirable in order to prevent over-treatment and collateral injury.

Typical hyperthermia dosimetry often relies on changes in imaging properties or temperature measurements based on the thermal distribution. Alternative dosimetric indicators can include the biomechanical properties...
of the tissue, reflecting the changes due to protein denaturation, coagulation, and tissue dehydration during hyperthermia treatments. Tissue stiffness can be probed by elastography modalities including MRI, ultrasound imaging, and optical coherence elastography (OCE), with OCE showing the highest displacement sensitivity (tens of nanometers). Magneto-motive optical coherence elastography (MM-OCE) is one type of OCE that utilizes MNPs as internal force transducers to probe the tissue stiffness. Therefore, we examined the feasibility of evaluating the hyperthermia dose based on the elasticity changes revealed by MM-OCE. Superparamagnetic MNPs were applied to ex vivo tissue specimens for both magnetic hyperthermia and MM-OCE experiments, where temperature and elastic modulus were obtained. A correlation between temperature rise and measured stiffness was observed. In addition, we found that with repetitive sequential treatments, tissue stiffness increased, while temperature rise remained relatively constant. These results potentially suggest that MM-OCE could indicate the irreversible changes the tissue undergoes during thermal therapy, which supports the idea for MM-OCE-based hyperthermia dosage control in future applications.

9722-38, Session 5

**Next generation in vivo optical imaging with short-wave infrared quantum dots (Invited Paper)**

Oliver T. Bruns, Thomas S. Bischof, Daniel K. Harris, Daniel Franke, Christopher J. Rowlands, Peter T. C. So, Massachusetts Institute of Technology (United States); Rakesh K. Jain, Massachusetts General Hospital (United States); Mounigi G. Bawendi, Massachusetts Institute of Technology (United States)

The short-wavelength infrared region (SWIR; 1000—2000 nm) provides several advantages over the visible and near-infrared regions for in vivo imaging. The general lack of autofluorescence, low light absorption by blood and tissue, and reduced scattering can render a mouse translucent when imaged in the SWIR region. Despite these advantages, the lack of a versatile emitter platform has prevented its general adoption by the biomedical research community. Here we introduce high-quality SWIR-emitting core/shell quantum dots (QDs) for the next generation of in vivo SWIR imaging. Our QDs exhibit a dramatically higher emission quantum yield (QY) than previously described SWIR probes, as well as a narrow and size-tunable emission that allows for multiplexing in the SWIR region. To demonstrate some of its capabilities, we use this imaging platform to measure the heartbeat and breathing rates in awake mice, as well as to quantify the lipoprotein turnover rates of several organs simultaneously in real time in mice. Finally, we generate detailed three-dimensional quantitative flow maps of brain vasculature by intravital microscopy and visualize the differences between healthy tissue and a tumor in the brain. SWIR QDs enable biological optical imaging with an unprecedented combination of deep penetration, high spatial resolution, and fast acquisition speed.

9722-39, Session 5

**Multi-harmonic nanoparticles for cell tracking (Invited Paper)**

Luigi Bonacina, Univ. de Genève (Switzerland)

Nanoparticles are increasingly employed as contrast agents for tissue imaging and diagnostics. One very recent development, yet little-known and strongly related to the evolution of ultrafast laser technology, is the use of so-called harmonic nanoparticles (HNPs). The latter designate a family of metal oxide crystals sharing the mutual characteristic of noncentrosymmetric lattice structure. This property determines their large nonlinear optical efficiency, and, in fact, in the last years they have been mostly investigated as localized sources for second harmonic generation in multi-photon microscopy.

After introducing the HNPs approach and highlighting its advantages with respect to other nanoparticles-based labelling strategies (quantum dots, up-conversion NPs) in terms of long term photo-stability, wavelength flexibility, and bio-compatibility, I will present some of HNPs most recent applications in the fields of regenerative medicine and cancer research.

I will then introduce a novel imaging protocol particularly adapted to multi-photon infrared (1.3 um) microscopy based on the co-localization of two harmonic signals simultaneously generated by a HNP. I will provide evidence that this simple scheme entails a major increase in selectivity against endogenous harmonic sources (collagen, lipids) for the identification of labelled cells embedded in thick samples. In particular, I will present the results of a study on metastases formation in lung tissue enabled by tracking cancer cells stained by Bismuth Ferrite HNPs intravenously injected in a mouse model.

9722-41, Session 6

**Combination of photothermal and photodynamic effects for cancer cell inactivation through surface plasmon resonance with Au nanoring based on two-photon absorption (Invited Paper)**

Chih-Ken Chu, Jen-Hung Hsiao, Jian-He Yu, Yi-Chou Tu, Chih-Kang Yu, Shih-Yang Chen, Po-Hao Tseng, Shuai Chen, Ye-an-Woei Kiang, Chih-Chung Yang, National Taiwan Univ. (Taiwan)
The surface plasmon (SP) resonance wavelength of Au nanoring (NRI) can be extended into the spectral range of >1000 nm for deeper tissue penetration. By using SP-enhanced two-photon absorption of a photosensitizer to generate singlet-oxygen, we can combine the photothermal effect through SP-enhanced absorption and the photodynamic effect for enhancing the efficiency of cancer cell inactivation. In this study, we use Au NRIs with the major SP resonance peak at 1066 nm for linking with the photosensitizer, AlPcS, and for inactivating oral cancer cells, SAS, through the excitation of a femtosecond laser at 1064 nm in central wavelength. The femtosecond laser generates strong SP resonance fields around Au NRIs for effective two-photon absorption of the linked AlPcS molecules to produce singlet-oxygen and inactivate cancer cells, which uptake the surface-modified Au NRIs, besides the photothermal inactivation effect. By comparing the inactivation results between Au NRI-AlPcS and Au NRI (without linked AlPcS), it is found that the laser threshold intensity for cancer cell inactivation with Au NRI-AlPcS is significantly lower than that with Au NRI alone. Although the use of a continuous laser of the same average power at the same wavelength as those of the femtosecond laser can also cause cancer cell inactivation, the corresponding threshold intensity is significantly higher.

9722-42, Session 6

**Membrane-targeting liquid crystal nanoparticles (LCNPs) for drug delivery**

Oakhil Kumar Nag, Christopher M. Spillmann, Jawad Naciri, James B. Delehanty, U.S. Naval Research Lab. (United States)

In addition to maintaining the structural integrity of the cell, the plasma membrane regulates multiple important cellular processes, such as endocytosis and trafficking, apoptotic pathways and drug transport. The modulation or tracking of such cellular processes by means of controlled delivery of drugs or imaging agents via nanoscale delivery systems is very attractive. Nanoparticle-mediated delivery systems mediate long-term residence (e.g., days), controlled release of the cargoes in the plasma membrane while simultaneously not interfering with regular cellular physiology and homeostasis. Our laboratory has developed a plasma membrane-targeted liquid crystal nanoparticle (LCNPs) formulation that can be loaded with dyes or drugs which can be slowly released from the particle over time. This talk will highlight the utility of these nanopreparations for membrane delivery and imaging.

9722-43, Session 6

**Nanometronomics: exploiting nanotechnology to optimize metronomic treatment of cancer** *(Invited Paper)*

Serena Mazzucchelli, Michela Bellini, Luisa Flandra, Marta Truffi, Maria A. Rizzuto, Manuela Nebuloni, Fabio Corsi, Davide Prosperi, Univ. degli Studi di Milano Bicocca (Italy)

The standard clinical protocols for cancer chemotherapy are based on the concept of “maximum tolerated dose” (MTD). However, prolonged time intervals between treatment cycles are required to allow for normal tissue recovery, during which neoangiogenesis and development of therapeutic resistance often occur. Recent discoveries in tumor biology suggest alternative strategies toward tumor eradication using targeted approaches that disengage the neoplastic microenvironment. This latter new concept, referred to as “metronomic” chemotherapy, redefines the therapeutic regimen to aim for prolonged responses rather than short-term tumor regression. In contrast to MTD regimens, low-dose metronomic (LDM) chemotherapy is characterized by the administration of a cytotoxic agent at lower, less toxic dose given at regular, more frequent time intervals. Unfortunately, most clinicians remain suspicious toward LDM chemotherapy, which they consider to be a palliative care rather than an upfront therapy.

The main disadvantages are low drug accumulation at the tumor and thus poor effectiveness against advanced-stage metastatic tumors. With the aim to improve the potential of metronomic paradigm, we investigated the use of H-ferritin (HFn) nanocages in delivering continuous low doses of cytotoxic doxorubicin (DOX) to a highly invasive breast cancer model. Mice bearing 4T1 cells were treated with placebo, free DOX or HFn-DOX for 21 days at about 1/3 of the standard clinical dosage. Our results demonstrate that LDM treatment with HFn-DOX is able to strongly affect the progression of the tumor and to alter the neovascularization process. In addition, HFn-DOX allows overcoming cardiotoxicity, one of the most severe side effects of DOX chemotherapy.

9722-44, Session 6

**Towards photodynamic therapy with ionising radiation: nanoparticle-mediated singlet oxygen generation**

Sandhya Clement, Wei Deng, Elizabeth Camilleri, Macquarie Univ. (Australia); Brian Wilson, Univ. Health Network (Canada); Ewa Goldys, Macquarie Univ. (Australia)

Photodynamic therapy (PDT) is a clinically approved method for the treatment of cancer by using singlet oxygen, a highly reactive oxygen generated from a photosensitizer drug upon photoactivation. Limited light penetration depth into the tissue means that PDT is unsuitable for deep tissue cancer treatments. This can be overcome by using X-ray/gamma rays activated nanoparticles able to trigger the photosensitizer drug and generate singlet oxygen. Additionally, inorganic nanoparticles interact more strongly with X- or gamma rays than the tissue, allowing to concentrate the effects of radiation near nanoparticle surface and they can also be molecularly targeted to cancer cells.

In this work we synthesized and characterized CeF3 nanoparticles, a well-known scintillator material. The nanoparticles were conjugated with Verteporfin, a photosensitizer drug by electrostatic interaction. We assessed the performance of CeF3 and the conjugates to generate singlet oxygen exposed to X-ray radiation. The X-ray singlet oxygen quantum yield of the nanoparticle-photosensitizer system was accurately quantified for the first time. This provided realistic estimates of the singlet oxygen dose taking into consideration the dose partition of the radiation between CeF3 and the tissue.

Furthermore, we investigated gold nanoparticle-photosensitizer systems. We confirmed that pure gold nanoparticles itself generate singlet oxygen which is attributed to plasmonic effects. We found enhanced singlet oxygen generation from gold-Rose Bengal conjugates and gold nanorod-verteporfin conjugates. These singlet-oxygen-generating nanomaterials add a new dimension to radiation-assisted PDT.

9722-46, Session 6

**VIVIT functionalized nanoparticles for inflammatory diseases treatment** *(Invited Paper)*

Miriam Colombo, Ivan Zanoni, Fabio Corsi, Francesca Granucci, Davide Prosperi, Univ. degli Studi di Milano Bicocca (Italy)

Innate immunity is the most ancient form of response to pathogens and it relies on evolutionary conserved signaling pathways. Nevertheless, increasing evidence suggests that factors that have appeared more recently in evolution, such as the Nuclear Factor of Activated T cell transcription factor family (NFATc), also contribute to innate immune response regulation in vertebrates. It has been recently observed that exposure to inflammatory stimuli, such as LPS, induces the activation of NFATc factors
in innate immune cells, including conventional dendritic cells (DCs), with a mechanism TLR4-independent involving instead CD14. In turn, NFAT contributes to the production of two vasoactive substances, IL-2 and PGE2, which are involved in the initiation of the inflammatory process. Inhibiting the NFAT pathway in innate immune cells is therefore an efficient way to down-modulate inflammation.

A highly specific nanostructured NFAT inhibitor active in the cells of innate immunity has been generated. This inhibitor has proven to be effective in reducing inflammation induced by LPS and other stimuli. The use of finely engineered iron oxide nanoparticles coated by an amphiphilic polymer functionalized with VIVIT peptide, overcomes several problems associated with systemic administration of drugs. In particular, the peptide reversible conjugation with nanoparticles improves the drug bioavailability and the targeting efficiency. VIVIT, which interferes with the activation of NFAT transcription factors, is released selectively in cell cytoplasm and is protected from protease degradation in vivo. VIVIT conjugated nanoparticle effectively interferes with cytosolic NFAT activation resulting in the arrest of inflammation signals.
Mechanism of eliciting host immunity against cancer cells treated with silicaphtalocyanine-based near infrared photoimmunotherapy (Invited Paper)

Hisataka Kobayashi, National Cancer Institute (United States)

Near infrared (NIR) photoimmunotherapy (PIT) is a new type of molecularly-targeted cancer photo-therapy based on conjugating a near infrared fluorophore to a monoclonal antibody (MAb) targeting cancer-specific cell-surface molecules. When exposed to NIR light, the conjugate induces a highly-selective necrotic/immunogenic cell death (ICD) only in receptor-positive, MAb-IR700-bound cancer cells. This cell death occurs as early as 1 minute after exposure to NIR light. Meanwhile, immediately adjacent receptor-negative cells including immune cells are unharmed. Therefore, we hypothesized that NIR-PIT could efficiently elicit host immunity against treated cancer cells. Three-dimensional dynamic quantitative phase contrast microscopy and selective plane illumination microscopy of tumor cells undergoing PIT showed rapid swelling in treated cells immediately after light exposure suggesting rapid water influx into cells, followed by irreversible morphologic changes such as bleb formation, and rupture of vesicles. Furthermore, biological markers of ICD including relocation of HSP70/90 and calreticulin, and release of ATP and High Mobility Group Box 1 (HMGB1), were clearly detected immediately after NIR-PIT. When NIR-PIT was performed in a mixture of cancer cells and immature dendritic cells, maturation of immature dendritic cells was strongly induced rapidly after NIR-PIT. In summary, NIR-PIT can induce necrotic/immunogenic cell death that promotes rapid maturation of immature dendritic cells adjacent to dying cancer cells. Therefore, NIR-PIT could efficiently initiate host immune response against NIR-PIT treated cancer cells growing in patients.

Fluorenyl benzothiadiazole and benzoselenadiazole near-IR fluorescent probes for two-photon fluorescence imaging

Kevin D. Belfield, New Jersey Institute of Technology (United States); Sheng Yao, Bosung Kim, Xiling Yue, Univ. of Central Florida (United States)

Imaging biological samples with two-photon fluorescence (2PF) microscopy has the unique advantage of resulting high contrast 3D resolution subcellular image that can reach up to several millimeters depth. 2PF probes that absorb and emit at near IR region need to be developed. Two-photon excitation (2PE) wavelengths are less concerned as 2PE uses wavelengths doubles the absorption wavelength of the probe, which means 2PE wavelengths for probes even with absorption at visible wavelength will fall into NIR region. Therefore, probes that fluoresce at near IR region with high quantum yields are needed. A series of dyes based on 5-thienyl-2,1,3-benzothiadiazole and 5-thienyl-2,1,3-benzoselenadiazole core were synthesized as near infrared two-photon fluorophores. Fluorescence maxima wavelengths as long as 714 nm and fluorescence quantum yields as high as 0.67 were achieved. The fluorescence quantum yields of the dyes were nearly constant, regardless of solvents polarity. These diazoles exhibited large Stokes shift (>114nm), high two-photon absorption cross sections (up to 2,800 GM), and high two-photon fluorescence figure of merit (FM , 1.04710-2 GM). Cells incubated on a 3D scaffold with one of the new probes (encapsulated in Pluronic micelles) exhibited bright fluorescence, enabling 3D two-photon fluorescence imaging to a depth of 100 μm.

Noninvasive imaging of multiple myeloma using near infrared fluorescent molecular probe

Walter J. Akers, Washington Univ. School of Medicine in St. Louis (United States); Deep Hathi, Haiying Zhou, Washington Univ. in St. Louis (United States); Monica Shokeen, Washington Univ. School of Medicine in St. Louis (United States)

Multiple myeloma is a plasma cell malignancy characterized by monoclonal gamopathy and osteolytic bone lesions. Multiple myeloma is most commonly diagnosed after pathologic fracture, in late disease stages. Early diagnosis and monitoring of disease status will improve quality of life and long-term survival for multiple myeloma patients from what is now a devastating and fatal disease. We have developed a near-infrared targeted fluorescent molecular probe with high affinity to the ?4?1 integrin receptor overexpressed by a majority of multiple myeloma cells as a non-radioactive analog to PET/CT tracer currently being developed for human diagnostics. A near-infrared dye that emits about 700 nm was conjugated two a high affinity peptidomimetic, LLP2A. Binding affinity and specificity for multiple myeloma cells was investigated in vitro by tissue staining and flow cytometry. After demonstration of sensitivity and specificity, preclinical optical imaging studies were performed to evaluate tumor specificity in murine subcutaneous and metastatic multiple myeloma models. The ?4?1-targeted molecular probe showed higher affinity for subcutaneous syngeneic tumors relative to an ?v?3 targeted fluorescent molecular probe. Importantly, tumor cells specific accumulation in the bone-marrow of metastatic multiple myeloma correlated with GFP signal from transfected cells. Ex vivo flow cytometry of tumor tissue and bone marrow further corroborated in vivo imaging data, demonstrating the specificity of the novel agent and potential for quantitative imaging of multiple myeloma burden in these models.

Pyraneryl-based near-IR fluorescent probes for tumor vascular imaging

Kevin D. Belfield, New Jersey Institute of Technology (United States); Xiling Yue, Univ. of Central Florida (United States); Alma R. Morales, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Grace W. Githaiga, Adam W. Woodward, Simon Tang, Univ. of Central Florida (United States); Junci Sawada, Masanobu Komatsu, Sanford-Burnham Medical Research Institute (United States); Xuan Liu, New Jersey Institute of Technology (United States)

A near-infrared dye that emits about 700 nm was conjugated to a monoclonal antibody (MAb) targeting a tumor vascular marker. Near infrared targeted fluorescent molecular probes were designed to report tumor vascularization. A near-infrared dye that emits about 700 nm was conjugated two a high affinity peptidomimetic, LLP2A. Binding affinity and specificity for multiple myeloma cells was investigated in vitro by tissue staining and flow cytometry. After demonstration of sensitivity and specificity, preclinical imaging studies were performed to evaluate tumor specificity in murine subcutaneous and metastatic multiple myeloma models. The ?4?1-targeted molecular probe showed higher affinity for subcutaneous syngeneic tumors relative to an ?v?3 targeted fluorescent molecular probe. Importantly, tumor cells specific accumulation in the bone-marrow of metastatic multiple myeloma correlated with GFP signal from transfected cells. Ex vivo flow cytometry of tumor tissue and bone marrow further corroborated in vivo imaging data, demonstrating the specificity of the novel agent and potential for quantitative imaging of multiple myeloma burden in these models.
Enhanced phosphorescent probes. These molecules brought about first demonstrations of two-photon phosphorescence lifetime microscopy (2PLM) of oxygen in vivo, providing new information for neuroscience and stem cell biology. However, current two-photon oxygen probes suffer from a number of limitations, such as sub-optimal brightness and high cost of synthesis, which dramatically reduce imaging performance and limit usability of the method. In this paper we discuss principles of 2PLM and address the interplay between the probe chemistry, photophysics and spatial and temporal imaging resolution. We then present a new approach to brightly phosphorescent chromophores with internally enhanced two-photon absorption cross-sections, which pave a way to a new generation of 2PLM probes.

9723-7, Session 2

Two-photon fluorescent sensor for K+ imaging in live cells

Binglin Sui, Xiling Yue, Bosung Kim, Univ. of Central Florida (United States); Kevin D. Belfield, New Jersey Institute of Technology (United States)

It is difficult to overstate the physiological importance of potassium for life as its indispensable roles in a variety of biological processes are widely known. As a result, efficient methods for determining physiological levels of potassium are of paramount importance. Despite this, relatively few K+-fluorescence sensors have been reported, with only one being commercially available. A new two-photon excited fluorescent K+-sensor is reported. The sensor is comprised of three moieties, a highly selective K+-chelator as the K+ recognition unit, a boron-dipyrromethene (BODIPY) derivative modified with phenylethynyl groups as the fluorophore, and two polyethylene glycol chains to afford water solubility. The sensor displays very high selectivity (>52-fold) in detecting K+ over other physiological metal cations. Upon binding K+, the sensor switches from non-fluorescent to highly fluorescent, emitting red to near-IR (NIR) fluorescence. The sensor exhibited a good two-photon absorption cross section, 500 GM at 940 nm. Moreover, it is not sensitive to pH in the physiological pH range. Time-dependent cell imaging studies via both one- and two-photon fluorescence microscopy demonstrate that the sensor is suitable for dynamic K+ sensing in living cells.

9723-8, Session 2

NIR and MR imaging supported hydrogel based delivery system for anti-TNF alpha probiotic therapy of IBD

Jelena M. Janjic, Duquesne Univ. (United States); Ales Berlec, Jožef Stefan Institute (Slovenia); Christina Bagia, Lu S. Liu, Duquesne Univ. (United States); Bratislav M. Janjic, Univ. of Pittsburgh (United States); Irena Jeric, Duquesne Univ. (United States); Michael Gach, Washington Univ. School of Medicine in St. Louis (United States); Borut Strukelj, Jožef Stefan Institute (Slovenia) and Univ. of Ljubljana (Slovenia)

Current treatment of inflammatory bowel disease (IBD) is largely symptomatic and consists of anti-inflammatory agents, immunosuppressives or antibiotics, whereby local luminal action is preferred to minimize systemic side-effects. Recently, anti-TNFf therapy has shown considerable success and is now being routinely used. We have previously reported the engineering of food-grade lactic acid bacterium Lactococcus lactis with the ability to bind on its surface TNFf, remove it from the intestine and prevent its availability to proinflammatory cells. Here we present a novel approach of using perfluorocarbon nanoemulsion containing hydrogels as imaging supported delivery systems for anti-TNF alpha probiotic therapy in IBD. The hydrogel composition is designed to allow in vivo monitoring of probiotic delivery to the lower colon and rectum. To further facilitate image guided therapy we engineered a food-grade lactic acid bacterium Lactococcus lactis capable of TNF-f-binding to...
simultaneously expresses infrared fluorescent protein (IRFP). Fluorescent imaging data demonstrates high bacteria loading in the hydrogel and complete release in 3 hours. Stability tests indicate that gels remain stable for at least 14 days showing no significant change in droplet size, zeta potential and pH. Flow cytometry analyses demonstrate the engineered bacteria binds TNFα in vitro upon release from the gel. Magnetic resonance and optical imaging show homogenous distribution of the bacteria in the gels and demonstrate the formulation imaging capacity. Clinical potential is also discussed.

9723-9, Session 2

**Application of fluorescent tracer agent technology to point-of-care gastrointestinal permeability measurement**

Richard B. Dorshow, Jeng-Jong Shieh, Thomas E. Rogers, MediBeacon, LLC (United States); Carla Hall-Moore, Nurmohammad Shaikh, Michael Talcott, Phillip I. Tarr, Washington Univ. School of Medicine in St. Louis (United States)

Gut dysfunction, often accompanied by increased mucosal permeability to gut contents, frequently accompanies a variety of human intestinal inflammatory conditions. These disorders include inflammatory bowel diseases (e.g., Crohn’s Disease) and environmental enteropathy and enteric dysfunction, a condition strongly associated with childhood malnutrition and stunting in resource poor areas of the world. The most widely used diagnostic assay for gastrointestinal permeability is the lactulose to mannitol ratio (L:M) measurement. These sugars are administered orally, differentially absorbed by the gut, and then cleared from the body by glomerular filtration in the kidney. The amount of each sugar excreted in the urine is measured. The larger sugar, lactulose, is minimally absorbed through a healthy gut. The smaller sugar, mannitol, in contrast, is readily absorbed through both a healthy and injured gut. Thus a higher ratio of lactulose to mannitol reflects increased intestinal permeability. However, several issues prevent widespread use of the L:M ratio in clinical practice. Urine needs to be collected over time intervals of several hours, the specimen then needs to be transported to an analytical laboratory, and sophisticated equipment is required to measure the concentration of each sugar in the urine.

In this presentation we show that fluorescent tracer agents with molecular weights similar to those of the sugars, selected from our portfolio of biocompatible renally cleared fluorophores, mimic the L:M ratio test for gut permeability. This fluorescent tracer agent detection technology can be used to overcome the limitations of the L:M assay, and is amenable to point-of-care clinical use.

9723-10, Session 3

**Vibrational imaging of glucose uptake activity in live cells and tissues by stimulated Raman scattering microscopy**

Fanghao Hu, Zhixing Chen, Luyuan Zhang, Yihui Shen, Lu Wei, Wei Min, Columbia Univ. (United States)

Glucose is consumed as an energy source by virtually all living organisms, from bacteria to humans. Its uptake activity closely reflects the cellular metabolic status in various pathophysiological transformations, such as diabetes and cancer. Extensive efforts such as positron emission tomography, magnetic resonance imaging and fluorescence microscopy have been made to specifically image glucose uptake activity but all with technical limitations. Here, we report a new platform to visualize glucose uptake activity in live cells and tissues with subcellular resolution and minimal perturbation. A novel glucose analogue with a small alkyne tag (carbon-carbon triple bond) is developed to mimic natural glucose for cellular uptake, which can be imaged with high sensitivity and specificity by targeting the strong and characteristic alkyne vibration on stimulated Raman scattering (SRS) microscope to generate a quantitative three dimensional concentration map. Cancer cells with differing metabolic characteristics can be distinguished. Heterogeneous uptake patterns are observed in tumor xenograft tissues, neuronal culture and mouse brain tissues with clear cell-cell variations. Therefore, by offering the distinct advantage of optical resolution but without the undesirable influence of bulky fluorophores, our method of coupling SRS with alkyne labeled glucose will be an attractive tool to study energy demands of living systems at the single cell level.

9723-11, Session 3

**Optimization of input parameters of acoustic-transfection for the intracellular delivery of macromolecules using FRET-based biosensors**

Sangpil Yoon, The Univ. of Southern California (United States); Yingxiao Wang, Univ. of California, San Diego (United States); K. Kirk Shung, The Univ. of Southern California (United States)

Engineering cells to modify cellular phenotype and function is an emerging topic for the generation of human induced pluripotent stem cells and gene editing using CRISPR/Cas9. However, the cell membrane is impermeable to macromolecules such as DNA, RNA, and proteins. Viral-vectors, electroporation, and cell-penetrating-peptide have been introduced to overcome this barrier. We have developed an ultrasound-based acoustic-transfection technique to induce the intracellular delivery of macromolecules. Ultrasound-based acoustic-transfection system consisted of an epi-fluorescence microscope and a custom-made high frequency ultrasonic transducer. A high frequency ultrasonic transducer was made of lithium niobate single crystal to have a center frequency of over 150 MHz. A aperture size and a focal distance of the transducer were 1 mm, resulting in focal diameter of less than 10 mm. Acoustic pulses were applied to a target single-cell to generate transient perturbation in cell membrane, through which macromolecule could enter. Acoustic pulses were controlled by four input parameters such as peak-to-peak voltage, pulse duration, pulse repetition frequency, and number of pulses. We have optimized input parameters and monitoring real-time Ca2+ concentration in a target single-cell using fluorescence resonance energy transfer (FRET)-based biosensors, which were previously transfected into HeLa cells. Optimized input parameters allowed the intracellular delivery of macromolecules with a single-cell level targeting capability and low cytotoxicity. The results suggest that the ultrasound-based acoustic-transfection technique is a versatile method for an efficient cargo delivery into a target cell.

9723-12, Session 3

**Water-soluble BIODIPY-based fluorescent probe for mitochondrial imaging**

Binglin Sui, Simon Tang, Adam W. Woodward, Bosung Kim, Univ. of Central Florida (United States); Kevin D. Belfield, New Jersey Institute of Technology (United States)

A new mitochondrial targeting fluorescent probe is designed, synthesized, characterized, and investigated. The probe is composed of three moieties, a BODIPY platform working as the fluorophore, two triphenylphosphonium (TPP) groups serving as mitochondrial targeting moiety, and two long highly hydrophilic polyethylene glycol (PEG) chains to increase its water solubility and reduce its cytotoxicity. As a mitochondria-selective fluorescent probe, the probe exhibits a series of desirable advantages with other reported fluorescent mitochondrial probes. It is readily soluble in aqueous media and emits very strong fluorescence. Photophysical determination experiments show that the photophysical properties of the
probe are independent of solvent polarity and it has high quantum yield in various solvents examined. The probe also has good photostability and pH insensitivity over a broad pH range. Results obtained from cell viability tests indicate that the cytotoxicity of the probe is very low. Confocal fluorescence microscopy colocalization experiments reveal that this probe possesses excellent mitochondrial targeting ability and it is suitable for imaging mitochondria in living cells.

9723-13, Session 3

**Graphene oxide: enhancer of bacterial growth or antimicrobial agent?**

Wen-Shuo Kuo, National Cheng Kung Univ. (Taiwan)

To explore the graphene oxide sheets applied in the field of biomedical nanotechnology, we examined whether graphene oxide-based materials (graphene oxide, graphene oxide-polyoxyalkylenamine, and graphene oxide-chitosan) stimulate or inhibit bacterial growth in detail. We found that it depended on whether the bacteria and graphene oxide-based materials were incubated with a nutrient at the very beginning step, a not being noticed but critical factor to decide the fortune of bacteria. Graphene oxide not only stimulated bacterial growth and microbial proliferation for Gram-negative and Gram-positive bacteria, but also might have augmented surface attachment for both types of bacteria. Once an external barrier composed of graphene oxide-based materials had formed around a bacterial surface, it suppressed nutrients essential to microbial growth and simultaneously produced oxidative stress, which caused bacteria to die, no matter possessing an outer-membrane-Gram-negative bacteria or lacking the outer-membrane-Gram-positive bacteria, even at high concentrations of biocompatible graphene oxide-polyoxyalkylamine. Moreover, because graphene oxide may act as a biofilm, we hypothesized that it would suppress the toxicity of low-dose chitosan.

9723-14, Session 4

**Photoluminescence quantum yields of PbSe and PbS QDs in the range of 1000 nm to 2000 nm (Invited Paper)**

Matthew C. Beard, Octavi E. Semonin, Justin C. Johnson, Ashley Marshall, Jianbing Zhang, Boris D. Chernomordik, National Renewable Energy Lab. (United States)

PbS and PbSe quantum dots (QDs) are promising strong infrared emitters. We have developed several synthetic routes to producing PbS and PbSe QDs with a variety of sizes such that the bandgap can be continuously tuned from 2000 to 1000 nm. We provide a simple and accurate synthetic route to reproducibly produce PbS QDs with a narrow size-distribution and high chemical yield. The different synthetic routes lead to differences in their surface chemistry and to differences in their air stability and photoluminescence quantum yields (PLQY). To characterize the PLQY we directly measured the PLQY IR-26 (a standard IR emitting organic dye) at a range of concentrations as well as the PLQY of PbS and PbSe QDs for a range of sizes. We find that the PLQY of IR-26 has a weak concentration dependence due to reabsorption, with a PLQY of 0.048 ± 0.002% for low concentrations, lower than previous reports by a full order of magnitude. We also find a dramatic size dependence for both PbS and PbSe QDs, with the smallest dots exhibiting a PLQY in excess of 60% while larger dots fall below 3%. A model, including nonradiative transition between electronic states and energy transfer to ligand vibrations, appears to explain this size dependence. These findings provide both a better characterization of photoluminescence for near infrared emitters. Halogen surface passivation provides both a larger PLQY (~ 30% improvement) as well as increased air stability.

9723-16, Session 4

**Setup for the power-dependent absolute quantum yield measurements of luminescent reporters in the VIS and IR spectral region: example of upconversion nanoparticles**

Christian Würth, Martin Kaiser, Marco Kraft, Bundesanstalt für Materialforschung und -prüfung (Germany); Verena Mühr, Stefan Wilhelm, Institut für Analytische Chemie-und Biosensorik (Germany) and Univ. Regensburg (Germany); Thomas Hirsch, Univ. Regensburg (Germany) and Institut für Analytische Chemie-und Biosensorik (Germany); Ute Resch-Genger, Bundesanstalt für Materialforschung und -prüfung (Germany)

Lanthanide-doped up-converting nanoparticles (UCNPs), are promising reporters for bioanalysis and theranostics, which areexcitable in the near infrared (NIR) by multiphoton absorption processes, and show multiple narrow emission bands in the visible (vis) and NIR, excellent photostability, and long luminescence lifetimes in the µs range. The rational design of brighter UCNPs requires an improved understanding of the radiationless deactivation pathways in UCNP, that are affected by size, surface chemistry, and microenvironment. In this respect, we discuss the experimental requirements on absolute measurements of the upconversion (UC) quantum yield (UCQY) and its excitation radiant power density dependence and present the design and characterization of unique integrating sphere setup for such measurements in the vis to IR spectral region including its calibration, the influence of the excitation beam profile and solutions to perform such measurements in aqueous media. Furthermore, we show systematic studies of the influence of UCNP size, surface ligands, and solvent on the photophysical key parameters characterizing the upconversion process such as the excitation power density dependence of UCQY, the up- and downconversion luminescence decay behavior of the different emission bands, the power dependent red-to-green intensity ratio, intensities of the individual emission bands, and their slope factors based on two series of UCNP. Focusing on the influence of the solvent and surface chemistry, we studied UCNPs from one batch modified with different surface ligands in organic solvents, water as well as in deuterium oxide. To demonstrate size effects, we present results from a size series dispersed in cyclohexane and in water.

9723-17, Session 4

**Quantum dot imaging depth and activatable quantum dot cancer probes in the near-infrared second optical window**

Sungjee Kim, Yebin Jung, Sanghwa Jeong, Pohang Univ. of Science and Technology (Korea, Republic of)

Quantum dot (QD) imaging capability was investigated by the imaging depth at the near-infrared second optical window (NIR-II, 1,000 to 1,400 nm) using time-modulated pulsed laser excitations to control the effective fluence rate. QD imaging depths in bovine liver and porcine skin tissues were extended by more than two times when the effective fluence rate was increased from 20 to 2,000 mW/cm². A QD sample was inserted into the abdomen of a live mouse, and the enhanced imaging capability was increased from 20 to 2,000 mW/cm². A QD sample was inserted into the abdomen of a live mouse, and the enhanced imaging capability was increased from 20 to 2,000 mW/cm². A QD sample was inserted into the abdomen of a live mouse, and the enhanced imaging capability was increased from 20 to 2,000 mW/cm².
High quality SWIR emitters enable novel in vivo imaging applications (Invited Paper)

Oliver T. Bruns, Thomas S. Bischof, Daniel Franke, Jessica A. Carr, Mounig Bawendi, Massachusetts Institute of Technology (United States)

The short-wavelength infrared region (SWIR; 1000—2000 nm) provides several advantages over the visible and near-infrared regions for in vivo imaging. The general lack of autofluorescence, low light absorption by blood and tissue, and reduced scattering can render a mouse translucent when imaged in the SWIR region. Despite these advantages, the lack of a versatile emitter platform has prevented its general adoption by the biomedical research community. Here we introduce high-quality SWIR-emitting core/shell quantum dots (QDs) for the next generation of in vivo SWIR imaging. Our QDs exhibit a dramatically higher emission quantum yield (QY) than previously described SWIR probes, as well as a narrow and size-tunable emission that allows for multiplexing in the SWIR region. To demonstrate some of its capabilities, we use this imaging platform to measure the heartbeat and breathing rates in awake mice, as well as to quantify the lipoprotein turnover rates of several organs simultaneously in real time in mice. Finally, we generate detailed three-dimensional quantitative flow maps of brain vasculature by intravital microscopy and visualize the differences between healthy tissue and a tumor in the brain. SWIR QDs enable biological optical imaging with an unprecedented combination of deep penetration, high spatial resolution, and fast acquisition speed.

Novel approach for non-invasive glucose sensing using vibrational contrast CD absorption measurements (Invited Paper)

Vladislav V. Yakovlev, Carlos Tovar, Brett H. Hoker, Georgi I. Petrov, Texas A&M Univ. (United States)

Noninvasive glucose sensing is a Holy Grail of diabetes mellitus management. Unfortunately, despite a number of innovative concepts and a long history of continuous instrumental improvements, the problem remains largely unsolved. Here we propose and experimentally demonstrate the first successful implementation of a novel strategy based on vibrational overtone circular dichroism absorption measurements. Such an approach uses a short-wavelength infrared excitation (1000—2000 nm), which takes the advantage of lower light scattering and intrinsic chemical contrast provided by the chemical structure of D-glucose molecule. We model the propagation of circular polarized light in scattering medium using Monte Carlo simulations to show the feasibility of such approach in turbid medium and demonstrate the proof of principle using optical detection. We also investigate the possibility of using ultrasound detection through circular dichroism absorption measurements to achieve simple and sensitive glucose monitoring.

TCSPC FLIM in the wavelength range from 800 nm to 1700 nm

Wolfgang Becker, Vladislav Shcheslavsky, Becker & Hickl GmbH (Germany)

Excitation and detection in the wavelength range above 800 nm is a convenient and relatively inexpensive way to increase the penetration depth of optical microscopy. Moreover, detection at long wavelengths avoids the problem that tissue autofluorescence contaminates the signals from endogenous fluorescence probes. FLIM at NIR wavelength may therefore be complementary to multiphoton microscopy, especially if the lifetimes of NIR fluorophores report biological parameters of the tissue structures they are bound to. Unfortunately, neither the excitation sources nor the detectors of standard confocal and multiphoton laser scanning systems are directly suitable for excitation and detection of NIR fluorescence. Most of these problems can be solved, however, by using ps diode lasers or Ti:Sapphire lasers at their fundamental wavelength, and NIR-sensitive detectors. With NIR-sensitive PMTs the detection wavelength range can be extended up to 900 nm, with InGaAs SPAD detectors up to 1700 nm. Here, we demonstrate the use of a combination of laser scanning, multi-dimensional TCSPC, and advanced excitation sources and detectors for FLIM at up to 1700 nm. The performance was tested at tissue samples incubated with NIR dyes. The fluorescence lifetimes generally get shorter with increasing absorption and emission wavelengths of the dyes. For the cyanine dye IR1061, absorbing around 1060 nm, the lifetime was found to be as short as 70 ps. Nevertheless the fluorescence decay could still be clearly detected. Almost all dyes showed clear lifetime changes depending on the binding to different tissue constituents.

Ag2S quantum dot: a new fluorescent nanoprobe in the second near-infrared window for in vivo imaging

Qiangbin Wang, Suzhou Institute of Nano-Tech and Nano-Bionics (China)

Fluorescent imaging in the second near-infrared window (NIR-II, 1.0—1.4 µm) is appealing due to minimal autofluorescence and negligible tissue scattering in this region, affording maximal penetration depth for deep tissue imaging with high feature fidelity. Herein, for the first time, we reported a new type of NIR-II QDs-Ag2S QDs and executed a series of bioapplication studies by using Ag2S QDs. The results show that, by using Ag2S QDs, the tissue penetration length can reach 1.2 cm, and the spatial and temporal resolution of the in vivo imaging can down to 25 µm and 50 ms, respectively, which are improved several to dozens of times in comparison with those using conventional fluorescence nanoprobes. Further, this advanced NIR-II fluorescence of Ag2S QDs were employed for high signal to noise ratio detection of tumor in vivo, dynamical monitoring the tumor angiogenesis, real-time stem cell tracking and regeneration in vivo, and imaging-guided surgery of glioma, etc.

Open-source multiprocessor software «KVAZAR» for biomolecular modeling

Anna Kolesnikova, Olga E. Glukhova, Mikhail M. Slepenchekov, Georgy V. Savostianov, N.G. Chernyshhevsky Saratov State Univ. (Russian Federation)

Open-source multiprocessor software «KVAZAR» has been developed. The software «KVAZAR» allows us to investigate the features of dynamic
process in biomacromolecular systems, including
• the self-assembly of a large biomolecular systems;
• the processes of organic and inorganic molecules manipulation on graphene;
• the physical and chemical processes in the intima of the arteries at the atomic and molecular-cellular level;
• the interaction of different proteins with cell membrane;
• the interaction of carbon structures with various biomolecules.

The software realizes molecular modeling methods of several classes, including empirical methods REBO/AIREBO, quantum tight binding method, coarse-grained model MARTINI and classical molecular dynamics. The software «KVAZAR» has a flexible, easily modifiable architecture, which will rapidly increase the capabilities of the software and thereby improve its competitiveness. The developed software package focused on the use of parallel hybrid architecture that combines various technologies of parallel programming (MPI, OpenMP, CUDA), that allows us to reduce several times the simulation time of physical and chemical processes at the atomic and molecular level. The advantage of the software «KVAZAR» in comparison with well-known foreign closed software is that software «KVAZAR» is open-source software. In this regard, the «KVAZAR» has the ability to the adaptation of the approaches, implemented in the program for solving specific scientific problems.

The functional capabilities of software «KVAZAR» are demonstrated in this paper at example of coarse-grained modeling of self-assembly of high-density lipoprotein in water at temperature 310K. According to numerical experiment data this process was completed during 300 ns.

9723-31, Session PMon

Theoretical investigation of interaction between the set of ligands and nicotinic acetylcholine receptor

Olga E. Glukhova, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Tatiana R. Prytkova, Chapman Univ. (United States); Dmitriy S. Shmygin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

Nicotinic acetylcholine receptors (nAChRs) are neuron receptor proteins that provide a transmission of nerve impulse through the synapses. They are composed of a pentameric assembly of five homologous subunits ($\gamma_7$ subunits for $\gamma$nACHr, for example), oriented around the central pore. These receptors might be found in the chemical synapses of central and peripheral nervous system, and also in the neuromuscular synapses. Transmembrane domain of the one of such receptors constitutes ion channel. The conductive properties of ion channel strongly depend on the receptor conformation changes in the response of binding with some molecule, i.e. acetylcholine. Investigation of interaction between ligands and acetylcholine receptor is important for drug design.

In this work we investigate theoretically the interaction between the set of different ligands (such as vanillin, thymoquinone, etc.) and the nicotinic acetylcholine receptor (primarily with subunit of the $\gamma$nACHr) by different methods and packages (AutodockVina, GROMACS, KVAZAR, HARLEM, VMD). We calculate interaction energy between different ligands in the subunit using molecular dynamic method. Based on obtained calculation results and using molecular docking method we found an optimal location of different ligands in the subunit.

9723-33, Session PMon

Predictive modeling of high density lipoprotein behavior on a few layer graphene undergoing nanoindentation by carbon nanotubes

Olga E. Glukhova, George V. Savostyanov, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

The interaction of biomacromolecules with carbon nanostructures arouses interest because of great prospects in the field of biotechnology, targeted drug delivery, the blood-brain barrier overcoming, the delivery of various substances through the membrane cell. In particular, it is established that graphene promotes the formation of vesicles formed by lipid layers and oxidized graphene promotes the self-organization of phospholipids on its surface. In this paper we carried out simulation of high density lipoprotein (HDL) biomacromolecule under external non-uniform mechanical load of a few layer graphene and single-walled carbon nanotube. The non-uniform load was achieved by a few layer graphene on one side and closed single-walled carbon nanotube on the opposite side. The tube was approaching to HDL on graphene deforming it. Two cases with velocities of the tube movement 20 and 5 m/s were considered. Coarse-grained molecular dynamics with application of force field MARTINI were used. All objects were solvated in water medium at T=310 K. As a result of a set of numerical experiment we determine regularities of HDL behavior.

The identification of the HDL behavior regularities under external mechanical loads will allow us to understand the lipoprotein possibility to transform for a passing through narrow channels, including gaps between endothelial cells of internal surface of vessel. So our results will allow us to gain new knowledge also about lipoproteins passing into arterial intima through the gaps between endothelial cells. This problem is very important to identify the mechanisms of occurrence of atherosclerosis.
Theoretical prediction of mutual influence between phospholipid and nanotube during their interaction

Mikhail M. Slepchenkov, Olga E. Glukhova, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

The interaction of carbon nanotubes (CNTs) with cells and microorganisms attracts interest since moment of CNT synthesis in macroscopic volume. Since moment of successful application of CNT as a cantilever needle of the atomic-force microscope the nanodentation of cells by nanotubes became actual. And with it, the interaction of tubes with phospholipid bilayer, which is a key component of the cellular membrane, constitutes the basis of indentation. Using hybrid model QM/MM in this paper we carried out investigation of interaction between phospholipid molecule and carbon nanotube during the indentation of high density lipoprotein (HDL). The object of investigation is armchair carbon nanotube with various diameters range from 0.5 to 1 nm. In a coarse of molecular dynamics study it is found that since the chirality of the tube (7,7) and diameter of 0.9 nm phospholipid partially penetrate into the cavity of the nanotube. However, the entire molecule does not fit into nanospace of tube (7,7), so part of the head and the second phospholipid tail remain outside the carbon nanostructures. Using semi-empirical PM6 method it is established that during the indentation process the charged structured molecule fragments forming the HDL create local electric field near CNT and continuously change electronic structure of CNT. However, the tube is not destroyed because the fields do not exceed the critical values of strength. The redistribution of the electron density on atom is observed in each time point.

A dual function theranostic agent for near-infrared photoacoustic imaging and photothermal therapy

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Theranostic, defined as combining diagnostic and therapeutic agents, has attracted more attention in biomedical application. It is essential to monitor diseased tissue before treatment. Photothermal therapy (PTT) is a promising treatment of cancer tissue due to minimal invasion, unharful to normal tissue and high efficiency. Photoacoustic tomography (PAT) is a hybrid nonionizing biomedical imaging modality that combines rich optical contrast and high ultrasonic resolution in a single imaging modality. The NIR wavelengths, usually used in PAT, can provide deep penetration at the expense of reduced contrast, as the blood absorption drops in the NIR range. Exogenous contrast agents with strong absorption in the NIR wavelength range can enhance the photoacoustic imaging contrast as well as imaging depth. Most theranostic agents incorporating PAT and PTT are inorganic nanomaterials that suffer from poor biocompatibility and biodegradability. Herein, we present a benzol[1,2-c;4,5-c’]bis[1,2,5] thiadiazole, based theranostic agent which not only acts as photoacoustic contrast agent but also a photothermal therapy agent. Experiments were performed on animal blood and organic nanoparticles embedded in a chicken breast tissue using PAT imaging system at ~ 803 nm wavelengths. Almost ten time contrast enhancement was observed from the nanoparticle in suspension. More than 6.5 time contrast enhancement was observed in tissue at 3 cm depth. Hela cell lines was used to test photothermal effect showing 90% cells were killed after 10 min laser irradiation. Our results indicate that the BBT - based nanoparticles are promising theranostic agents for PAT imaging and cancer treatment by photothermal therapy.

Synthesis and spectroscopic evaluation of PbS quantum dots emitting at 1300 nm for optimized imaging in optical window II

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Contrast agents for optical imaging have traditionally been designed for use in the near-infrared (NIR) spectral range (700-900 nm, Optical Window I) where absorption and scattering of tissue are relatively low. Recent advances in imaging instrumentation and understanding of tissue optical properties have revealed another window beyond 1000 nm known as Optical Window II or the extended Near Infrared (exNIR) which possesses improved tissue transparency. In this work, we present a method to synthesize highly fluorescent quantum dots emitting at 1300 nanometers, the optimal wavelength for tissue transparency. The quantum dots were synthesized in organic solvents, and a method of transforming them into water using two different mercapto-acids is discussed. Optical characterizations such as absolute quantum yield and the fluorescence lifetime are presented.

High specificity ZnO quantum dots for diagnosis and treatment in bacterial infection

Min Zhang, China Pharmaceutical Univ. (China); Zhiyu Qian, Nanjing Univ. of Aeronautics and Astronautics (China); Yueqing Gu, China Pharmaceutical Univ. (China)

The significant mortality and morbidity made bacterial infection to be an arising medical and public concern worldwide, which made early infection diagnosis and effective antibiotic importantly. In this work, we developed a fluorescent nano-probe MPA@ZnO-PEP by conjugating SiO2-stabilized ZnO quantum dot (ZnO@SiO2) with an bacteria-targeting peptide, and capped with MPA, a near infrared (NIR) dye. The nanoprobe MPA@ZnO-PEP exhibited excellent fluorescence property with the emission of 560 nm and 780 nm, and could discriminate the bacterial infection from sterile inflammation with high specificity both in vitro and in vivo. The biocompatibility of MPA@ZnO-PEP was verified by MTT assay. By decorating MPA@ZnO-PEP with a kind of antibiotic methicillin, a theranostic nanoparticle MPA/Met@ZnO-PEP was successfully developed, which exhibited enhanced antibacterial activity. More importantly, MPA/Met@ZnO-PEP demonstrated significant improved capability to combat with the anti-methicillin-resistant-bacteria (MRSA) infection, as a result of the increased cell membrane permeation mediated by MPA/ZnO@SiO2-PEP. These results suggest that the as-prepared MPA/ZnO@SiO2-PEP holds great potential to realize efficient non-invasive early diagnosis of bacterial infections, providing important guiding information for treatment, and can be employed as promising candidates for drug carriers of effective bacterial-targeting therapy.

Real time detection of bcl-2 mRNA expression in living cell using gold nanoparticle beacon

Qiumei Zhou, China Pharmaceutical Univ. (China); Zhiyu
Bcl-2 protein, one of the anti-apoptotic members in Bcl-2 family proteins, has a close relationship with drug resistance of tumor cells. Bcl-2 mRNA expression is a promising biomarker for the prediction of drug-resistance. However, the traditional technical process in clinic is complicated and cannot perform the real-time detection of mRNA in living single cells. In the present study, the expression of Bcl-2 mRNA was analyzed based on gold nanoparticle beacon in tumor cells. Firstly, gold nanoparticles was synthesized and optimized. The Bcl-2 beacon sequence was screened using RNA structure 5.2 software and ascertained it based on BLAST, and the hairpin DNA modified by fluorescein isothiocyanate (FITC) at the 5’end and thiol at the 3’end was synthesized. Then, Bcl-2 molecular beacon were characterized by UV-vis and fluorescence spectroscopy. The cytoxicity of Bcl-2 molecular beacon on L02, K562 and K562 /Adr cells were investigated by MTT assay. The result suggested that Bcl-2 molecular beacon was low inherent cytoxicity. Finally, Laser scanning confocal microscope and Flow Cytometry data showed that the quenching efficiency was up to 90%, which was consistent with the results of qRT-PCR measurement and Western Blot. In summary, Bcl-2 molecular beacon designed in this study is a reliable agent for detection Bcl-2 mRNA expression in living tumor cells, a promising strategy for in guiding patient treatment and management in individualized medication.

Hyperspectral imaging in shortwave infrared: from stain-free microscopy to deep tissue imaging (Invited Paper)

Mikhail Y. Berezin, Washington Univ. School of Medicine in St. Louis (United States)

Recent advances in relatively unexplored short wave infrared (SWIR) range from 800-1600 nm detectors make wide-field imaging in this spectral range attractive to biology. The distinct advantages of SWIR region over the visible and near infrared (NIR) in tissue analysis are two-fold: (i) high abundance endogenous chromophores (i.e. water and lipids) enable tissue component differentiation based on wavelength-dependent absorption properties and (ii) the weak scattering of tissue permits better resolution of imaging in thick specimens. When combined with high spectral resolution, SWIR imaging produces a spectroscopic image, where every pixel corresponds to the entire high-resolution spectrum. This hyperspectral (HS) approach provides rich information about the relative abundance of individual chromophores and their interactions that contribute to the intensity and location of the optical signal. The presentation discusses the challenges in the SWIR-HS instrument design and data analysis and demonstrates some of the promising applications of this technology in life science and medicine.

Golden optical window III from 1600 to 1870 for deep imaging of brain and other tissues (Invited Paper)

Lingyan Shi, Laura A. Sordillo, Adrian Rodriguez-Contreras, Robert R. Alfano, The City College of New York (United States)

This presentation introduces three new optical windows in the NIR and demonstrates their potential for deep brain tissue imaging with contrast agents and compared them with the conventional therapeutic window from 650 nm to 950 nm. The “Golden Optical tissue window II” from 1600 to 1870 has the longest penetration length. In the past, the near-infrared (NIR) radiation has been employed using one- and two-photon fluorescence imaging for probes at wavelengths from 650 to 950 nm (optical tissue window I) for deep tissue imaging; however, the longer wavelengths in NIR have been overlooked due to a lack of suitable NIR imaging detectors. This research describes and introduces three new optical windows in the NIR and demonstrates their potential for deep brain tissue imaging using proper probes. The transmittances and the attenuation lengths are measured in rat brain tissue in the second (II, 1100-1350 nm), third (III, 1600-1870 nm), and fourth (IV, centered at 2200 nm) NIR optical tissue windows. The relationship between transmittances and tissue thickness is measured and compared with the ballistic and diffusion theory. Due to a reduction in scattering from Mie scattering in NIR and minimal absorption, window III is shown to be the best for deep imaging for one , two and three photon imaging, and window II and IV show similar better potential for deep imaging than window I.

Absolute fluorescence measurements > 1000 nm: setup design, calibration and standards (Invited Paper)

Ute Resch-Genger, Christian Würth, Jutta Pauli, Soheil Hatami, Martin Kaiser, Bundesanstalt für Materialforschung und -prüfung (Germany)

There is an increasing interest in optical reporters like semiconductor quantum dots and upconversion nanophosphors with emission > 1000 nm for bioanalysis, medical diagnostics, and safety barcodes and hence, in reliable fluorescence measurements in this wavelength region, e.g., for the comparison of material performance and the rational design of new nanomaterials with improved properties [1-4]. The performance of fluorescence measurements > 800 nm and especially >1000 nm is currently hampered by the lack of suitable methods and standards for the simple determination of the wavelength-dependent spectral responsivity of fluorescence measuring systems and the control of measured emission spectra and intensities [3-5]. This is of special relevance for nanocrystalline emitters like quantum dots and rods as well as for upconversion nanocrystals, where surface states and the accessibility of emissive states by quenchers largely control accomplishable quantum yields and hence, signal sizes and detection sensitivities from the reporter side. Here, we present the design of an integrating sphere setup for the absolute measurement of emission spectra and quantum yields in the wavelength region of 650 to 1600 nm and its calibration as well as examples for potential fluorescence standards from different reporter classes for the control of the reliability of such measurements [5]. This includes new spectral fluorescence standards for the wavelength region of 650 nm to 1000 nm as well as a set of quantum yield standards covering the wavelength region from 400 nm to 1000 nm.

Nanodiamond as a multi-role fluorescent marker for bioimaging

Brian R. Patton, Martin J. Booth, Univ. of Oxford (United Kingdom)

Nanodiamond has shown much promise as a fluorescent marker for bioimaging applications. Its size, biocompatibility and ability to be functionalised make it suitable for a wide range of imaging modalities and sample types. In addition, defects such as the nitrogen vacancy are sensitive to electric and magnetic fields, opening the possibility of in vivo measurement of biologically generated fields at the cellular scale. In order to implement nanodiamond biosensing, it is important to target the nanoparticles to structures of interest, image them efficiently and perform quantum optical measurements to correlate emission with the local electromagnetic environment. We are developing microscopes that will allow superresolution imaging of individual nanodiamonds deep in tissue.
to correctly locate them in respect to the local anatomy and subsequently perform sensing measurements. We will present here the current status of our research programme which aims to utilise the NDs to image neural structures and activity in fruit fly (Drosophila Melanogaster) brains. In particular, we are interested in the improvements to imaging quality, sample viability and field-sampling rate that can be achieved by incorporating adaptive optics in our microscopes. By using spatial light modulators or deformable mirrors we can correct the wavefront distortion induced by the optically inhomogenous tissue we wish to image through. We will demonstrate the improvement in signal to noise and resolution that can be achieved when imaging with aberration-corrected superresolution techniques in fly tissue.

9723-26, Session 8
Fluorescent silica nanoparticles containing covalently bound dyes for reporter, marker and sensor applications
Gabor Patonay, Maged M. Henary, Gala Chapman, Kyle Emer, Georgia State Univ. (United States)
Silica nanoparticles have proven to be useful in many bioanalytical and medical applications. Combining the properties of silica nanoparticles and fluorescent dyes that may be used as chemical probes is relatively easy by simply soaking silica nanoparticles in a solution of the dye of interest. Under proper conditions the entrapped dye can stay inside the silica nanoparticle for several hours resulting in a useful probe. In spite of the relative durability of these probes, leaching can still occur. A much better approach is to synthesize silica nanoparticles that have the fluorescent dye covalently attached to the structure of the silica nanoparticle. This can be achieved by using modified TEOS during the synthesis. The molar ratio of TEOS and modified TEOS will determine the fluorescent dye load in the silica nanoparticle. Dependent on the chemical stability of the reporting dye either the reverse micellar (RM) of the Stroeber method can be used for silica nanoparticle synthesis. If dye stability allows the RM is preferred as it results in a much easier control of the silica nanoparticle reaction itself. Dependent on the functional groups present in the reporting dye to be used to prepare the modified TEOS and its spectral properties, the silica nanoparticle can be used for many applications such as pH sensor, metal ion sensors, etc. In addition surface activated silica nanoparticles with reactive moieties are also excellent reporters or they can be used as bright fluorescent labels. Many different fluorescent dyes can be used to synthesize silica nanoparticles including NIR dyes. Several bioanalytical applications will be presented, probes that can be used in vivo or in vitro and studying amoeba phagocytosis.

9723-27, Session 8
Synthesis and energy transfer within carbon-based fluorescent rare earth nanoparticles and nanocomposites
Brian G. Yust, Mircea Chipara, Aaron Saenz, The Univ. of Texas Rio Grande Valley (United States)
Recently, there has been a great deal of interest in fluorescent and upconverting rare earth-based nanoparticles for biomedical imaging and photodynamic therapy applications. While many of the widely explored upconverting contrast agents are comprised of fluoride or oxide crystal structures, very little work has been done to investigate the up- and downconversion emission in rare earth-doped carbon nanocomposites. Of particular interest, graphene-UCNP nanocomposites and sesquicarbide nanoparticles may offer a wide range of new applications when coupled with the extraordinary optical properties of rare earth-doped systems, such as potential use as nano-transducers. Carbon-based nanocomposites and sesquicarbores doped with rare earth elements were synthesized using the microwave and solvothermal methods with additional brief high temperature heat treatments. They were then characterized by XRD, visible and NIR excitation and emission spectroscopy, as well as Raman spectroscopy. Tuning of the emission manifold ratios was explored through different compositions and size. Also, energy transfer between the emitting ions and the electronic states of the host structure was explored. Finally, cytotoxicity was tested, and cellular uptake of these nanomaterials was performed with confocal microscopy.

9723-28, Session 8
Nanoparticle-enhanced x-ray therapy for cancer
Renat R. Letfullin, Rose-Hulman Institute of Technology (United States); Colin E. W. Rice, Univ. of Minnesota, Twin Cities (United States); Thomas F. George, Univ. of Missouri-St. Louis (United States)
Photothermal therapies of nanophotohyperthermia and nanophotothermolysis utilize light absorbptive 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 a 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 applications, 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: (1) The high penetration depth of X-rays through biological media makes non-invasive treatments very feasible. (2) 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. (3) X-ray sources are already common throughout the medical industry, making future implementation on existing equipment possible. This paper uses the 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.

9723-29, Session 8
Anisotropic silver nanoparticles: sorption and desorption of cationic porphyrins
Anna G. Gyulkhandanyan, Anna A. Zakoyan, Institute of Biochemistry (Armenia); Robert K. Ghazaryan, Yerevan State Medical Univ. (Armenia); Aram G. Gyulkhandanyan, Institute of Biochemistry (Armenia); Marina A. Sheyryan, Yerevan State Univ. (Armenia); Grigor V. Gyulkhandanyan, Institute of Biochemistry (Armenia)
The main mechanism for enhancing of photodynamic inactivation (PDI) of microorganisms is increasing the local concentration of the photosensitizer in the immediate vicinity of bacterial cell wall by using nanoparticles. Among many types of nanoparticles-nancontainers used to enhance the effectiveness and targeted delivery of photosensitizers, nanoparticles of anisotropic silver occupy a special place. They possess a number of valuable and rare qualities: special optical properties caused by surface plasmon resonance, a highly developed surface, catalytic activity, a
high-capacity of electric double layer and others, as well as a unique antimicrobial and antiviral properties. To achieve a maximum reinforcement of PDI of microorganisms it is necessary desorption of porphyrins from nanoparticles. We have studied interaction of cationic porphyrins and Zn-metalloporphyrins with different peripheral groups and have shown that the formation of nanocomposites is mainly due to the electrostatic interaction between the positively charged groups of porphyrins with the surface of anisotropic nanoparticles of silver (binding more than 70%). By absorption and fluorescence spectroscopy has shown that under the action of monovalent and divalent salts (NaCl and CaCl2) desorption of porphyrins and metalloporphyrins from silver nanoparticles completely occurs, whereas the action of different intensity of light on desorption is not affected. Sequential titrations of solutions of nanocomposites by salts (0.05 to 0.5 M) showed that complete desorption of porphyrins occurs at concentration of 0.2 M NaCl, whereas for CaCl2 at concentration 0.1 M. The efficiency and perspectivity usage of such nanocomposites for PDI of Gram (+) and Gram (-) microorganisms is shown.
Plasmonic nanostars can be functionalized with a range of heterobifunctional maleimide-PEGs (e.g., density PEGylation (1-5 PEG molecules/nm²). Furthermore, SiGNS probes afford diverse surface chemistry modifications, as we have demonstrated multiplexing capacity of up to nine resonant and non-resonant SERS reporters (785 nm excitation laser). Additionally, our SiGNS probes allow for the detection of multiple genetic leukemia biomarkers.

9724-3, Session 1
Simultaneous detection of multiple biomarkers by means of SERS on polymer nanopillar gold arrays

Carlo F. Morasso, Silvia Picciolini, Dora Mehn, Fondazione Don Carlo Gnocchi (Italy); Paola Pellacani, Plasmore S.r.l. (Italy); Gerardo R. Marchesini, Biosypher Ltd. (United Kingdom); Renzo Vanna, Alice Gualerzi, Marzia Bedoni, Fondazione Don Carlo Gnocchi (Italy); Franco Marabelli, Univ. degli Studi di Pavia (Italy); Furio Gramatica, Fondazione Don Carlo Gnocchi (Italy)

In the field of cancer research there is an increasing need for highly sensitive, accurate and reproducible technologies which would allow the detection of very low concentrations of biomarkers associated to the onset of a disease and the responsiveness to therapy. Surface-Enhanced Raman Spectroscopy (SERS) is emerging as a very promising tool for its higher sensitivity, specificity and better multiplexing capability compared to the conventional analytical methods.

Here, we present a new sensor based on the use of SERS on a specifically designed 2D solid substrate, in order to improve the stability of the system and to obtain a regularly distributed array of hot spots.

In our study, a nanostructured surface made of polymeric pillars embedded in a gold layer is tested in a biochemical assay for the simultaneous detection of multiple genetic leukemia biomarkers.

The special fabrication process combining soft lithography and plasma deposition techniques allowed us to tailor the structural parameters of the crystal surfaces to tune the plasmonic resonance spectral position close to the excitation wavelength of the light source maximizing the enhancing properties of the substrate.

On the optimized surface, the sensor was built as a sandwich assay between the surface, functionalized with thiol-modified oligonucleotides, and gold nanoparticles, labeled with different Raman reporters. Thanks to the combination of the favorable properties of our SERS substrate and the use of nanoparticles, we were able to detect simultaneously a leukemia biomarker (WT1 gene) and a housekeeping gene with low picomolar sensitivity.

9724-4, Session 1
Plasmonic nanostructures for bioanalytical applications of SERS

Mehmet Kahraman, Gaziantep Üniv. (Turkey); Sebastian Wachsmann-Hogiu, Univ. of California, Davis (United States)

Surface-enhanced Raman scattering (SERS) is a potential analytical technique for the detection and identification of chemicals and biological molecules and structures in the close vicinity of metallic nanostructures. We present a novel method to fabricate tunable plasmonic nanostructures and perform a comprehensive structural and optical characterization.
of the structures. Spherical latex particles are uniformly deposited on glass slides and used as templates to obtain nanovoid structures on polydimethylsiloxane surfaces. The diameter and depth of the nanovoids are controlled by the size of the latex particles. The nanovoids are coated with a thin Ag layer for fabrication of uniform plasmonic nanostructures. Structural characterization of the surfaces is performed by SEM and AFM. Optical properties of these plasmonic nanostructures are evaluated via UV/Vis spectroscopy, dark field microscopy, and SERS. The sample preparation step is the key point to obtain strong and reproducible SERS spectra from the biological structures. When the colloidal suspension is used as a SERS substrate for the protein detection, the electrostatic interaction of the proteins with the nanoparticles is described by the nature of their charge status, which influences the aggregation properties such as the size and shape of the aggregates, which is critical for the SERS experiment. However, when the solid SERS substrates are fabricated, SERS signal of the proteins that are background free and independent of the protein charge. Pros and cons of using plasmonic nano colloids and nanostructures as SERS substrate will be discussed for label-free detection of proteins using SERS.

9724-5, Session 1
SERS sensing of sub-nanoliter analyte on diatom biosilica using inkjet printing
Yuting Xi, Xianming Kong, Paul LeDuff, Gregory L. Rorrer, Alan X. Wang, Oregon State Univ. (United States)

Diatoms are photosynthetic micro-organisms that create their own skeletal shells of hydrated amorphous silica, called frustules, which is a kind of inexpensive nature resource and exhibit hierarchical nano-scale photonic crystal features. In our previous work, we have demonstrated that diatom biosilica with self-assembled plasmonic nanopaticles can be used as ultra-sensitive, low-cost substrates for surface-enhanced Raman scattering (SERS) sensing. The enhancement comes from the periodic pores of diatom frustules that could enhance localized surface-plasmon resonances of Ag NPs. Interestingly, we find that that each diatom frustule has a dimension around 10-20 μm and can be used as a miniaturized biosensor due to the unique morphology and nanofluidic channel effect of the frustule. To take advantage of such nature-created micro-scale biosensors, we develop a novel strategy to dispense sub-nanoliter volume of analyte into each individual frustule by inkjet printing, which provides the possibility of accurate point-of-detection and miniature amount of analyte consumption. In our experiment, each analyte droplets can be controlled within 50 picoliter and multiple droplets can be precisely delivered to the same frustule within a few seconds. Our experimental results show up to 4x higher SERS signals of DTNB compared with those from conventional colloidal SERS substrates, which provides the possibility of on-chip multiplex biosensing.

9724-7, Session 1
Gold nanostructuring by soft UV-nanoimprint lithography for surface-enhanced Raman scattering applications: directional and enhancement localization properties
Jean-François Bryche, Lab. Charles Fabry (France) and Univ. Paris-Sud 11 (France); Raymond Gillibert, Univ. Paris 13 (France) and HORIBA Jobin Yvon (France) and Lab. Charles Fabry (France); Mitreadeep Sarkar, Lab. Charles Fabry (France) and Institut d’Optique Graduate School (France); Grégory Barbillon, Institut d'Électronique Fondamentale (France) and Univ. Paris Sud 11 (France); Ryohei Yasukuni, Univ. Paris 13 (France); Aurore Olivéro, Lab. Charles Fabry (France) and HORIBA Jobin Yvon (France) and Univ. Paris 13 (France); Frédéric Hamouda, Univ. Paris-Sud 11 (France) and Institut d’Électronique Fondamentale (France); Mondher Besbes, Lab. Charles Fabry (France) and Institut d’Optique Graduate School (France); Julien Moreau, Institut d’Optique Graduate School (France) and Lab. Charles Fabry (France); Marc Lamy de la Chapelle, Univ. Paris 13 (France); Bernard Bartenlian, Institut d’Électronique Fondamentale (France) and Univ. Paris-Sud 11 (France); Michael T. Canva, Lab. Charles Fabry (France) and Univ. de Sherbrooke (Canada)

Biological detection needs large nanostructured areas and Soft UV-Nanoimprint Lithography can provide it at low cost and for mass production. Gold nanodisks arrays were obtained by this technique with diameters varying from 100 to 500 nm with a periodicity of 400 and 600 nm. SERS intensity was investigated and performed at three different wavelengths (633, 660 and 785 nm). Directional SERS measurements (depending of incidence angle of light) demonstrated that the SERS intensity increased up to an order of magnitude when Bragg mode is excited and is confirmed by simulations. Surface localization of the molecules is also studied.

9724-6, Session 1
SERS detection and targeted ablation of lymphoma cells using functionalized Ag nanoparticles
Qian Yao, Fei Cao, Beijing Univ. of Technology (China); Chao Feng, Beijing University of Technology (China); Yan Zhao, Xiuhong Wang, Beijing Univ. of Technology (China)

Non-Hodgkin’s lymphoma (NHL), a heterogeneous group of malignancies of the lymphoid tissue, is a prevalent cancer worldwide with a high mortality rate. Because most NHLs are of B-cell origin, the detection and identification of CD20, a kind of the B-cell surface antigens, is crucial for the immunotherapies of lymphoma. However, traditional detection technologies, such as fluorescence and chemiluminescence, are often relatively time-consuming and not suitable for the detection of living cells or tissues. As an optical detection technique, surface-enhanced Raman scattering (SERS) has the potential to mitigate these shortfalls. SERS offers many advantages, including sensitivity, high levels of multiplexing, robustness and ability to perform detection in blood and other biological matrices. Up to date, several SERS probes have been successfully demonstrated for targeting cancer cells, including lymphoma cells.

In this presentation, we designed and synthesized a multifunctional SERS nanoprobes, which can target, detect and eliminate lymphoma cells in vitro within one single platform. Ag nanoparticles were first modified with p-mercaptobenzoic acid, followed by association of anti-lymphoma drug Rituximab. The p-mercaptobenzoic acid molecule can function both as a SERS reporter and a linker. Since Rituximab is an antibody drug that specifically targets CD20(+) B cell lymphoma cells, the such designed nanoprobe can specifically target and ablate CD20(+) lymphoma cells, meanwhile it also allows SERS detection of lymphoma cells. Our preliminary results indicated that the nanoprobe retained the antibody activity of Rituximab by ADCC assay. It effectively inhibited the growth of human Burkitt’s Lymphoma Raji and Daudi cells. It also allows single cell detection of live lymphoma Raji or Daudi cell. This nanoprobe holds great potential for non-invasive detection and ablation of lymphoma cells in vivo.

9724-8, Session 2
New avenues for confocal surface plasmon microscopy (Invited Paper)
Mike G. Somekh, Suejit Pechprasarn, Shen Hong, Wai-Kin
Plasmonics in Biology and Medicine XIII

Chow, Jingkai Meng, The Hong Kong Polytechnic Univ. (Hong Kong, China)

We have previously demonstrated the use of a defocused confocal microscope for measuring the properties of surface plasmons on a localized scale.

In this paper we discuss some recent advances in this technology. We demonstrate how the system can operate as an interferometer so that both the amplitude and phase of the surface plasmon can be measured. We present results that show that by illumination part of the microscope back focal plane with a spiral phase distribution we can produce an interferometer which removes the need for phase stepping and can recover the amplitude and phase of the surface plasmon in a single shot. We show how the non-confocal microscope can be used to perform sensitive measurement of multipoint real time binding on a microscopic scale.

In addition to modifications of the microscope itself we construct different structures to support surface waves with both TE and TM incident polarization. We show how we can switch between these polarization states and demonstrate how they have different properties. We present results to show how the different surface wave modes are sensitive to the dipole orientation of materials deposited on the surface and argue that this provides a powerful means to perform local measurement of structure.

9724-9, Session 2

Plasmonic engineering of near-fields attains ultrasensitive super-resolved molecular detection (Invited Paper)

Donghyun Kim, Yonsei Univ. (Korea, Republic of)

Surface-enhanced nanoplasmonic structures can create locally amplified electromagnetic near-fields as a consequence of evanescent field localization on metallic substrates. The creation of localized fields has been investigated in many past works of research because the approach allows the potential to improve detection sensitivity and resolving power in various molecular sensing and imaging applications. Use of plasmonic field localization is described for label-free biosensing based on colocalization of near-field and target distribution. Also described is the improvement of resolving power that has drawn tremendous attention for imaging molecular processes typically impossible to observe under diffraction limit. While emerging approaches have been extremely successful to produce super-resolved images, we explore alternative approaches based on nanoplasmonics by which achievable resolution may be customized to fit the specific imaging needs and at the same time a conventional optical system may be used. Feasibility studies performed on visualizing internalization of virus particles, sliding microtubules and bacterial motility on random and periodic nanopatterns will be presented. Enhancement of axial resolution for the detection of intracellular protein distribution is also reported by extraordinary light transmission using graded plasmonic nanoapertures.

9724-10, Session 2

Near-, middle-, and far-field dipolar interactions in gold nanoparticle arrays

Vira V. Kravets, Anatoliy O. Pinchuk, Univ. of Colorado at Colorado Springs (United States)

The resonance wavelengths of coherent electron oscillations in chains of gold 50 nm diameter nanoparticles are calculated. Results are compared to the experimental values, extracted from the particles light extinction spectra. Distances between the particles in the chain are varied from 100 to 1000 nm, and the incident light polarized in plane of the sample is gradually changed from parallel to perpendicular to the chain of the particles. Our calculations are based on the near-, middle-, and the far-field dipole interactions. Such theoretical model is different from the most widely-used two-component (near- and far-field) interaction model, and as we found describes our experiment with less than 2% error.

Arrays of noble metal nanoparticles are of interest for plasmonics, nanonanics, photovoltaics, and biochemical applications. They are widely used as biosensors and molecular rulers. Over the last decade, interest has turned towards the localized surface plasmon resonance (LSPR) in single-nanoparticle sensors. Benefits of such an approach include simplicity (it does not require momentum-matching geometry), versatility on the nanoscale level, and the possibility of single-molecule detection. While single-nanoparticle sensors offer a better sensitivity down to single protein-receptor binding, a high degree of sensor miniaturization tends to result in a worse detection limit in terms of surface coverage. A solution lies in the use of an array of nanoplasmonic sensors, each of which is capable of resolving single protein binding events. Present study provides a background for biosensing and molecular ruler applications.

9724-11, Session 2

Detecting rare CA125-immune cell binding in ovarian cancer using plasmonic gold nanoparticles

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Ovarian cancer, if found early, is considered a highly survivable disease, with a 5-year survival rate greater than 90%. Frustratingly, however, over 70% of patients are diagnosed at advanced stages with less than 30% of patients surviving beyond 5 years. While increasing evidence has emerged suggesting an immunomodulatory effect of CA125, these studies have been hampered by both the low level of CA125-immune cell binding events (100-500 events/cell), as well as the endogenous autofluorescence from immune cells. In this work, we describe a plasmonic gold nanoparticle (AuNP) based imaging toolkit to detect and quantify low levels of CA125-immune cell binding in whole blood. AuNPs were functionalized with anti-CA125 antibodies, and their enhanced light scattering and immune-cell binding was captured using a high-resolution dark-field imaging system. CA125-immune cell binding densities were evaluated through spectral color shifts as a result of AuNP plasmon coupling. With the addition of immune cell lineage fluorescent markers, we were also able to simultaneously identify which immune cell subtypes bound CA125. The presented toolkit will be used to evaluate patient samples to determine the correlation of CA125-immune cell binding and patient diagnosis. We anticipate the findings of this work to have a major impact on the current state of disease monitoring and open the door for improved early detection methods for ovarian cancer.

9724-12, Session 2

Photothermal inactivation of bacteria on plasmonic nanostructures

Greggy M. Santos, Felipe Ibanez, Fusheng Zhao, Debora Rodrigues, Wei-Chuan Shih, Univ. of Houston (United States)

The risks of contracting a hospital-acquired (nosocomial) infection can be as high as 15.5%. Among the major factors that can contribute to the risk of bacterial infection are the type of patient’s disease, surgical procedures, and urinary tract infections (use of catheters). The most important trait of a disease-causing bacterium, responsible for the nosocomial infections,
is its antibiotic resistance. This adaptation allows the bacteria to survive treatment with antibiotics and, frequently, cause death to patients. Considering pathogenic bacteria continuously evolve to be resistant to an increasing number of commercial antibiotics, it is essential to propose reliable and instantaneous methods to inactivate bacteria. Exposure to high temperatures is a well-known method to inactivate microorganisms. This method is commonly used to sterilize laboratory materials and medical instrumentation. High temperatures are achieved by using conventional sterilization equipment, such as dry ovens or autoclaves. These methods are proven effective, but require longer sterilization durations (minutes to hours) to completely inactivate microorganisms. In this paper, we demonstrate the inactivation of bacteria on nanoporous gold disks (NPGD). Upon excitation with incident light, the localized surface plasmon resonance (LSPR) of the NPGD enhances the local electric field within its nanoporous networks. The LSPR results in radiative and non-radiative processes that have been utilized for a wide range of applications such as Raman and fluorescence enhancement and photothermal molecular release. It was shown that the irradiated NPGD are excellent photothermal agents for heating the surrounding media and can inactivate various types of bacteria within 1 minute.

9724-13, Session 3

**Wavelength-scanning surface plasmon resonance imaging for real-time detection of biomolecular interactions in parallel (Invited Paper)**

Junle Qu, Yonghong Shao, Shenzhen Univ. (China)

No Abstract Available

9724-14, Session 3

**Bimodal instrumentation to improve plasmonic biodetection**

Aurore Olivéro, Lab. Charles Fabry (France) and HORIBA Scientific (France); Julien Moreau, Lab. Charles Fabry (France); Jean-François Bryche, Lab. Charles Fabry (France) and Institut d’Électronique Fondamentale (France); Raymond Gillibert, Chimie, Structures, Propriétés de Biomatériaux et d’Agents Thérapeutiques (France) and HORIBA Scientific (France); Mitradeep Sarkar, Lab. Charles Fabry (France); Grégory Barbillon, Institut d’Électronique Fondamentale (France); Emmanuel Maillart, HORIBA Scientific (France); Marc Lamy de la Chapelle, Chimie, Structures, Propriétés de Biomatériaux et d’Agents Thérapeutiques (France); Bernard Bartonlian, Institut d’Électronique Fondamentale (France); Michael T. Canva, Lab. Charles Fabry (France) and Univ. de Sherbrooke (Canada)

To address major health concerns as the early diagnosis of cancer or the prevention of food contamination, diseases that affect millions of people worldwide, the need to detect trace molecules has subsequently emerged. A powerful way to perform biomolecular detection in real time and at a high throughput resides in the method of Surface Plasmon Resonance imaging (SPRI). Yet, reaching trace molecule detection implies pushing forward the current limits of label-free biosensing techniques that are particularly restrained by non-specific interactions due to the biofunctionalization of the samples.

To overcome the issue of non-specificity in order to reveal the presence of molecules at a very low concentration in complex media, we designed a unique bimodal optical instrument coupling SPRi to Surface Enhanced Raman Scattering (SERS) on a common nanostructured substrate. The adding of SERS characterization of molecules firstly captured and quantified by SPRI provides the spectral footprint of the molecular targets, thus identifying them without ambiguity.

The nanostructured substrate used simultaneously for SPRi and SERS is composed of an array of gold nanocylinders deposited on a continuous gold film. Such a substrate supports a new hybrid plasmonic mode disclosed by our numerical simulations and experimentally characterized. We proved that this hybrid mode improves biodetection by creating a better confinement of the evanescent wave, thus rendering the SPR sensing more robust towards environmental perturbations, and by amplifying the enhancement factor of the SERS measurement.

9724-15, Session 3

**Time-lapse scanning surface plasmon microscopy of living adherent cells with a radially polarized beam**

Lotfi Berguiga, Laura Streppa, Ellse Boyer-Prosver, Francesca Ratti, Evelyne Goillot, Cristina E. Martinez-Torres, Ecole Normale Supérieure de Lyon (France); Anne Devin, Institut de Biochimie et Génétique Cellulaires (France); Laurent Schaeffer, Alain Arneodo, Françoise Argoul, Ecole Normale Supérieure de Lyon (France)

We introduce a high resolution scanning surface plasmon microscope for long term imaging of living adherent mouse myoblast cells. The coupling of a high numerical aperture objective lens with a fibered heterodyne interferometer provides both enhanced sensitivity and long term stability.

This microscope is taking advantage of the plasmon resonance excitation and its amplification of the electromagnetic field in near-field distance to the gold coated coverslip. We discuss the principle of image formation and image contrast optimization. V(Z) curves corresponding to the variation of the reflected field versus the defocalisation of the objective lens. Adherence and motility of living C2C12 myoblast cells is followed for 48 hours, showing that the dynamics of these cells changes after 15 hours, concomitantly to a modification of the gold substrate property by the deposition of extracellular matrix compounds by the adherent myoblasts.

This plasmon enhanced evanescent wave microscopy is particularly interesting in the context of cell adhesion, since it can be performed without staining of the biological sample. Finally on a fixed myoblast cell, we show that local index variations can also be retrieved from a finite set of V(X,Y,Z) maps, highlighting local fiber-like structures (cytoskeleton filaments) that act as guiding wave elements. When the acto-myosin network is disrupted by blebbistatin addition, these elongated structures disappear, confirming their nature and their origin in unperturbed cells. This method is further extended to the characterisation of myotubes (obtained by differentiation of myoblasts), to show that these cells have a drastically different adhesion program on flat surfaces.

9724-30, Session PMon

**Reusable tin plasmonic nanostructures for intracellular delivery**

Alexander J. Raun, Nabila Saklayen, Christine M. Zgrabik, Daryl I. Vulis, Marlinna Madrid, Evelyn L. Hu, Eric Mazur, Harvard Univ. (United States)

Developing a method to efficiently deliver drugs and biomolecules such as DNA into cells is an important area of biomedical research. This form of intracellular delivery relies on porating cells’ membranes to allow exterior molecules to efficiently enter the cell while maintaining high viability. Various techniques, including viral methods, electroporation, and...
optoporation, can perform intracellular delivery but come with significant drawbacks such as high cell death, low throughput, and low efficiency. We present a new laser-based delivery method that uses a pulsed laser to excite plasmonic Titanium Nitride (TiN) nanostructures for cell poration and offers high efficiency, viability, and throughput. This research explores the use of TiN as a plasmonic material for these laser-activated nanostructures due to its high robustness and thermal stability. We investigate different fabrication conditions to maximize plasmonic enhancement and stability after prolonged laser exposure. We deliver dye molecules, siRNA, and microspheres to cells to quantify poration efficiency and viability by imaging the target cells at defined time intervals post laser irradiation. Additionally, we use scanning near-field optical microscopy (SNOM) and scanning electron microscopy (SEM) techniques to study nanostructure damage and plasmonic characteristics. Overall, TiN presents a strong opportunity for use in future biomedical devices for intracellular biomolecular delivery and regenerative medicine.

9724-31, Session PMon
Enhancement of scattering from nanoparticles using substrate effect
Krishnendu Chakraborty, Abhay K. Tiwari, Manoj M. Varma, Murugesan Venkatapathi, Indian Institute of Science (India)

Scattering cross-section of metal nanoparticles is enhanced due to Localized Surface Plasmons Resonance (LSPR) permitting the observation of single metal nanoparticles as small as 40 nm using dark-field microscopy. Single particle resolved measurements allow the study of reactions happening on the nanoparticle surface involving an ultra-low number of reactant molecules to understand stochastic effects in reactive systems. Here we report a method to enhance the intensity of resonantly scattered light by using appropriately designed substrates. Specifically, we show that by using a multi-layer dielectric substrate with its high reflectance window spanning the LSPR resonance position, one can increase the intensity of scattered light by nearly an order of magnitude. We took three substrates namely Silicon, glass and the multilayer dielectric mirror. Disk shaped gold nanostructures with sizes ranging from 80 nm – 300 nm were fabricated using electron beam lithography on all three substrates. Sizes of individual nanostructures were determined by atomic force microscopy (AFM) and the dark-field image of each nanostructure was taken with an optical microscope. It was observed that the intensity of light scattered by single nanoparticles was roughly an order magnitude larger than that from Silicon and glass substrates. We used a numerical scheme based on Discrete Dipole Approximation to computationally validate our results. The numerical results matched the experiments quite well. The substrate enhanced scattering signal will useful to improve the signal to noise ratio in single particle resolved measurements.

9724-33, Session PMon
Effect of atmospheric pressure plasma on antimicrobial activity of cotton fabrics dyed with Zataria multiflora Bios
Soudabeh Hajahmadi, Najafabad Branch, Islamic Azad Univ. (Iran, Islamic Republic of)

Nowadays it is very important to finish almost all garments with antimicrobial treatment, many of the synthetic antimicrobial agents for textiles cause environmental deterioration and health problems for consumers, therefore bio materials which inhibit the growth of microorganism without causing health are becoming more and more attractive. Zataria multiflora Boiss. is a popular and medicinal plant with a remarkable antibacterial and antioxidant activity. The present study was carried out to determine the antimicrobial properties of cotton fabrics treated with atmospheric pressure plasma (APP), dyed with ZM extracts as a natural antimicrobial finishing. Cotton fabric was treated with argon or air plasma at atmospheric pressure. The antimicrobial activity of Zataria multiflora extract improve remarkably after application APP treatment on cotton fabrics against some common micro-organisms that could growth on textiles, gram negative (Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa) and gram positive (Staphylococcus aureus, Bacillus subtilis) bacteria compare with untreated swatches. Durability of antimicrobial activity of ZM on fabrics to light (color fastness) also measured and discussed. All treated species illustrated very effective antimicrobial properties, more than 94% microbial reduction in all bacterial population. Therefore, APP treatment improve the antimicrobial activity of ZM on fabrics and can be used as bio-engineering method for produce antibacterial textile and consider it as a durable finishing for household or hospital consumption.

9724-34, Session PMon
A novel fast speed wavelength-scanned technology in wavelength interrogation SPR imaging sensors
Yonghong Shao, Youjun Zeng, Kaiqiang Chen, Shenzhen Univ. (China); Jianan He, Dayong Gu, Shenzhen International Travel Health Care Ctr. (China) and Research Institute of Disease Control and Prevention (China); Junle Qu, Shenzhen Univ. (China); Ho-Pui A. Ho, The Chinese Univ. of Hong Kong (Hong Kong, China)

Surface plasmon resonance (SPR)-based biosensing is one of the most advanced label-free, real-time detection technologies. Traditional wavelength interrogation SPR imaging technologies scan an incidence wavelength normally using a monochromator based on the rotating grating. This kind of mechanical scan method results in the very slow measurement speed, as limits applications of this kind of SPR sensors. In this paper, we present a novel fast wavelength-scanned technology suitable for fast wavelength interrogation SPR imaging sensors. A liquid crystal tunable filter (LCTF) is used as a fast wavelength scanner. 50ms per wavelength and passband width of 10nm are realized with no any mechanical moving part. Moreover, a feedback loop is used to automatically track the movement of the SPR dip. By combining the wavelength-scanning near the SPR dip and the feedback loop, a fast wavelength-scanned SPR imaging is realized and a dynamic process of the interaction between rabbit anti-mouse IgG and mouse IgG is monitored with the time resolution of less than one second. As far as we know this is the fastest wavelength interrogation SPR imaging sensor ever reported in the literature.

9724-35, Session PMon
Simultaneous trapping-and-detecting surface-enhanced Raman spectroscopy by self-aligned hot-spots
Soonwoo Hong, On Shim, Hyosung Kwon, Korea Univ. (Korea, Republic of); Yeonho Choi, Korea Univ. College of Health Sciences (Korea, Republic of)

Surface-enhanced Raman spectroscopy (SERS) is a rapidly emerging technique as a label-free and sensitive detection system. However, it is still a challenge to match the enhanced area with an illuminated spot because SERS measurements occur in the area of light excitation, and the magnitude of the enhancement factor can be influenced by which SERS substrates are used. Here, we demonstrated a new technique that converges SERS with plasmonic trapping. Using plasmonic trapping, we could trap gold nanoparticles to the desired position so that hot-spots are fabricated onto that aimed position which is directly connected to matching the hot-spots with the detection sites. Both fabricating hot-spots and measuring Raman signal can be performed at the same time. Using our technique, we could
detect Rhodamine 6G (R6G) to 100 nM, and the magnitude of its signal increased 40 fold than the magnitude of the initial signal over time. We also categorized three different modes of our technique by the difference of Raman signal intensity and the morphology of the attached particles. To further verify, trapping force and trapping potential were simulated by the finite element method because it is critical to improve the reproducibility of the Raman signal. We believe that our innovative technique will open new prospects in the field of biosensors.

9724-36, Session PMon
Nano structured plasmonic devices for immunodiagnostics, multiplexed label free biosensing and imaging
Divya Sharma, R. P. Dwivedi, Shoolini Univ. (India); Santosh Kumar, Ashish Bisht, DIT Univ. (India)

The past few decades have envisaged and drafted the use of optical biosensors into the label free and multiplexed biosensing exploring the surface plasmon polaritons(SPR), which have turned into a gold standard on the commercial basis. Their applications allow very quick and high sensitive monitoring of the biomolecular interactions in much more real time without any kind of fluorescent labeling but there lies an underlying problem with them. They are bulky and are difficult in scaling up for high throughput detection which likewise forms drawbacks or limitations in the currently used commercial SPR systems. SPR also prevents the use of high numerical aperture optics high throughput detection The Interferometric analysis on the biosensing frontier offers the detection without any expensive and robust labeling. So the integration of compact non-linear plasmonic structure and microfluidics, holds a great promise to develop very fast, cheap, portable devices for and rural health care applications. Also, there comes a promise in device miniaturization and cost effectiveness. The integration of plasmonic chips utilizing nonlinear interferometry with functional microfluidic platform for the preparation of sample, would result in economic settings in case of diagnostic testing, decrease of size and reduction of cost of biosensing devices and adding up the prospective approach delivered by multiplexed immunoassays for immunodiagnostics and potential to expand into point-of-care(POC) diagnostics as well.

9724-37, Session PMon
Fabrication of tunable plasmonic 3D nanostructures for SERS applications
Ayse Ozbay, Handan Yuksel, Ramazan Solmaz, Mehmet Kahraman, Gaziantep Univ. (Turkey)

Surface-enhanced Raman scattering (SERS) is a powerful technique used for characterization of biological and non-biological molecules and structures. Since plasmonic properties of the nanomaterials is one of the most important factor influencing SERS activity, tunable plasmonic properties (wavelength of the surface plasmons and magnitude of the electromagnetic field generated on the surface) are crucial in SERS studies. SERS enhancement can be maximized by controlling of plasmonic properties of the nanomaterials. In this study, a novel approach for the template to fabricate tunable plasmonic 3D nanostructures based on combination of soft lithography and nanosphere lithography. First, spherical latex particles having different diameters are uniformly deposited on glass slides with convexity assembly method. The experimental parameters for the convective assembly are optimized by changing of lateral velocity and NP concentration, stage velocity and NPs volume dropped between to glass slides. Structural characterization of thin films is performed by atomic force microscopy (AFM). Colloidal suspensions are concentrated by centrifugation to obtain thin films by the deposition of NPs on a regular glass slide with the convective assembly. The experimental parameters for the convective assembly are optimized by changing of NP concentration, stage velocity and NPs volume dropped between to glass slides. Structural characterization of thin films is performed by atomic force microscopy (AFM) and scanning electron microscopy (SEM). SERS is also used for the optical characterization of the prepared thin films of NPs. Rhodamine 6G and aminothiophenol (ATP) are used as probe molecules to evaluate SERS activity of the thin films depending on the type and concentration of NPs. The results demonstrate that, SERS performances of the thin films are dependent on not only the type of NPs but also the concentration of NPs used to obtain thin films. The thin film having highest SERS activity could be used for the SERS based immunoassays for the detection and identification of bacteria.

9724-38, Session PMon
Development of SERS substrates for immunoassay applications
Ökkes Celik, Mehmet Kahraman, Gaziantep Univ. (Turkey)

Surface-enhanced Raman scattering (SERS) is an emerging technique for the detection and identification of biological structures. SERS based immunoassay methods are mostly used for the specific detection and identification of bacteria. In this study, SERS substrates are developed with deposition of synthesized spherical 15 nm gold nanoparticles (AuNPs) and 50 nm silver nanoparticles (AgNPs) on regular glass slides with convective assembly method for SERS based immunoassay for the detection and identification of bacteria. The synthesized NPs are characterized by UV-vis absorption spectroscopy, dynamic light scattering (DLS) and atomic force microscopy (AFM). Colloidal suspensions are concentrated by centrifugation to obtain thin films by the deposition of NPs on a regular glass slide with the convective assembly. The experimental parameters for the convective assembly are optimized by changing of NP concentration, stage velocity and NPs volume dropped between to glass slides. Structural characterization of thin films is performed by atomic force microscopy (AFM) and scanning electron microscopy (SEM). SERS is also used for the optical characterization of the prepared thin films of NPs. Rhodamine 6G and aminothiophenol (ATP) are used as probe molecules to evaluate SERS activity of the thin films depending on the type and concentration of NPs. The results demonstrate that, SERS performances of the thin films are dependent on not only the type of NPs but also the concentration of NPs used to obtain thin films. The thin film having highest SERS activity could be used for the SERS based immunoassays for the detection and identification of bacteria.

9724-39, Session PMon
Aptamer conjugated silver nanoparticles for the detection of interleukin 6
Andrea K. Locke, Nicole Norwood, Haley L. Marks, Monika Schechinger, Texas A&M Univ. (United States); George W. Jackson, BioTex, Inc. (United States) and Base Pair Biotechnologies, Inc. (United States); Duncan Graham, Univ. of Strathclyde (United Kingdom); Gerard L. Cote, Texas A&M Univ. (United States)

Dengue fever emerged in the 1950s and is now endemic in the tropics and subtropics, putting about 40% of the world’s population at risk. Up to 100 million infections, including 22,000 deaths, occur annually by mosquito transmission, as there is not one vaccine for the four dengue viruses. More serious forms of infection, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), can be fatal, but proper and timely management can reduce mortality to less than one percent. Interleukin 6 (IL-6) production in serous forms of infection, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), can be fatal, but proper and timely management can reduce mortality to less than one percent. Interleukin 6 (IL-6) production in the serum has been shown to significantly increase in patients with DHF/DSS, especially during the first few days post-infection. Therefore, an assay that detects the presence and concentration of IL-6 in the blood could improve early detection of DHF/DSS and considerably reduce mortality. In the past couple of years a number of aptamer sequences and sequence pairs have been selected and characterized for targeting interleukin-6, some of which additionally inhibit protein signaling. 2 Binding these highly specific aptamers to spectral tagging elements such as metallic nanoparticles and dyes allows for the optical monitoring of the nanoparticles’ aggregation response to the protein, using techniques such as absorbance, fluorescence and Raman spectroscopy. Specifically we present a sandwich-type assay using two IL6-specific aptamers that have been PEGylated and thiolated for easy immobilization onto silver nanoparticles. These particles were mixed together and exposed to varying levels of IL6 protein, and their
degree of aggregation was analyzed using multiple spectroscopies and nanocharacterization techniques.

9724-40, Session PMon

Surface-enhanced Raman spectroscopy on metal-dielectric-metal nanohole arrays using long-range surface plasmons to produce a sustained electric field depth profile

David Galvan, Qiuming Yu, Univ. of Washington (United States)

Surface-enhanced Raman spectroscopy (SERS) is exceedingly adept for biomedical applications due to its low limit of molecular detection and the detailed chemical information obtained from SERS measurements. However, SERS is inherently a near-field effect extending ~5 nm from the sensing surface, making it incompatible with bioactive coatings for use in biomedical, homeland security and food safety applications. In the present work, a new class of SERS-active substrates was designed to incorporate long-range surface plasmons, with the aim of extending the detection region further into the sample medium. The substrates under investigation consisted of a metal-dielectric-metal structure, with the dielectric polymer (Teflon AF) having a refractive index matched to water. Finite-difference time-domain simulations were used to design the nanohole arrays and investigate the electric field distribution as a function of nanohole diameter, pitch and depth of the resonant cavity. Specially designed ‘long-range’ SERS (LR-SERS) substrates were shown to have an electromagnetic enhancement factor (EF) of 6.37 x 10^5, which is ~4.5x greater than a conventional substrate. Moreover, at a distance of 25 nm from the surface, the conventional EF had decayed to 6.67 x 10^2, whereas the LR-SERS substrates maintained an EF of 3.62 x 10^4. The signal of a Raman reporter molecule, rhodamine 6G, was then investigated as a function of distance from the gold surface. LR-SERS substrates displayed strong signals from rhodamine 6G at a distance of 25 nm from the surface, indicating the successful generation of long-range surface plasmons in the array.

9724-41, Session PMon

A high sensitive surface plasmon resonance sensor using a genetically engineered M-13 phage

Hyerin Song, Wonguen Kim, Kyujung Kim, Jin-Woo Oh, Pusan National Univ. (Korea, Republic of)

A genetically engineered M-13 phage which has a specific attachment to the target and performs like a liquid crystal was adapted to the surface plasmon resonance (SPR) sensor for enhancing the sensitivity and selectivity. The sensitivity enhancement of the M-13 phage based sensor was decided by the orientation of the phage alignments which is proved in this research by experiments and simulation. The integration of a specific high density binding peptide (i.e., His-Pro-Gln, or HPQ) appeared on the surface of the M-13 phage results in high selectivity to streptavidin which is well binding with target protein, biotin. Consequently, we experimentally measured resonance peak shifting through SPR sensor system and confirmed that resonance peak shifting resulting from around 100 aM of biotin was efficiently detected in real time using the most sensitive and selective phage film which aligned directionally. The developed HPQ phage-based SPR sensor has advantage of the simplicity of phage self-templating compared with other relatively complex lithography techniques. In addition, the functionalized M-13 bacteriophages allow the developed SPR sensor to show immediate improvements in sensitivity and selectivity.

9724-42, Session PMon

Near-field localization by two dimensional metallic nano-post arrays with ultrashort light pulses

Hongki Lee, Donghyun Kim, Yonsei Univ. (Korea, Republic of)

Locally amplified near-fields can be induced with nanostructures within a sub-diffraction-limited volume, which is useful for biomedical imaging and sensing applications. Employment of field localization in the biomedical applications based on pulsed light sources necessitates understanding of spatial and temporal field characteristics near nanostructures. For this purpose, we consider in this work metallic nano-post arrays of three different shapes (circular, rhombic, and triangular) to localize near-fields. They were modeled to be located on an ITO film and a quartz substrate with periods changing from 300 to 900 nm in a step of 200 nm. The size changes from 50 to 250 nm which corresponds to the radius for the case of circular nano-posts and the distance between center and vertices for equilateral rhombic and triangular nano-posts. Numerical calculation of near-fields at the top of nano-posts was performed with finite difference time domain method when the Gaussian pulses at center wavelengths of 532, 633, and 850 nm were normally incident. Near-fields localization occurred mainly at vertices of nano-posts. Triangular nano-posts were of primary interest for they tend to show the strongest field intensity within a diffraction limited field-of-view. The observed fields on the triangular vertices were enhanced by 7.85, 51.54, and 7268 times when the center wavelengths were 532, 633, and 850 nm respectively. Their temporal peaks were delayed by 2.05, 4.03, and 14.49 fs, which indicates the correlation between field enhancement and time delay associated with electron damping process. It was shown that with rhombic and triangular nano-posts fields can be localized below 10 nm in size on vertices and their signal-to-noise ratio increased with a larger period.

9724-16, Session 4

Switching and logic manipulation of droplets and dielectric nanoparticles in micro-nanofluidics system (Invited Paper)

Guanghui Wang, Nanjing Univ. (China); Ho-Pui A. Ho, The Chinese Univ. of Hong Kong (Hong Kong, China); Xuping Zhang, Nanjing Univ. (China)

Switching and logic manipulation are very important for large scale integration of lab-on-a-chip system. In this talk, we demonstration this kind of manipulation in centrifugal microfluidics and silicon photonics based nanofluidics system.

1. Switching and logic manipulation in centrifugal microfluidics

Centrifugal microfluidics is a promising option for the lab-on-a-chip and point-of-care (PoC) diagnostics applications. For traditional chip on LOAD, it always has one state and monomorphic function. In order to make the chip on centrifugal platform to be configurable and lead to large scale integration, we proposed a binary centrifugal microfluidics platform based on a novel mechanic structure. The periodical switching of state would provide a “clock” signal for sequence of droplet binary logic operations. With the binary states platform and the “clock” signal, the chip becomes configurable and these upon function units can perform real digital logic operation. We demonstrate droplet generation and a series of droplet logic operations, such as binary valving, droplet routing and digital addressable droplet storage. With the switching ability of this platform, multiple solutions or functions could be integrated within a single chip. It is a promising approach for lab-on-a-chip large scale integration.

2. Switching and logic manipulation in silicon photonics based nanofluidics

We also numerically demonstrate the switching and logic manipulation in silicon photonics based nanofluidics. The gradient force and the scattering
force, which induced by the evanescent field of waveguide mode or induced by the localized mode of metal structures, contribute to the trapping and transportation of nanoscale biological samples. This hybrid chip has two layers, the upper one for nanofluidic channels and the beneath one for nanophotonic structures as an optical actuator. Then the logic operation of light wave in nanophotonics layer is mapping up to the operation of sample in nanofluidics layer. In this way, we proposed several function units for the switching and logic manipulation of nanoscale biological samples including particle switching with a wavelength division multiplexing (WDM) tree splitter, nano-optical convey belt on silicon waveguide and particle switching based on phase modulation with a ring-assisted Mach-Zehnder interferometer.

9724-17, Session 4

Plasmonic filter array for on-chip near-infrared spectroscopy

Erwen Li, Alan X. Wang, Oregon State Univ. (United States)

Infrared (IR) spectroscopy is a widely used technique for chemical and biological sensing as it detects and identifies various analytes by probing their unique molecular vibration signatures, which provides a label-free and even recognition-free detection mechanism compared with other optical methods such as fluorescence or refractive index sensing. However, commercial IR spectroscopy systems are large, heavy and expensive. Therefore, an on-chip IR spectrometer is highly desirable for portable and cheap spectroscopy sensing system. Surface plasmonic nanostructures are attractive due to their capability in light manipulation. In this paper, we designed and experimentally demonstrated a plasmonic filter array using Au subwavelength gratings for NIR spectroscopy. We fabricated multiple Au gratings with different periods on a single glass substrate. The Au gratings are designed to perform as narrowband optical transmission filters in the bandwidth of interest, with typical full wavelength half maximum around 10 nm. The transmission peak of the filters can be tuned by changing the grating period. Using a broadband light source, we can extract the spectra information by measuring the transmitted power of each plasmonic filter. A thin Su-8 film is spin-coated onto the Au grating for solid-phase micro-extraction. Xylene is chosen as analyte in our experiment. Due to the field enhancement of the surface plasmon polaritons at the Au/Su-8 interface, the NIR absorption of xylene in the Su-8 sensing phase is also increased. The hydrophobic Su-8 layer also eliminates the strong interference from water. The NIR spectroscopy of xylene is measured from 1500 to 1700 nm wavelength range.

9724-18, Session 4

Novel zwitterionic non-fouling modification on plasmonic optofluidic system toward real-time therapeutic monitoring

Fang Sun, Shaoyi Jiang, Qiuming Yu, Univ. of Washington (United States)

In the past decades, surface-enhanced Raman spectroscopy (SERS) has emerged as a promising analytical technique for environmental monitoring, food safety and pharmaceutical analysis. Reliable biosensing based on SERS platforms in complex biological media such as whole blood and plasma is still challenging due to matrix interference and nonspecific adsorption of proteins. Background noise from matrix interference could mask the signals from target analytes because a bare SERS-active surface lacks of selectivity. Nonspecific adsorption of proteins could impede the adsorption of target analytes to SERS-active substrate surfaces. In this work, we developed a novel surface coating on SERS-active substrate surfaces to enable the detection of drug molecules in human blood plasma. The grafted dense layer of zwitterionic polymer brush can effectively resist protein adsorptions from the whole blood plasma and shield the interfering species while still allow small analytes to diffuse to the SERS-active surface. As a proof of concept, an anti-cancer drug, doxorubicin was quantitatively detected and dynamically monitored in plasma by using an optofluidic system with the SERS-active substrate surface functionalized with zwitterionic polymer. We demonstrated that the system was exceptional stable and can response to drug level changes. This new technique could be potentially used for the detection and dynamically monitoring of a variety of therapeutic drugs in real-time.

9724-19, Session 4

Fluorescence intensity enhancement mechanism in presence of plasmonic nanoparticles

Sumana Das, Brahmamanandam Javvaji, Krishna H. Villa, Akshata Arikady, Rohit Sali, Gopalkrishna M. Hegde, D. Roy Mahapatra, Indian Institute of Science (India)

Fluorescence is an important tool in bio-imaging, bio-sensing, diagnostics, DNA sequencing and genomics. Its high sensitivity and selectivity in detection of target molecule makes it useful in lab-on-chip microfluidic devices. However, the detection of sample using fluorescence is limited by its low brightness, relatively high background signal from the sample and a low photostability. Recently, various methods have been explored to enhance the fluorescence intensity, such as modulating the morphology of nanoparticles, increasing the diameter of nanoparticles, which is very much in need for the understanding of various biologically relevant mechanisms. Among these methods, plasmon-enhanced fluorescence strategy has attracted considerable attention. In the present experimental study, we show fluorescence intensity enhancement in presence of Au nanoparticle of diameter. Varying size of nanoparticle is used to study the enhancement effect of fluorescence. The present study is focused on nanoparticle interaction with cells and on optimization strategies to maximize the fluorescence enhancement at the vicinity of the nanoparticles, for important applications such as fluorescence-based biochip platforms. Result was also correlated ZnO nanoparticle effect on fluorescence enhancement, which has different optoelectronic properties compared to Au nanoparticles. Electromagnetic wave field model is employed to simulate the effect of gold nanoparticle on cell with the assumption that the nanoparticles are a collection of discrete dipoles, which are ordered with the fluorescence molecules on cell wall. Photon counts are estimated considering the nanoparticle absorption coefficient, dielectric constants and quantum efficiency for emitted field centred at the emission wavelength of the fluorescence molecule.

9724-20, Session 5

Photoacoustic investigation of gold nanoshells for bioimaging applications

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Gold nanoshells (AuNS) are nanoparticles that have a thin gold shell surrounding a dielectric core. They exhibit plasmon excitation in the near-infrared regime, with relatively strong optical extinction coefficient compared to standard gold nanoparticles. Optical transmission through tissue is optimal in the near-infrared, enabling many biomedical imaging applications in-vivo. The optical properties of the AuNS can be tuned by engineering its physical and chemical properties. Photoacoustic microscopy (PAM) was used to characterize AuNS particles. Our system is capable of both a qualitative and quantitative photoacoustic analysis using a focused laser with ultra-high frequency (UHF) transducers up to 1200 MHz. Lateral and axial resolutions up to 1?m can be achieved, based on the transducer parameters. The nanoshell size was 350 nm with a polystyrene (PS) core and Au shell was prepared by sol gel method. The
Au shell was formed by successive bonding of Au nanoparticles, 1 to 2 nm in diameter, onto PS spheres which exhibited surface charge between 0.1 and 2.0 milliequivalent/gram. Two types of samples containing self-assembled monolayers of Au shells were prepared for PAM studies: (A) sparsely covered and (B) fully encapsulated. The sparsely covered AuNS contained a monolayer of Au nanoparticles of size 1 to 2 nm whereas sample B contained a completely covered Au shell of thickness 15 nm. These samples exhibited an optical extinction peak at 533 nm and 675 nm respectively. Using a PAM with a 200 MHz transducer and a 532 nm pulsed laser, the peak PA signal from sample A and B was 0.12 ± 0.14 mV and 1.27 ± 0.18 mV per fluence (mJ/cm²) respectively. The fully encapsulated shell exhibited a 10X increase in PA signal due to both inter- and intra-plasmonic couplings. This study demonstrates how AuNS can be characterized using PAM, with many potential applications in biomedical imaging.

9724-22, Session 5
Plasmonic nanohole arrays on Si-Ge heterostructures: an approach for integrated biosensors
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Nanohole array surface plasmon resonance (SPR) sensors offer a promising platform for label-free biosensing. Integrating nanohole arrays with group-IV semiconductor photodetectors could enable low-cost CMOS-compatible, disposable biosensors that can be combined with integrated circuitry for continuous monitoring of biochemicals and fast sensor data processing. Such an integrated biosensor could be realized by structuring a nanohole array in the contact metal layer of a photodetector.

Here, we investigate the effect of adding a nanohole array to an Al film on the near-infrared absorbance of a Si-Ge photodiode structure. The vertical Si-Ge photodiode structure, grown by molecular beam epitaxy, consisted of doped Ge layers sandwiched between doped Si layers. The Ge layers simultaneously form a waveguide with Si as the cladding. Nanohole arrays were structured into the 100 nm thick Al layer through e-beam lithography and dry etching. A 50 nm SiO2 layer between the Al and Si-Ge layers were fabricated by the reduction of cobalt salt using trioctylphosphine as a surfactant and oleic acid as a stabilizer. We observe superparamagnetic response of Co nanoparticles with mean diameter ~ 8 nm at room temperature and suppression of magnetization at low temperatures measured with SQUID magnetometer (MPMS, Quantum design). Structural characterization with transmission electron microscopy shows hexagonal close packing lattice. Aggregation of particles changes magnetic response and strongly reduces the quality of plasmon resonance. We observe plasmonic materials in the ultraviolet spectral range does not consider Co as a promising candidate. Moreover, it is a common belief that the quality of the plasmon resonance of Co is quite low, which follows, in particular, from the experimental data for permittivity of bulk cobalt. Here we show that Co nanoparticles with high quality crystal structure and spin polarization support an excellent plasmon resonance at about 275 nm, which is comparable with the noble metal in the visible range. While the plasmon resonance is due to the conduction electrons oscillations the interband transitions affect the plasmon resonance quality. Since the d-band is responsible for magnetic properties as well there is an obvious link between magnetic and plasmonic responses. Colloidal magnetic cobalt nanoparticles were fabricated by the reduction of cobalt salt using trioctylphosphine as a surfactant and oleic acid as a stabilizer. We observe superparamagnetic response of Co nanoparticles with mean diameter ~ 8 nm at room temperature and suppression of magnetization at low temperatures measured with SQUID magnetometer (MPMS, Quantum design). Structural characterization with transmission electron microscopy shows hexagonal close packing lattice. Aggregation of particles changes magnetic response and strongly reduces the quality of plasmon resonance.

Plasmonic fano resonance sensing system using gold nanosphere and J-aggregates
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No Abstract Available
High-resolution imaging of cells using metal clad waveguides (MCWG) (Invited Paper)

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Surface plasmon resonance (SPR) is a powerful label-free approach for real-time monitoring of changes in biological cell activity induced by external agents such as hormones, pharmacological agents, and toxins. Receptors at the cell surface recognize biomolecules in the surrounding media, triggering distinct intracellular events such as cell contraction and detachment. Such morphological changes induce refractive index changes at the metal-dielectric interface detectable by SPR. In this work, we demonstrate cell/substrate interface imaging based on metal clad waveguides (MCWG) as an alternative to SPR. MCWGs allow for a higher measurement sensitivity and deeper evanescent field penetration depth compared to SPR. Our setup is based on a high numerical aperture microscope objective allowing us to discriminate individual cell activity within the evanescent field at high spatial resolution. We evaluated the performance of our system by detecting individual cell-cell responses within a cell monolayer exposed to endotoxins. Our findings indicate that the sensor signal during toxin exposure originates from the appearance of intracellular gaps. As with high-resolution SPR imaging, lateral resolution in MCWG-based imaging is limited along the direction of light propagation by the finite longitudinal decay length of the lossy guided modes. Our work demonstrates a numerical method to improve lateral resolution in high-resolution MCWG imaging based on combining multiple images of a sample acquired with distinct light excitation directions. Since SPR is a special case of metal-clad waveguides, this work is broadly applicable to this type of structure.

Plasmon-enhanced sensing in ZnO nanostructures

Fang Xu, Zhiwen Kang, Jiajie Chen, The Chinese Univ. of Hong Kong (Hong Kong, China)

Semiconductor oxide sensors are widely used in many applications including environment control, chemical process control, national security, personal safety and medical diagnosis. The device primarily operates by modifying its conductance upon exposure to target gaseous molecules under elevated temperature conditions. The requirement of constant heating makes the device not suitable for low-power situations and portable systems. In this project, we investigate the use of plasmonic localization effects for inducing local heating or light-activated charge transfer processes in the sensing material in order to achieve plasmon-assisted gas detection. Localized surface plasmon resonance (LSPR) is the collective electron oscillations in metal nanoparticles (NPs) excited by incident light. LSPR-enhanced performance has been reported for solar cells, photocatalytics, photodetectors and LEDs. The fabrication of 3-D nanostructured ZnO based sensor devices is reported. The ZnO nano-tetrapods were synthesized by thermal evaporation of Zn powder. The choice of this material is based on several known advantages including its catalytic effects for redox reactions, high sensitivity and good selectivity to ethanol vapor.

Plasmonic dual-cladding fiber probe for bio-sensing with integrated parasitic effects compensation

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Surface plasmon resonance based fiber sensors are particularly attractive for the detection of bio-chemicals in clinical applications because they combine high sensitivity with the advantages of optical fibers, such as reduced invasive impact, absence of electrocution risk and simplified insertion into the patient body through catheteres. Today, however, their use is still mainly confined to lab demonstrators and, furthermore, essentially using transmission-based setups, which have feeding and collecting fibers at the opposite sides of the transducer; this is a simple and robust solution but it introduces severe limitations in many practical cases. Aiming at bio-medical applications, the paper presents an innovative plasmonic fiber probe that: i) exploits a reflection-based setup to develop a sensing tool that can be inserted into catheters; ii) makes use of large core fibers to simplify mating and thus to ease the realization of disposable sensing tips; iii) exploits a dual cladding geometry to allow using the inner cladding to guide the sensing signal and inscribing a Bragg grating in the fiber core to be used to compensate for temperature and strain-induced transducer spectral shifts. The latter characteristic has called for the development of a special combiner with feed-through to couple feeding/receiving fibers with the inner cladding of the transducer fiber and the compensation fiber with its core. Some prototypes have been fabricated and fully characterized in terms of spectral resolution, FWHM notch peak, sensitivity, etc. Preliminary results have demonstrated the viability of the proposed solution, although it still requires optimizations.

Portable multichannel surface plasmon resonance imaging (SPRI) device

Chih Han Chen, Hsin-Yuan Chuang, How-Foo Chen, National Yang-Ming Univ. (Taiwan)

Surface plasmon resonance imaging (SPRI) is a technique utilized in biosensing field for decades. Due to its characteristics as label-free, high-sensitivity, and multi-spots or multi-channels detection in real time, this technique has been used widely in various areas, such as drug development, life science research, molecular affinity study, and heavy metal ion detection. However, applications are majorly limited by the size of the instrument and the cost: The current commercial products are usually bulky and expensive, including the instrument and the consumable testing chips. When the size becomes compact, the sensitivity is also significantly deteriorated. For the purpose of point of care (POC) biosensor, a disposable polymer-molding prism with two parabolic side surfaces is employed for SPRI. A compact SPRI biosensor downsized to a form factor of 20 cm "15 cm"5 cm is proposed in this study. As an innovated SPRI biosensor, the compact size and lower cost assure applications from laboratory examination to clinical use while the sensitivity is not scarified. In this demonstration, we integrated the CCD detection system and multichannel fluidic system into this device that allows users to quickly screen various samples simultaneously. A 635 nm laser beam and 1-D translation stage are used for SPRI excitation and incident angle tuning. By changing the position of a right-angle mirror
mounted on the 1-D linear translation stage, we can control the incident angle in the range of 30 degrees. Thus, a large dynamic range of the system and the sensitivity of 10^-6 RIU can be obtained. Moreover, with the disposable prism design, we can eliminate the risk of cross contamination in a cost effective manner compared to commercial products.

9724-29, Session 6

**Trapping of plasmonic nanocrystals for surface-enhanced Raman scattering via microbubble generation on random gold nano-island substrate**

Zhiwen Kang, Fang Xu, Jiajie Chen, Ho-Pui A. Ho, The Chinese Univ. of Hong Kong (Hong Kong, China)

Plasmonic nanocrystals have attracted extremely interests because of their fascinating optical properties for energy localization, local activation, sensing, and surface-enhanced Raman scattering (SERS). Trapping such nanocrystals is of importance for the purpose of realizing in situ or in vivo manipulation and detection. In general, optical tweezers is regarded as an excellent tool because of its non-invasive characteristic, but it is still not very feasible to trap plasmonic nanocrystals due to the requirement of high laser power consumption and large NA (numerical aperture) objective for producing strong gradient force. In this work, we use random gold nano-island substrate (AuNIS) to optothermally generate microbubble based on the plasmonic absorption activated local heating effect, and then utilize such microbubble generation for trapping of plasmonic nanocrystals. The trapping happens at the location where the bottom of the microbubble makes contact with the substrate, and also where the target nanoparticles are dragged in with the convective flow, and a balance between the surface tension forces and pressure forces gives rise to the trap. With the trapping of plasmonic nanocrystals taking place, SERS is demonstrated as one of the possible applications. The used laser power density is as low as 300 µW/µm² and only a 20X objective (NA=0.45) is used in the system. Two kinds of Raman reporters [4-mercaptobenzoic acid (4-MBA) with –SH bond, and Rhodamine B (RhB) without –SH bond] are used in the measurements. The minimum concentrations of detection can achieve 10^-12 mol and 10^-7 mol for the 4-MBA and RhB, respectively.
9725-1, Session 1

**Cellular biosensing using optical spectroscopy (Invited Paper)**

Adam Wax, Duke Univ. (United States)

The interaction of light with biological cells can provide a unique tool for studying their biophysical properties. Optical spectroscopy of biological cells can reveal detailed information on their structure and dynamics in a way that is not possible with traditional microscopy techniques. Histological evaluation can only obtain a snapshot of the activity of individual cells, relying instead on large ensembles to develop a picture of their temporal evolution. On the other hand, short optical spectroscopy can be applied to cells with little to no preparation and can enable studies of the same live cells at extended time intervals. Our research group has developed a suite of optical spectroscopic tools to assess the structure and function of biological cells and modulation due to the onset of disease. The wavelength dependence of the interaction of cells with light provides information of cell features through elastic scattering across the visible and near infrared spectrum. Alternatively, the angular dependence of scattered light can also be used to reveal cell properties. We will discuss how both modes of elastic scattering can be used to evaluate cell status. Finally, the recent advances in the use of optical phase imaging to create contrast in nearly transparent biological cells will also be discussed as related to the role of this modality in biosensing.

9725-2, Session 1

**Single exosome detection in serum using microtoroid optical resonators**

Judith Su, California Institute of Technology (United States)

Recently exosomes have attracted interest due to their potential as cancer biomarkers. We report the real-time, label-free sensing of single exosomes in serum using microtoroid optical resonators. We use this approach to assay the progression of tumors implanted in mice by specifically detecting low concentrations of tumor-derived exosomes. Our approach measures the adsorption of individual exosomes onto a functionalized silica microtoroid by tracking changes in the optical resonant frequency of the microtoroid. When exosomes land on the microtoroid, they perturb its refractive index in the evanescent field and thus shift its resonance frequency. Through digital frequency locking, we are able to rapidly track these shifts with accuracies of better than 10 attometers (one part in 10^11). Samples taken from tumor-implanted mice from later weeks generated larger frequency shifts than those from earlier weeks. Control samples taken from a mouse with no tumor generated no such increase in signal between subsequent weeks. Analysis of shifts from tumor-implanted mouse samples show a distribution of unitary steps, with the maximum step having a height of -1.2 fm, corresponding to an exosome size of 44 ± 4.8 nm. This size range corresponds to that found by performing nanoparticle tracking analysis on the same samples. Our results demonstrate development towards a minimally invasive tumor “biopsy” that eliminates the need to find and access a tumor.

9725-3, Session 1

**Detection of esophageal cancer cell by photoelectrochemical Cu2O/ZnO biosensor**

Chao-Hsin Hsu, Cheng-Hsuan Chu, National Chung Cheng Univ. (Taiwan); Weichung Chen, I-Chen Wu, Ming Tsang Wu, Kaochsiung Medical Univ. (Taiwan); Chie-Tong Kuo, National Sun Yat-Sen Univ. (Taiwan); Raymond Chien-Chao Tsiang, Hsiang-Chen Wang, National Chung Cheng Univ. (Taiwan)

We have demonstrated a Cu2O/ZnO nanorods (NRs) array p-n heterostructures photoelectrochemical biosensor. The electrodeposition of Cu2O at pH 12 acquired the preferably (111) lattice planes, resulting in the largest interfacial electric field between Cu2O and ZnO, which finally led to the highest separation efficiency of photogenerated charge carriers. High verticality ZnO nanorods by seed layer and thermal annealing assist the hydrothermal growth. The optimized Cu2O/ZnO NRs array p-n heterostructures exhibited enhanced PEC performance, such as elevated photocurrent and photoconversion efficiency, as well as excellent sensing performance for the sensitive detection of four strains of different races and different degree of cancer cell which made the device self-powered. We got spectral response characteristics and operating wavelength range of biosensor, and to verify the biological characteristics of cancer cells wafer react with different stages of cancer characterized by a cancer measured reaction experiment.

9725-4, Session 1

**Biodynamic Doppler imaging of subcellular motion inside 3D living tissue culture and biopsies (Invited Paper)**

David D. Nolte, Animated Dynamics, Inc. (United States)

Biodynamic imaging is an emerging 3D optical imaging technology that probes up to 1 mm deep inside three-dimensional living tissue using short-coherence dynamic light scattering to measure the intracellular motions of cells inside their natural microenvironments. Biodynamic imaging is label-free and non-invasive. The information content of biodynamic imaging is captured through tissue dynamics spectroscopy that displays the changes in the Doppler signatures from intracellular constituents in response to applied compounds. The affected dynamic intracellular mechanisms include organelle transport, membrane undulations, cytoskeletal restructuring, strain at cellular adhesions, cytokinesis, mitosis, exo- and endo-cytosis among others. The development of 3D high-content assays such as biodynamic profiling can become a critical new tool for assessing efficacy of drugs and the suitability of specific types of tissue growth for drug discovery and development. The use of biodynamic profiling to predict clinical outcome of living biopsies to cancer therapeutics can be developed into a phenotypic companion diagnostic, as well as a new tool for therapy selection in personalized medicine. This invited talk will present an overview of the optical, physical and physiological processes involved in biodynamic imaging. Several different biodynamic imaging modalities include motility contrast imaging (MCI), tissue-dynamics spectroscopy (TDS) and tissue-dynamics imaging (TDI). A wide range of potential applications will be described that include process monitoring for 3D tissue culture, drug discovery and development, cancer therapy selection, embryo assessment for in-vitro fertilization and artificial reproductive technologies, among others.
9725-5, Session 2

Fabrication and characterization of silicon nitride directional coupler interferometer for sensing aptamer hybridization

Kyohe Okubo, Ken Uchiyamada, Kiyoshi Asakawa, Masatoshi Yokokawa, Hiroaki Suzuki, Univ. of Tsukuba (Japan)

We report the use of a silicon nitride directional coupler (DC) for biosensing in order to improve sensitivity compared with our previous epoxy polymer (SU-8) based DC sensor. The DC detects changes in the bulk refractive index (RI) as relative output near-field patterns using a CCD camera. Two parallel waveguides separated by a distance form the DC. FDTD simulation provided a suitable geometry of the waveguide that allows monomodal light guiding, low-loss connection angle between the waveguide and coupler, and high sensitivity toward the local RI change. In addition, meander-line asymmetric waveguides were employed to make the total DC length longer and to integrate the DC in (100 μm)x2 square area. The modeling and prediction provide an ideal design of the waveguides so that the wave fronts propagating in the curved waveguides are controlled following the Fermat’s principle, resulting in the successful cascade-connection of the phases in the separated DC segments thus high sensitivity in total. The chip is fabricated by using the conventional photolithographic technique, such as electron beam writing and reactive ion etching. End-fire coupling between the waveguide and visible light of 632.5 nm wavelengths was carried out to obtain signal dependence on DC length. Optical intensities measured at the output ports agreed well to the corresponding fitting curve and the curve calculated based on the coupled mode theory. Preliminary test to demonstrate biosensing performance revealed that monitoring of aptamer hybridization obtains a significant net shift from the baseline.

9725-6, Session 2

High-sensitivity high-throughput chip based biosensor array for multiplexed detection of heavy metals

Hai Yan, The Univ. of Texas at Austin (United States); Naimei Tang, Swapnajit Chakravarty, Omega Optics, Inc. (United States); Ray T. Chen, The Univ. of Texas at Austin (United States) and Omega Optics, Inc. (United States)

Heavy metals released into the environment via industrial activities belong to a class of persistent environmental toxins that lead to various health hazards. The heavy metal ions in water can be detected reliably by the binding reactions between metal-chelate and specific antibody. However, existing detection techniques rely on fluorescent labels or are hard to multiplex. Here we propose an on-chip label-free detection approach based on silicon photonic crystal (PC) microcavities. The PC microcavities are combined by multimode interferometer (MMI) to form a sensor array that allows simultaneous detection of multiple biochemical reactions. Slow light effect in the PC microcavity enhanced the interaction between the light and the analyte, resulting in high sensitivity and low detection limit (ppb level). The integration with a silicon chip also facilitates the integration with microfluidics and peripherals to form an automated test system for field studies. We experimentally demonstrate the detection of heavy metal ions, uranium and cadmium, in environmental samples with sensitivities down to 1 ppb. Specific antibody is immobilized on the sensor surface and the optical spectrum of the sensor is obtained from a testing system using broadband light source and optical spectrum analyzer. Then the environmental sample containing metal-chelate is flowed onto the surface through microfluidic channels. The specific binding between the metal-chelate and the antibody was quantified by the resonance wavelength shift in the optical spectrum. Specificity of the detection is also verified by using control samples that do not contain heavy metal ions.

9725-7, Session 2

Label-free detection of protein molecules secreted from an organ-on-a-chip model for drug toxicity assays

Andres W. Morales, The Univ. of Texas at San Antonio (United States); Yu S. Zhang, Julio Aleman, Brigham and Women’s Hospital (United States) and Harvard-MIT Health Sciences and Technology (United States); Parissa Alreasool, Brigham and Women’s Hospital (United States) and Harvard-MIT Health Sciences and Technology (United States) and Tufts Univ. (United States); Mehmet R. Dokmeci, Ali Khademhosseini, Brigham and Women’s Hospital (United States) and Harvard-MIT Health Sciences and Technology (United States) and Harvard Univ. (United States); Jing Yong Ye, The Univ. of Texas at San Antonio (United States)

Clinical attrition is about 30% from failure of drug candidates due to toxic side effects, costing the pharmaceutical industry greatly and slowing drug development. This partly originates from animal models inaccurately representing human physiology. There is a clear unmet need for drug toxicity assays using human-based models in complementary to traditional animal models before expensive clinical trials. Organ-on-a-chip techniques developed in recent years allow creating a variety of human organ models mimicking different human physiological conditions. However, it is extremely challenging to monitor the transient and long-time response of the organ models to drug treatments for drug toxicity tests. Firstly, when an organ-on-a-chip model interacts with drugs, some protein molecules may be released into the medium due to certain drug effects, but the amount of the protein molecules is limited, as an organ tissue grown using the organ-on-a-chip technique is typically miniaturized. Secondly, traditional fluorescence techniques cannot be utilized for real-time monitoring of the concentration of the protein molecules, because the protein molecules are continuously secreted from the tissue and it is practically impossible to achieve fluorescence labeling in the dynamically changing environment. Therefore, direct measurements of the secreted protein molecules with a label-free approach is required for this application. In this paper, we report the development of a photonic crystal-based biosensor for label-free assays of secreted protein molecules from a liver-on-a-chip model. Ultrahigh sensitivity and specificity have been demonstrated.

9725-8, Session 2

Preliminary measurement results of biotinylated BSA detection of a low cost optical cavity based biosensor using differential detection

Peter Cowles, Cody Joy, Tony Bujana, DongGee Rho, Seunghyun Kim, LeTourneau Univ. (United States)

We report a Fabry–Pérot based biosensor using a novel differential detection method for point-of-care applications. Two laser diodes allow for multiplexing capability along with the ability to enhance the responsivity using differential detection. The laser wavelengths are chosen so that the optical intensities of two lasers change monotonically with opposite slopes upon the immobilization of desired biomarkers. Since both wavelengths of light travel along the same path, variations due to the path will be present in both wavelengths and thereby reduced by the differential detection calculation. The biosensor is created through microfabrication processes that establish two silver nanomirrors on either side of a cavity fashioned by bonding patterned SU-8 with a nanolayer of PMMA. The cavity width,
PMMA thickness, and silver thickness have been optimized to achieve a large change in differential value with a large fabrication tolerance. The cavity is prepped for functionalization by exposing the PMMA layer to ultraviolet light and then treating the surface with a mixture of 1-ethyl-3-3-dimethylaminopropyl carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) which allows for Avidin binding. Biotinylated BSA will be introduced to the sample and the intensities of the laser diodes will be measured by an sCMOS camera. A change in the differential value will correlate to the binding of BSA. Initial concentration of Biotinylated BSA for detection is set initially to 1.36 ug/mL and is reduced to limit of detection. In this presentation, we will discuss simulation results, fabrication procedures, functionalization procedures, and preliminary measurement results for biomarker detection.

9725-9, Session 2

A miniaturized optoelectronic system for rapid quantitative label-free detection of harmful species in food

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The development of miniaturized and more powerful analytical tools for Point-of-Need (PoN) applications has become an intensely active research field. Sensors combining photonic transducers and microfluidic components have emerged as an answer to the insatiable demand for miniaturized tools of increased performance and speed of detection. Despite the recent advances, optical sensors still face a major hurdle that has so far hindered their evolution into truly viable products for PoN applications: the inability to couple light to the waveguides on-chip in a way that is effective, reliable, cost-efficient and that allows for small size and portability.

We present an all-silicon, fully-integrated optoelectronic platform of multi-analyte, label-free, real-time and high-sensitivity detection capability. The platform is comprised of planar waveguides shaped as Broad-Band Mach-Zehnder Interferometers and self-aligned to VIS-NIR light-sources, all monolithically integrated on the same silicon chip and fabricated with standard microelectronic/micromanufacturing processes. The integrated nature of the chip allows to functionalize each transducer independently with different recognition biomolecules enabling real-time multi-analyte tests. The platform has been successfully employed to detect allergens in milk and rinsing water samples, mycotoxins in beer samples, as well as of pesticides in wine and grape samples following competitive immunoassay formats. The analytical sensitivities achieved for all analytes were lower than those set as allowable by EU legislation with analysis times ranging from 5-30 min depending on the assay. Moreover, the bio-functionalized chips could be regenerated and used for the assay repetitively without significant loss of analytical sensitivity, thus further reducing the analysis cost.

9725-10, Session 3

A nanoplasmonic electrical-field enhanced resonating device for detection of multiple biotargets from whole saliva, blood and serum (Invited Paper)

Utkan Demirci, Stanford Univ. (United States)

Micro and nano-scale technologies have great potential on medicine and biology. Particularly, recent advances in biosensing technologies provide notable impact on diagnostics, therapy monitoring, and cell manipulation areas. However, wide dynamic range, label-free detection, clinical sample processing, assay time, and cost are still considerable challenges for developing a biosensing platform. Thus, these bottlenecks hinder both their broad applications and translation from laboratories to clinics and point-of-need settings. Here, to address all these obstacles on a single platform, we present a portable Nanoplasmonic Electrical-field Enhanced Resonating Device (NE2RD), which generates hot-spots on a 3-D oriented gold nanoparticle surfaces. NE2RD provides an unprecedented detection for multiple biotargets (protein biomarkers, drugs, allergens, viruses, bacteria, and eukaryotic cells) from clinical matrices such as whole saliva, blood and serum without sample preprocessing steps under various conditions of temperature, pH and ionic strength, and reports results within 30 minutes. This platform demonstrates a sensitivity down to 400 fg/mL and five orders of magnitude linear dynamic range broader than ELISA. These significant advantages in dynamic range, detection limit, and portability could potentially guide broadly applicable platforms for scientific discovery and precision medicine at point-of-need settings.

9725-11, Session 3

Surface enhanced Raman optical activity for characterization of aqueous protein mixtures

Clint Perlaki, Quan Liu, Nanyang Technological Univ. (Singapore)

Future development of tissue engineering requires new technology to perform rapid and accurate protein detection. Methods used today, such as Western blotting, cannot selectively quantify proteins with the rapidity necessary for monitoring and regulating complex protein signaling within
Engineered tissue. As such, we explore surface enhanced Raman optical activity (SEROA) to provide tissue engineering with a fast, non-destructive, and non-invasive protein detection platform currently nonexistent. One major challenge in traditional Raman scattering (RS) is proteins generate extremely similar spectra making selective quantification highly inaccurate. In contrast, the power of Raman optical activity is its sensitivity to proteins’ secondary and tertiary structure—an essential property that can uniquely identify one protein from another, but seldom exploited for protein characterization.

We explore the use of SEROA and surface enhanced Raman spectroscopy (SERS) on a set of proteins individually and in mixtures using a custom optical setup. These data are used to quantify the concentration of each protein within the mixture to demonstrate the enhanced quantitative power of SEROA over traditional SERS. Our study suggests the unique potential of SEROA as an effective tool for protein characterization.

9725-12, Session 3

Hybrid nanoparticle-nanocup array structure with a tunable sensitivity for colorimetric biosensing

Sujin Seo, Gang Logan Liu, Univ. of Illinois at Urbana-Champaign (United States)

Colorimetric detection is cost-effective and user-friendly when used for sensing target analytes without a need of bulky and expensive equipment. The extraordinary transmission phenomena through plasmonic periodic nanoholes achieve colorimetric sensing by detecting color changes of transmitted light associated with the refractive index variation. The application of the nanohole arrays, however, is relatively restricted due to a limited sensitivity as well as the multiple transmission peaks in the visible light range. In order to improve the sensitivity bounded by the underlying nanostructures, hybrid nanoparticle (NP) – nanocup array substrates are developed for tuning the sensitivity. An ultrasensitive colorimetric and spectroscopic detection of the antigen-antibody binding is achieved by a three-dimensional (3D) self-assembly of Au NPs on the Au nanocup arrays. The NPs arranged in circle exhibits colorimetric properties. Utilizing the periodic nanocup arrays fabricated by a replica molding, the NPs were self-assembled on the sidewall of the nanocup by an electrostatic attraction through a self-assembled thiolated linker molecule. These 3D self-assembled NPs in each nanocup result in strong localized electric field by the plasmonic coupling between the NPs and the nanocup structure. The strong electric field allows to sense small refractive index change by molecular adsorption or binding. A precise control over the number of the NPs assembled inside the nanocup region reveals a linear relationship between the number of NPs and the sensitivity. Engineering the number of plasmonic hot spots is a key to increasing colorimetric sensitivity at a low cost for a refractive index based chemical and bio-sensing.

9725-14, Session 4

Plasmonic biosensor for label-free malachite green detection

Suyan Qiu, Fusheng Zhao, Gregg M. Santos, Wei-Chuan Shih, Univ. of Houston (United States)

Malachite green (MG), a well-known triphenylmethane dye, exhibits good antibacterial, antifungal and antiparasitic functions. It is used as a good veterinary drug to resist fungal and parasitic infections with high effectiveness in aquaculture due to its low cost and ready availability. However, MG is highly restricted or banned to use in fish farming in many countries by taking into account their potential carcinogenic and mutagenic properties. Despite its potential health threat, there were hundreds of notifications related to the illegal use of MG under the European Union Rapid Alert System for Food and Feed system between 2003 and 2013, and has been frequently reported in recent years also. Therefore, a sensor detection limit of 2 ppb for the sum of MG and its reduced leuco-form has been proposed by the European Union. It is suggested that more robust and reliable analytical techniques need to be developed to detect MG residues in aquatic products. In the present study, we show that the recently developed nanoporous gold (NPG) disks are novel and high-performance plasmonic substrates. In the sensing application, we demonstrate a label-free plasmonic biosensor for MG detection relying on the advantages of NPG disks such as high-density hot spots and large surface area. Further, we employed a capture scaffold which promotes highly-specific interactions with MG molecules via π-π stacking and electrostatic interaction. We have demonstrated highly-sensitive, highly-specific detection of MG molecules beyond the current state-of-the-art.

9725-15, Session 4

Dual-mode bioenabled nano-plasmonic sensors for biological and chemical detection

Xianming Kong, Kenny Squire, Yuting Xi, Paul LeDuff, Gregory L. Rorrer, Alan X. Wang, Oregon State Univ. (United States)

Optical sensors play pivotal roles in biological and chemical detection as they provide ultra-high sensitivity, label-free sensing capability, and unrivaled molecular specificity by probing the vibrational bands of the analyte molecules. Recent research progress of nano-photonic sensors relies on rationally designed structures such as photonic crystals and plasmonic nano-antennas to enhance the detection sensitivity, which requires expensive top-down semiconductor fabrication processes. In this paper, we described bioenabled nano-plasmonic sensors through the integration of diatom biosilica with self-assembled silver nanoparticles. Diatoms are photosynthetic marine micro-organisms that create their own skeletal shells of hydrated amorphous silica, called frustules, which possess photonic crystal-like hierarchical micro- & nano-scale features. Our research

9725-13, Session 4

Plasmonic paper: an emerging analytical platform for highly sensitive biosensors (Invited Paper)

Srikanth Singamaneni, Washington Univ. in St. Louis (United States)

Plasmonic biosensors hold enormous potential for the development of low-cost, label-free, point-of-care biodiagnostics. However, two major challenges need to be overcome to reap the benefits of this class of biosensors: (i) state-of-the-art plasmonic biosensors either offer limited sensitivity or are impractical for real-world applications due to their poor stability and excessive cost; (ii) these biosensors rely on natural antibodies, which are high-cost and impose severe limitations in handling, storage and device integration. We demonstrate that a common filter paper can be transformed into a plasmonic sensing platform for highly sensitive and selective detection of trace levels of chemical and biological analytes. We also demonstrate that short peptides as biorecognition elements compared to larger antibodies as target capture agents offer several advantages. Using a bioplasmonic paper device, we demonstrate the selective and sensitive detection of the cardiac biomarker troponin I. The smaller sized peptide provides higher sensitivity and a lower detection limit using a bioplasmonic paper device. Furthermore, the excellent shelf-life and thermal stability of peptide-based plasmonic biosensors, which precludes the need for special storage conditions, makes it ideal for use in resource-limited settings. We also demonstrate plasmonic biosensors based on artificial antibodies by molecularly imprinting the plasmonic nanotransducers. Apart from significantly lowering the cost, these developments are critical for translating plasmonic sensors to point-of-care and resource-limited settings.

9725-17, Session 5

Dual-mode bioenabled nano-plasmonic sensors for biological and chemical detection

Xianming Kong, Kenny Squire, Yuting Xi, Paul LeDuff, Gregory L. Rorrer, Alan X. Wang, Oregon State Univ. (United States)

Optical sensors play pivotal roles in biological and chemical detection as they provide ultra-high sensitivity, label-free sensing capability, and unrivaled molecular specificity by probing the vibrational bands of the analyte molecules. Recent research progress of nano-photonic sensors relies on rationally designed structures such as photonic crystals and plasmonic nano-antennas to enhance the detection sensitivity, which requires expensive top-down semiconductor fabrication processes. In this paper, we described bioenabled nano-plasmonic sensors through the integration of diatom biosilica with self-assembled silver nanoparticles. Diatoms are photosynthetic marine micro-organisms that create their own skeletal shells of hydrated amorphous silica, called frustules, which possess photonic crystal-like hierarchical micro- & nano-scale features. Our research
shows that such hybrid plasmonic-biosilica nanostructures can improve the detection limit by several orders of magnitude compared with conventional colloidal nanoparticle sensors. The enhanced sensitivity comes from the optical coupling of the guided-mode resonance of the diatom frustules and the localized surface plasmons of the silver nanoparticles. Additionally, the rich chemistry of the diatom biosilica and the nano-corrugated frustule with large surface-to-volume ratio can offer extremely high surface functionality for molecule binding and analyte concentration. Two optical sensing mechanisms based on surface-enhanced Raman scattering (ERS) and surface plasmonic resonances (SPRs) are currently under development. We will demonstrate that the bioenabled nano-plasmonic sensors can increase the SERS signals of Raman dyes by more than 12? and enhance the immunoassay sensitivity using standard antibody-antigen binding by two orders of magnitude. We also applied the nano-plasmonic biosensors for food safety sensing and water contamination monitoring.

9725-17, Session 5
Optofluidic nanotweezer methods for characterizing nanoparticles and viruses (Invited Paper)
David Erickson, Cornell Univ. (United States)

Direct measurements of the strength of particle interactions are critical for characterizing the stability and behavior of colloidal and nanoparticle suspensions. Current techniques are limited in their ability to measure piconewton scale interaction forces on sub-micrometer particles due to signal detection limits, thermal noise, and throughput. We have recently developed a technique for making direct mechanical measurements of the force and work associated with the steric and electrostatic effects that stabilize colloidal nanoparticles. "Nanophotonic Force Microscopy", as we call it, is unique in that it uses statistical methods to provide direct measurements of these forces at the individual particle scale, while still being sufficiently high-throughput to produce meaningful population level data. In this talk I will introduce the technology, its advantages, and some of the major uses. Specific case studies will include label-free monitoring of binding of individual antibodies onto single viruses and the measurement of the strength of nanoparticle coatings used for steric stabilization.

9725-18, Session 5
An optofluidic FRET laser using quantum dots as donor
Qiushu Chen, Univ. of Michigan (United States); Alper Kiraz, Koç Univ. (Turkey); Xudong Fan, Univ. of Michigan (United States)

An optofluidic FRET (Fluorescence resonance energy transfer) laser can be formed by putting FRET donor-acceptor pairs inside a microcavity acting as gain medium. This integration of optofluidic laser and FRET mechanism provides novel research frontier, including sensitive biochemical analysis and novel photonic devices, such as on chip coherent light source and bio-tunable lasers. In this work, we demonstrate an unprecedented optofluidic FRET laser using quantum dots (QDs) as donor. With a capillary-based ring resonator, we achieve lasing from Cy5 as acceptor in QD-Cy5 FRET pair when excited at 450 nm within the QD absorption band, far away from Cy5 absorption maximum. We also investigate the output efficiency of our QD FRET laser in comparison with the directly excited dye laser. It is revealed that regardless of the high absorption cross section of QDs in the blue and near UV range, the threshold pump intensity of the QD FRET laser is not significantly reduced. Theoretical analysis is performed to address the phenomenon observed. Our study reveals the differences between QDs and organic dye molecules as donor in optofluidic FRET laser operation. Discussion is made on the strategies for achieving FRET laser with QDs for low threshold, high efficiency and high sensitivity. Given the unique photophysical properties of QDs, we believe that the demonstrated capability of QDs serving as donor in FRET laser will greatly improve the versatility of the optofluidic laser technology and accelerate the application of this technology in ultra-sensitive biochemical analysis and novel on-chip devices.

9725-19, Session 5
Optofluidic lasers and their applications in bioanalysis (Invited Paper)
Xudong Fan, Univ. of Michigan (United States)

The optofluidic laser is an emerging technology that integrates microfluidics, miniaturized laser cavity, and laser gain medium in liquid. It is unique due to its biocompatibility, thus can be used for unconventional bioanalysis, in which biointeraction or process takes place within the optical cavity mode volume. Rather than using fluorescence, the optofluidic laser based detection employs laser emission, i.e., stimulated emission, as the sensing signal, which takes advantage of optical amplification provided by the laser cavity to achieve much higher sensitivity.

In this presentation, I will first introduce the concept of optofluidic laser based bioanalysis. Then I will discuss each of the three components (cavity, gain medium, and fluids) of the optofluidic laser and describe how to use the optofluidic laser in bioanalysis at the molecular, cellular, and tissue level. Finally, I will discuss future research and application directions.

9725-20, Session 5
Hybrid optofluidic integration (Invited Paper)
Holger Schmidt, Univ. of California, Santa Cruz (United States)

Optofluidic integration of photonic and fluidic functions on a single chip-scale system is attracting a lot of interest for biological and chemical analysis with numerous analytic and diagnostic applications. We have developed a liquid-core waveguide-based approach for ultrasensitive detection of individual biological particles on a chip. In addition to ultrasensitive on-chip detection, this optically planar platform is ideally suited for integration with additional functionalities. I will describe the silicon-based platform and key applications such as amplification-free detection of clinical virus samples. I will then discuss next-generation hybrid integration with nanopores and soft PDMS chips to add electronic sensing and sample preparation capabilities.

9725-21, Session 6
Rapid detection of interleukin-8 at sub pg/ml concentration using magnetic modulation biosensing
Jasenka Verbar, Orr Hadass, Paul Olivo, MagBiosense, LLC (United States); Amos Danielli, Bar-Ilan Univ. (Israel)

Rapid, highly sensitive, and non-invasive detection of biomarkers is crucial in many areas of medicine. Often, even slight changes in the biomarker concentration can be clinically significant, which presents the need for assays that can detect very low quantities of biomarkers. Previously, we presented a novel platform to measure low concentrations of proteins that can be applied to a point-of-care device. The platform, termed Magnetic Modulation Biosensing (MMB), uniquely combines magnetic- and fluorescent-labeling of biomarkers. Chemokine interleukin-8 (IL-8) is a very useful biomarker in many areas, such as cancer, inflammation, prostatitis, and pyleonephritis. We use a two-site 'sandwich' immunoassay with paramagnetic beads conjugated to IL-8 antibodies. When IL-8 attaches to the beads, a biotinylated IL-8 antibody binds to a different IL-8 epitope.
Then, a fluorescent dye, such as phycoerythrin, is linked to the antibody via avidin/biotin interaction. Thus, the amount of emitted fluorescence is directly related to the amount of IL-8 detected. An alternating magnetic field gradient is used to concentrate the magnetic beads to a small volume, causing an increase in the fluorescent signal. The beads are set in periodic motion in and out of a laser beam, enabling the differentiation of background fluorescence from the oscillating IL-8 bound fluorescent signal. The modulation eliminates the need for washing steps, making a simpler assay that is useful for point-of-care applications. A dose response of IL-8 in buffer was performed, with a calculated level of detection of 0.04pg/ml with p<0.01. Additionally, we demonstrated that MMB has a 6-log dynamic range.

9725-22, Session 6

**Minimizing DNA microarrays to a single molecule per spot: using zero-mode waveguide technology to obtain kinetic data for a large number of short oligonucleotide hybridisation reactions**

Jens Sobek, Hubert Rehrauer, Ralph Schlapbach, ETH Zürich (Switzerland)

Single molecule interaction studies were performed by using a modified commercial DNA sequencer (RS2, Pacific Biosciences, Menlo Park). The optical part of the sequencer consists of a confocal microscope allowing parallel excitation and detection of fluorescence in 160000 zero-mode waveguide nanostructures on the SMRT (single-molecule real-time) chip surface. Short oligonucleotide probes covalently immobilized at the chip surface interact with dye labeled short oligonucleotides giving rise to fluorescence traces which allow calculation of association and dissociation rate constants. Kinetic data are compared with results from measurements applying different technologies, including surface plasmon resonance, interferometry, and thermophoresis.

In an extension of this method we show that hybridization properties of hundreds to thousands of different oligonucleotides can be investigated in parallel on a single chip. This can be achieved by immobilization of a mixture of oligonucleotides on a SMRT cell, the measurement of hybridization with an analyte oligonucleotide, and finally the determination of oligonucleotide structure in each ZMW by subsequent sequencing. This new method allows the generation of large sets of kinetic data. As an example, it enables the evaluation of hybridization properties of a perfect match oligonucleotide and additionally hundreds of related mismatch oligonucleotides in a single measurement.

9725-23, Session 6

**Novel label-free biosensing technology for monitoring of aqueous solutions**

Florian Kehl, ETH Zürich (Switzerland); Robert Bielecki, Optics Balzers AG (Liechtenstein); Stephane Follonier, Ctr. Suisse d’Electronique et de Microtechnique SA (Switzerland); Denis Dorokhin, Optics Balzers AG (Liechtenstein)

Waste water, drinking water and other industrial water sources are more and more/increasingly polluted with a large variety of contaminants, such as pesticides or residuals of pharmaceuticals. These compounds can impact human and animal organisms and lead to serious health issues. Today, in order to analyze the presence and quantity of the abovementioned micropollutants, samples are typically sent to specialized centralized laboratories and their processing may take up to several days. In order to meet the demand for continuous and consistent monitoring of aqueous solutions we propose a novel label-free technology system comprising proprietary chip and reader device designs.

The core of the system is constituted by a planar-grated-waveguide (PGW) chip. Label-free biosensors, based on PGWs are sensitive to effective refractive index changes caused by the adsorption of biomolecules (micropollutants) onto the sensor surface or due to refractive index changes of the bulk solution.

The presented reader device operates with a novel readout concept based on a scanning MEMS mirror for the angular interrogation of input grating couplers at a high repetition rate. The reader has fully integrated optics, electronics and fluidics and at the same time consumes limited energy (portable, field use ready).

In the recent experiments, the effectiveness of the technology has been demonstrated with various liquids and bioassays showing (i) an excellent refractometric sensitivity with a limit of detection towards effective refractive index changes of $\Delta n_{eff} \leq 2 \times 10^{-7}$, and (ii) the capability to perform affinity measurements for large (>150 kDa) and small (<250 Da) molecules.

9725-24, Session 6

**Thermo-optical tuning of cascaded double micro-ring resonators for dynamic range enhancement**

Prashanth R. Prasad, Shankar Kumar Selvaraja, Manoj M. Varma, Indian Institute of Science (India)

Silicon microrings offer a promise for realization of high performance, compact and low cost refractive index sensors. Measurement of spectral shifts of ring spectra can directly be linked with the change in the bulk or surface refractive index surrounding the ring. While this scheme is straightforward, it requires expensive, high resolution spectrometer measurement instruments for achieving acceptable limit of detection. An elegant solution to this problem is to use two microrings in series-cascade to convert spectral shift to intensity change. The intensity output in this configuration depends on the overlap of spectra of the two rings. This scheme provides high sensitivity owing to the Vernier effect. However, main drawback of this method is its low detection range. We show that the detection range of series-cascaded double-ring sensors can be enhanced by about ten times by tuning the spectrum of one of the two rings, while the other ring is used to probe the analyte. The extent of tuning required can be readily obtained using thermo-optic effect. In order to demonstrate this concept experimentally, we have fabricated cascaded double ring structures where a heating element is placed adjacent to one of the rings covered with an oxide spacer layer. We were able to achieve a spectral tuning of about 6 pm/mW using our device design, operating in the 1310 nm wavelength band, with a single mode rib waveguide (220 nm SOI, 70 nm etch depth, 600 nm width). Further improvements in the device design can provide better performance.

9725-26, Session 6

**Performance limitations of label-free sensors in molecular diagnosis using complex samples**

Manoj M. Varma, Indian Institute of Science (India)

Label-free biosensors promised a paradigm involving direct detection of biomarkers from complex samples such as serum without requiring multistep sample processing typical of labelled methods such as ELISA or immuno-fluorescence assays. Label-free sensors have witnessed decades of development with a veritable zoo of techniques available today exploiting a multitude of physical effects. It is appropriate now to critically assess whether label-free technologies have succeeded in delivering their promise with respect to diagnostic applications, particularly, ambitious goals such as early cancer detection using serum biomarkers, which require low limits of detection (LoD). Comparison of nearly 120 limits of detection (LoD)
values reported by labelled and label-free sensing approaches over a wide range of detection techniques and target molecules in serum revealed that labeled techniques achieve 2-3 orders of magnitude better LoDs. Data from experiments where labelled and label-free assays were performed simultaneously using the same assay parameters also confirm that the LoD achieved by labelled techniques is 2 to 3 orders of magnitude better than that by label-free techniques. Furthermore, label-free techniques required significant signal amplification, for e.g. using nanoparticle conjugated secondary antibodies, to achieve LoDs comparable to labelled methods substantially deviating from the original “direct detection” paradigm. This finding has important implications on the practical limits of applying label-free detection methods for molecular diagnosis. A simple mathematical model is presented to explain the observed gap in LoD between label-free and labelled detection methods and provides insights on the means to bridge this gap.