Technical Summaries

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Hemispherical Stokes polarimeter for early cancer diagnosis

P. Lemaillet, J. C. Ramella-Roman, The Catholic Univ. of America (United States)

Early diagnosis of melanoma is essential to provide the best treatment before skin cancer reaches metastasis. Analysis of the roughness of skin can provide valuable information to the physician for diagnosis or aid in the decision making of the physical removal. For this purpose, we present the development of a hemispherical imaging Stokes polarimeter designed to monitor skin cancer based on roughness assessment of the epidermis. The setup is composed of 16 out-of-plane polarized light illuminations tubes composed of a three color LED and a vertical polarizer, a Stokes polarimeter composed of 2 LCD retarders, a reference vertical polarizer and a fast acquisition camera. Calibration of the Stokes polarimeter uses a set of well-known input polarization states. A roughness gold standard and a facet model describing the principal angle of polarization of the analyzed light as a function of the angle of incidence are used to position each illumination polarizer. A set of phantoms mimicking the optical properties of skin at 633 nm as well as skin roughness were built using wax as a bulk material, titanium dioxide as scatterer and a black dye as the absorber. Images of the phantoms are presented and analyzed using a facet model.

Non-invasive in-vivo micro-Raman spectroscopy of a murine skin tumor model reveals cancer-specific spectral biomarkers

H. Wang, N. Huang, J. Zhao, The BC Cancer Agency Research Ctr. (Canada); H. Lui, The Univ. of British Columbia (Canada); M. Korbelik, H. Zeng, The BC Cancer Agency Research Ctr. (Canada)

We developed a micro-Raman spectrometer system for use in differentiating tumor lesions from normal skin and tested the system in an in vivo animal model. The axial resolution and lateral resolution of the system were measured to be 8.6 m and 2.2 m, respectively. Raman spectra with good signal-to-noise ratio (SNR) were obtained within 15 seconds under 27 mW of excitation light exposure to the skin surface. A study of 494 Raman spectra from 24 mice revealed different spectral patterns at different depths and between normal and tumor bearing skin sites. A peak at 899 cm-1 (possibly from proline or fatty acids) and one with higher intensity in the 1325 - 1330 cm-1 range (assigned to nucleic acids) were correlated with the presence of tumors and could potentially be used as biomarkers for skin cancer detection. PCA (principal component analysis) based spectral diagnosis performed on the murine tumor model achieved diagnostic sensitivity of 95.8% and specificity of 93.8%. These results encourage us to develop further the use of confocal Raman spectroscopy as a clinical tool for non-invasive human skin biochemical analysis, particularly in relation to skin cancer.

Enhancement methods of laser photon density in soft tissue: tissue temperature, laser modulation frequency, and their combination

C. Yeo, H. Park, D. Kang, B. Jung, Yonsei Univ. (Korea, Republic of)

Low-level laser therapy (LLLT) accomplished a remarkable improvement in clinic to date. However, the efficacy of LLLT in deep tissue layer may be negatively affected by strong tissue light scattering property which limits photon density. In order to enhance the photon density in deep tissue layer, this study suggests two independent methods and a combination method: 1) Temperature effect in tissue was studied with ex vivo porcine skins. Laser transmissions were quantitatively measured as a function of temperature from 20° to 0° at 5° decrement; 2) Diode laser in pulse mode was irradiated to porcine skin sample at various modulation frequencies of 10, 250, 500, 750, and 1kHz. Diffusion images were acquired with a CCD camera and analyzed; 3) The combination method of two methods was also evaluated. Results demonstrated 1) a linear increase in peak intensity of laser beam profiles, which demonstrates decreasing of µs, 2) increment of transmission in lower frequency than higher frequency, which means enhancement of photon density, and 3) better enhancement of photon density in the combination method than independent two methods. In conclusion, this study implies that both cooling and pulse-modulation methods in soft tissue may be effective methods to increase photon density in low-level laser therapy.
UV doses and skin effects during psoriasis climate therapy

L. L. Randeberg, E. L. P. Larsen, Norwegian Univ. of Science and Technology (Norway); L. T. N. Nilsen, The Norwegian Radiation Protection Authority (Norway); A. L. Krogstad, Oslo Univ. Hospital (Norway)

Psoriasis is a common autoimmune disease with inflammatory symptoms affecting skin and joints. One way of dealing with psoriasis is by controlled solar UV exposure treatment, climate therapy, as performed at the Norwegian Health Centre at Gran Canaria. However, this treatment has to be optimized and dangerous side effects such as erythema and an increased risk of skin cancer should be minimized.

A radiation transfer model used in previous studies (LibRadTran) was expanded to cover the whole year and adapted to estimate the daily UV doses obtained by the patient. The modelled UV doses were used as a basis to suggest sun exposure guidelines for the patients to optimize the effectiveness of their treatment. These guidelines were created with respect to weather conditions, daily and yearly UV radiation variations. This theoretical work is further supplemented by exploring the possibilities of applying diffuse skin reflectance measurements (in the visible and near infrared part of the spectrum) for the assessment of the skin changes during the climate therapy treatment. To refer the skin measurements to a specific UV intake the UV dose is to be measured with personal dosimeters worn by the patients. In this way the treatment and sun exposure effects will be recorded. These recordings and measurements will give a better scientific basis for further optimization of the treatment effect.

Real-time laser speckle imaging during port-wine stain laser treatment

O. Yang, B. Yang, S. Nelson, K. M. Kelly, B. Choi, Beckman Laser Institute and Medical Clinic (United States)

Port wine stain (PWS) birthmarks are vascular malformations seen in ~12,000 births a year. Since the majority of cases are found in highly visible regions on the face and neck, PWS can have a profound effect on an individual's psychosocial development. In addition to its obvious cosmetic detriments, facial PWS lesions have also been linked to a higher propensity for glaucoma and seizures, thereby compounding the need for a cure. Currently laser therapy only helps ~60% of the patients with complete disappearance a rarity.

The primary reason for the low success rate is a high degree of subjectivity involved in patient treatments such as selection of laser wavelength, pulse duration, and number of pulses, which depend on patient age, PWS size/location, and skin-type. One of the few canons in laser treatment of PWS is the need to achieve photococagulation which seals off PWS blood vessels, resulting in cessation of blood flow.

Laser-induced purpura is a common index used to assess photococagulation; however, laser-induced hemoglobin diffusion into the perivascular space can also manifest itself as purpura without photococagulation. Therefore a more definitive prognostic indicator is needed in order to confirm photococagulation. Laser speckle imaging (LSI), has the potential to fill this diagnostic void.

We have developed a graphics processing unit (GPU) based real-time LSI system that allows us to acquire real-time blood flow data. Access to real-time data gives the physician access to blood flow information at a time when treatment protocol can still be modified to yield more successful results.

Fluorescence lifetime imaging of skin cancer

R. Patelay, C. B. Talbot, I. H. Munro, Imperial College London (United Kingdom); G. Breunig, JenLab GmbH (Germany); K. Koenig, JenLab GmbH (Germany) and Saarland Univ. (Germany); Y. Alexandrov, S. Warren, M. A. Neil, P. M. W. French, Imperial College London (United Kingdom); A. Chu, Imperial College Healthcare NHS Trust (United Kingdom) and Imperial College London (United Kingdom); G. W. Stamp, The Royal Marsden Hospital (United Kingdom) and Imperial College London (United Kingdom); C. W. Dunsby, Imperial College London (United Kingdom)

The diagnosis of skin cancer often requires invasive biopsies. We are investigating the use of two photon microscopy (TPM) and fluorescence lifetime imaging as a non-invasive method for diagnosis. Fluorescence intensity imaging and spectroscopy have previously been used to distinguish malignancy in the skin, however, the use of fluorescence lifetime imaging microscopy (FLIM) in this field has been limited to-date. We have used a commercially available, clinically licensed system (DermalInspect®) to excite tissue autofluorescence in ex vivo skin cancer samples using a tunable Ti:Sapphire femtosecond pulsed laser (720nm-920nm). This system was modified to collect time resolved FLIM images using time correlated single photon counting in up to four spectral channels, together with paired steady state measurements of the fluorescence emission spectrum at each depth in the sample.

We characterise cellular autofluorescence using both manual and novel automatic image segmentation methods allowing fluorescence lifetimes to be calculated for each cell in each spectral channel. This technique was applied to images obtained from a range of skin cancer lesions ex vivo, including nodular Basal Cell Carcinomas (nBCC) (n=3, 441 cells) and dysplastic naevi (n=4, 401 cells). Clear morphological differences could be detected between diagnostic categories using fluorescence intensity images. In addition, we demonstrated a statistically significant increase in mean lifetime of nBCCs in comparison to dysplastic naevi. (2.01ns±0.28 vs 1.64ns±0.35) in the emission spectral channel 300-500nm. These results support the potential of FLIM and TPM as clinically useful modalities to better distinguish between benign and malignant tissue.
Temperature-dependent refractive index of fatty tissue measured by optical coherence tomography

H. Lim, T. E. Milner, The Univ. of Texas at Austin (United States)

Optical coherence tomography (OCT) has been used in various diagnostic imaging applications. OCT may also be utilized to estimate important optical properties of tissues including absorption coefficient, reduced scattering coefficient, attenuation coefficient and refractive index. Some physical properties (e.g., optical attenuation) of lipid-rich tissue have been reported to have an anomalous temperature dependence compared to other tissues. Lipid structures in tissue have been observed to demonstrate dynamic morphological changes as temperature is varied. As thermotherapy and cryosurgeries targeting lipid-rich tissues are more widely practiced, further studies on temperature dependent optical properties may provide investigators useful data.

In this experiment, we measured the group refractive index of lipid tissue while varying temperature from -6 to 37°C while recording OCT images. Rodent subcutaneous and visceral fat were imaged in vitro by OCT with a center wavelength of 1,300nm. Group refractive index of the lipid-rich tissue was derived by computing the ratio of optical path length and physical path length. Measured group refractive index of lipid-rich tissue at 20°C was 1.460 +/- 0.0027. Knowledge of the temperature dependence of group refractive index of lipid-rich tissues may give insight aiding development of OCT guided laser and cryo-therapeutic procedures.

Collateral damage-free debridement using 193 m ArF laser

J. J. Wynne, IBM Thomas J. Watson Research Ctr. (United States); J. M. Felsenstein, Private Dermatologist (United States); R. Trzcinski, D. Zupanski-Nielsen, D. P. Connors, IBM Thomas J. Watson Research Ctr. (United States)

In 1983, Lane et al irradiated the skin of live guinea pigs with 193 nm radiation from an ArF excimer laser, as well as with 248 nm radiation from a KrF excimer laser. They discovered that 193 nm laser radiation failed to remove (ablate) tissue after bleeding commenced. In contrast, 248 nm radiation continued to ablate tissue, despite bleeding. 193 nm radiation (at 6.4 eV) is strongly absorbed by aqueous salt solution, as found in blood, through the process of electron photodetachment from hydrated chloride ions (Cl-), with a characteristic resonance absorption maximum at 190 nm. Such an electronic excitation does not produce heat. This process depletes the laser fluence sufficiently to suppress further ablation of protein and lipids in tissue and/or blood.

We apply this knowledge to propose a novel technique to debride necrotic tissue associated with burns, decubitus, stasis, and neuropathic ulcers, without causing collateral damage to adjacent viable tissue. Results will be presented demonstrating that excimer laser irradiation of charred pig skin, in vitro, debrides burn eschar, while leaving the underlying skin free from damage.

Experiments are now underway to determine the optimum 193 nm ArF laser fluence for debriding necrotic erosions, in vivo, while sparing viable tissue sterile and free of collateral damage. Biopsies and cultures of the irradiated residual tissue will demonstrate such tissue suitable for skin grafting with minimal risk of infection. The viability of an actual skin graft will validate the effectiveness of this technique.

Reference: Randall J. Lane, Ralph Linsker, James J. Wynne, Abel Torres, and Roy G. Geronemus, Ultraviolet-Laser Ablation of Skin, Archives of Dermatology 121, pp. 609-617, May 1985

High-resolution multimodal clinical multiphoton tomography of skin

K. Koenig, Saarland Univ. (Germany)

CE-marked clinical multiphoton systems for 5D imaging of human skin with subcellular resolution, 10 nm spectral resolution, and 250 ps temporal resolution are in clinical use in Europe, Asia, and Australia. These tomographs provide optical biopsies with submicron resolution based on two-photon excited autofluorescence (NAD(P)H, flavoproteins, keratin, elastin, melanin, porphyrins) and second harmonic generation by collagen. Photons are collected by time-resolved single photon counting. The novel tomographs are employed for the early detection of malignant melanoma, atopic dermatitis, and the analysis of treatment effects as well as for the intratissue detection of cosmetic and pharmaceutical components including sunscreen nanoparticles. Novel developments include the combination with ultrasound, optical coherence tomography, CARS, and diffuse reflectance.

Characterizing variability in Raman Spectra of benign lesions toward cancer detection in skin

I. J. Pence, C. A. Patil, E. Vargis, B. Caldwell, D. L. Ellis, A. Mahadevan-Jansen, Vanderbilt Univ. (United States)

Clinical diagnosis of malignant and benign skin lesions is often difficult because of the subjective nature of visual inspection and the potential for sampling error in biopsy. Raman Spectroscopy has demonstrated the potential to perform non-invasive classification of skin lesions, however, the high level of physiological and anatomical variability in benign skin can complicate optical diagnosis. A thorough understanding of benign lesion’s variability both between patients and within a single patient may lead to improved diagnostic outcomes. Here, we present a fiber-optic probe-based 785nm Raman Spectroscopy study of 164 patients with benign lesions, which included seborrhoeic keratosis, actinic keratosis, basal cell carcinoma, squamous cell carcinoma, dysplastic nevus, congenital nevus, angioma, dermatofibroma, sebaceous hyperplasia, and solar lentigo. Measurements were made of both the lesions and adjacent or contralateral normal skin. Diagnosis of the lesions was performed by dermatologists through visual inspection of patients prior to data collection. We report an analysis of the spectral variability of normal skin and common benign lesions. Characterization of these classes of skin is a critical first step in the formation of a non-malignant spectral database that will serve as the basis for future comparisons with malignant lesions.

In-situ and in-vivo imaging of microneedle insertion into human skin using optical coherence tomography

B. Pova?ay, Medizinische Univ. Wien (Austria); S. Coulman, A. P. Alex, M. Pearnton, Cardiff Univ. (United Kingdom); B. Hofer, Medizinische Univ. Wien (Austria); C. O’Mahony, Tyndall National Institute (Ireland); W. Drexlert, Medizinische Univ. Wien (Austria); J. C. Birchall, Cardiff Univ. (United Kingdom)

Microneedles are being developed to minimize pain and injury to the skin, reduce costs and simplify injection procedures. The main goal for vaccination and other treatment applications is the optimization of penetration depth and reliability of transport as well as the fast sealing of the formed conduits. Optical coherence tomography (OCT) is a non-invasive imaging method that is well established in ophthalmology and gains momentum for quasi-instantaneous histology-level examination in
clinical environments. In the current study an OCT-system operating at 20 kHz line acquisition rate (a 262 megavoxel volume is raster scanned within 13 s), 800 nm central wavelength and 160 nm bandwidth, achieves axial and transverse resolutions of <4 and <6 µm. It is able to visualize all epidermal layers as well as the papillary dermis and most portions of the reticular dermis, depending on the skin type. Image resolution and contrast degrades with imaging depth due to scattering at this wavelength. However, microneedles aim to reach the dermal-epidermal junction only, where highly immune-competent cells can interact with the formulation to avoid direct contact to the vascular system. This work unveils significant differences between multiple needle types and their impact on human skin of multiple subjects and locations. Furthermore a comparison with the gold standard of histology or standard hypodermic needles shows much smaller remaining microchannels in either case due to self-sealing of living tissue and underlines the necessity of in vivo monitoring.

7883A-15, Session 3
Comparison of skin responses from macroscopic and microscopic UV challenges
I. Seo, P. R. Bargo, M. Chu, E. C. Ruvolo, Jr., N. Kollias, Johnson & Johnson CPPW (United States)
The traditional phototest to determine the sensitivity of skin to ultraviolet radiation has been performed in skin areas ranging 1 cm² and 1 in². There have been reports that the apparent sensitivity of the skin, expressed as erythema, decreases for areas of the order of 1 mm² and smaller. In this study, we investigated the responses of human skin to solar-simulated radiation with a beam diameter of 200 mm (0.03 mm²). Twelve human subjects of skin phototype I-IV were exposed to solar-simulated radiation on their upper inner arm or on their lower back with a series of doses in increments of 20% in order to determine the threshold dose to induce a minimal perceptible erythema response (MED). Each dose was delivered with a liquid light guide of 8 mm diameter and with quartz optical fibers of 200 mm diameter. The resulting skin responses were evaluated visually and investigated with a reflectance confocal microscope and imaging. The erythema response to the microscopic challenge was always diffuse with no clear boundaries extending to several times the exposed site diameter at doses greater than 2MED. The pigment responses induced by UV exposure were observed both by video microscope and confocal microscope on 5-7 days after exposure. Unlike the erythema response, pigment responses to the microscopic challenge were always confined within the irradiated sites and the produced melanin is non-uniformly granulated and different from constitutive melanin in size and shape. The skin returned to normal appearance from the microscopic challenge after two weeks of exposure while change in appearance for the larger areas persisted for several weeks to months. This new modality of testing provides the possibility to study skin at the microscopic level with a rapid recovery following challenge.

7883A-17, Session 4
Skin autofluorescence variation with body sites characterized by fluorescence excitation emission matrix (EEM) spectroscopy
J. Zhao, The Univ. of British Columbia (Canada) and The BC Cancer Agency Research Ctr. (Canada); F. Feng, The Univ. of British Columbia (Canada); H. Zeng, The Univ. of British Columbia (Canada) and The BC Cancer Agency Research Ctr. (Canada); D. I. McLean, H. Lui, The Univ. of British Columbia (Canada)
Background: Autofluorescence spectra of skin depend on a combination of the excitation/emission wavelengths as well as the physiological and pathological state of the skin. Previous studies showed that aged skin and diseased skin exhibit characteristic changes in skin autofluorescence. However, most of these studies were limited to a few excitation or emission wavelengths. Variations in skin autofluorescence due to anatomic body site have never been systematically examined. Objective: The objective of this study was to systematically characterize the in vivo autofluorescence properties of human skin at various sites using fluorescence excitation emission matrix (EEM) spectroscopy. Materials and Methods: In this study, we compared skin autofluorescence properties among 10 anatomic skin sites using a high-precision spectrofluorometer based on two double-grating monochromators. The measurement sites included the forehead, cheek, nose, neck, palm, thumbnail, hand, forearm, arm and mid-back. The excitation and emission wavelengths were 260 - 450 nm and 300 - 700 nm with 5 nm steps, respectively. To date twenty-six healthy subjects participated in this study with average age of 34 (21- 74) years old, 18 male and 8 female. Results and Conclusions: The major skin fluorophores can be easily identified from the 2D contour plots of the EEM skin autofluorescence spectra, including tryptophan, collagen, elastin and NADH peaks. On average, facial skins showed strong tryptophan fluorescence and measurable porphyrin fluorescence; palm and nail skins show strong tryptophan and keratin fluorescence; the rest show strong tryptophan and/or collagen/elastin fluorescence. These results are useful for non-invasively profiling the biochemical of skin and provide a basis for future interpretation of autofluorescence in skin disease.
Non-invasive multimodal confocal imaging of squamous cell carcinoma in mice

A. N. Yaroslavsky, Univ. of Massachusetts Lowell (United States); P. A. Mroz, Harvard Medical School (United States) and Massachusetts General Hospital (United States); V. A. Neel, Massachusetts General Hospital (United States)

Background and objective: As the incidence of nonmelanoma skin cancers continues to rise, the development of noninvasive methods for their detection becomes increasingly more important. In this contribution we present and correlate to histopathology reflectance and fluorescence confocal images acquired in vivo and noninvasively from squamous cell carcinomas (SCC) developed in SENCAR mice.

Materials and Methods: In total, 20 SENCAR mice were used for the experiments. The tumors were induced in 6-8 weeks old male mice using 7,12-dimethylbenz[a]anthracene. Carcinogenesis was promoted by topical application of 12-O-tetradecanoylphorbol-13-acetate twice a week. After 20 - 25 weeks of promotion all mice developed multiple papillomas and SCC lesions. Prior to imaging all mice were anesthetized. 0.25 mg/ml aqueous MB was injected around cancerous areas. In vivo noninvasive imaging was performed using a multimodal confocal system. Reflectance images were acquired at 658 and 785 nm. Fluorescence images were excited at 658 nm and registered between 690 and 710 nm. The system provided the lateral resolution of 0.6 µm and axial resolution of 7 µm. Imaging results were compared to the respective H&E histopathology.

Results and Conclusions: The imaging system was capable of acquiring high-quality multimodal images of SCC down to the depths of 250 µm. Both reflectance and fluorescence images exhibited patterns close to those of human SCC and correlated well with histopathology. Squamous cell carcinoma of SENCAR mouse is suitable for modeling human SCC.

Next generation Er:YAG fractional ablative laser

B. Nussbaumer, Pantec Engineering AG (Liechtenstein); A. Heinrich, A. Vizhanyo, P. Krammer, S. Summer, S. Gross, T. Bragagna, C. Böhler, Pantec Biosolutions AG (Liechtenstein)

The P.L.E.A.S.E.® Professional is a portable bench top fractional ablative laser system emitting at 2.94 µm (Er:YAG). The wavelength is on the main water absorption peak in the infrared and allows accurate and painless removal of the outer skin layer. The new miniaturized diode pumped laser is a small, lightweight and cost efficient device that allows a fourfold reduction in volume and weight compared with standard lamp pumped lasers based devices. The main applications are in conventional and aesthetic dermatology with or without a topical drug. The device allows precise skin microporation with depth control, fundamental requisite in transdermal drug delivery applications. The microporation process yields the high repetition rate of up to 500 Hz which allows single pore drilling while the incorporated beam deflection unit makes the device flexible in pore density. Furthermore, by during the process changing of the laser parameters it is possible to bring heat into the skin layers to increase the rejuvenation effect.

The P.L.E.A.S.E.® Professional has been used to deliver large molecular weight biopharmaceuticals through the skin barrier. With FSH (follicle stimulating hormone) a protein of 32 kDa size entered stably and reproducibly across the skin from a patch in the blood circulation and has shown excellent bioavailability, safety and tolerability in phase I clinical trials. For controlled drug release into the systemic circulation the device has to create defined micro pores in the stratum corneum and therefore it incorporates a skin layer detection, which guarantees that the micropores reach the desired layers.
Conference 7883A: Photonics in Dermatology and Plastic Surgery

7883A-22, Session 4

Comparative study of skin wrinkle assessment: computer-assisted 2D image analysis and μ-CT
S. Eom, Y. Bae, C. Ko, H. S. Kim, B. Jung, Yonsei Univ. (Korea, Republic of)

As life expectancy has been increased, quantitative assessment and evaluation of skin surface, especially morphological changes of the skin wrinkle, plays an important role in the medical and cosmetic fields. This study attempts to analyze facial expression lines from different modalities and deduce quantitative diagnostic data in order to confirm the clinical usefulness of the methodology of 2D image processing for wrinkle assessment. Silicon replicas were used to sample facial skin surface wrinkle at the regions of nasolabial groove, corner of the eye and forehead from every twenty healthy Korean males and females with ages ranging from 20 to 50 years old. Intuitively, we evaluate diagnostic parameters such as total facial skin wrinkle length, average depth of the furrows, wrinkle interval and the facial wrinkles through cross-sectioned replica using μ-CT and microscopy to verify the usefulness of μ-CT for skin wrinkle assessment. Finally, the same parameters were also extracted from 2D image and μ-CT methods. In the computer-assisted 2D image processing method, “shape factor” and “shade-correction” algorithm was mainly applied in order to extract pre-defined wrinkle like objects. The μ-CT extraction facial skin wrinkle parameters by calculating the straight line distance between two points and 3D image of the replicas was reconstructed using commercial software. There is statistically meaningful correlation between the data from μ-CT and 2D images. In this study, we propose facial skin wrinkle evaluation methodology and confirm clinical usefulness of 2D image processing method by comparing the data with the one from μ-CT.

7883A-23, Session 5

Low-cost/high-efficiency lasers for medical applications in the 14XX-nm regime
E. McIntyre, J. J. Callahan, D. Bean, L. Yanushefski, SemiNex Corp. (United States)

Laser therapy is becoming an increasingly popular method of treating numerous dermatological conditions. The widespread use of these devices is often limited by the cost and size. Low cost, portable lasers would expand the laser market further into homes, general practitioners, dermatologists, plastic surgeons, and 3rd world countries. There are numerous light devices currently on the market for hair removal and growth, acne reduction, and wrinkles. These devices are varied, from LEDs to intense pulsed light (IPL) to lasers. One particular disease is leishmaniasis, caused by a parasite carried by sand flies, most often occurring in third world countries. While there are drug therapies available, they sometimes require hospitalization for several days and are very expensive. An RF device has been FDA approved for treatment of leishmaniasis, but costs about $20,000 which is too expensive for widespread use. Since the method is heating the lesion, the same effect could be achieved using an infrared laser. Diode lasers have the capability to be produced in mass quantity for low cost, as shown by the ubiquity of diode lasers in the telecom industry and household appliances. Unfortunately, many diode lasers suffer from poor efficiency, particularly in wavelengths for dermatology. Advances are being made to improve wall plug efficiency of lasers to reduce waste heat and increase output power. In this paper, those efforts being made to develop manufacturing partners to lower the cost while increasing the production volume of long wavelength lasers will be discussed along with performance data and clinical results.

7883A-24, Session 5

Motion correction in spatial frequency domain imaging: optical property determination in pigmented lesions
J. Q. Nguyen, R. B. Saager, Beckman Laser Institute and Medical Clinic (United States); D. J. Cuccia, Modulated Imaging, Inc. (United States); K. M. Kelly, D. J. Hsiang, Univ. of California, Irvine (United States); A. J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

Spatial Frequency Domain Imaging (SFDI) is a relatively new method of optical interrogation that allows for a wide-field and non-contact probing of turbid media. By combining ‘structured illumination’ projections with a camera-based imaging system, subsurface optical-properties and chromophore concentrations of in-vivo skin can be quantified. In particular, we are in the process of characterizing malignant melanoma and benign pigmented lesions. While SFDI has proven to be a useful tool in various research endeavors, there is still the important challenge of developing a clinically deployable SFDI system. Part of this challenge is related to the development of strategies for effective compensation of subject motion, which is critical for recording high fidelity optical properties. In order to address this issue, we have developed a motion correction procedure that utilizes canny edge-detection to reposition image-sets into a singular region-of-interest during data processing. Stationary phantom measurements are then used to correct for the light intensity roll-off that occurs when the targeted lesion is moved through an area illuminated the divergent planar light-source used by the SFDI instrumentation. Finally the collected image-sets are processed by a modified demodulation formula based on an averaged correction factor. By comparing the results of this adjusted processing method with data gathered by the current SFDI processing system, we are able to systematically characterize the impact of motion variables on SFDI measurements.

7883A-25, Session 5

In-vivo analysis of human skin anisotropy by polarization-sensitive optical coherence tomography
S. Sakai, Kanebo Cosmetics Inc. (Japan); M. Yamanari, Y. Lim, Univ. of Tsukuba (Japan); N. Nakagawa, Kanebo Cosmetics Inc. (Japan); S. Makita, Y. Yasuno, Univ. of Tsukuba (Japan)

An understanding of skin anisotropy is crucial for plastic surgeons, as the severity of scar formation is known to be associated with the direction of the incision. Some cleavage lines, such as Langer’s lines and relaxed skin tension lines (RSTLs), have been proposed as keys to understanding skin anisotropy. Skin anisotropy is another important issue in cosmetic science, as it reportedly changes with age. Collagen, a dominant dermal structural protein, forms a fibrous structure believed to play an important role in skin anisotropy. There have been few reports, however, on the relationship between the running of collagen fiber and the direction of the cleavage line. This issue presents a challenge, as it is difficult to correctly evaluate the direction of collagen fiber in excised skin without internal stress. We have focused in vivo evaluation of the direction of collagen fiber. Collagen fiber has birefringence, a property analyzable in skin in three dimensions by high-speed polarization-sensitive optical coherence tomography (PS-OCT). Here we used PS-OCT for an in vivo analysis of anisotropic changes in the dermal birefringence of mechanically stretched human skin. The dermal birefringence of the forehead increased significantly when the skin was stretched in parallel with the RSTL and decreased significantly when the skin was stretched perpendicularly to the RSTL. En-face images of dermal birefringence revealed that stretching in parallel with the RSTL promoted the formation of a macro rope-like collagen structure. These results suggest that PS-OCT enables the in vivo evaluation of skin anisotropy.

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TEL: +1 360 676 3290 · +1 888 504 8171 · customerservice@spie.org
Intravital multiphoton tomography as an appropriate tool for non-invasive in-vivo analysis of human skin affected with atopic dermatitis

V. Huck, Westfälische Wilhelms-Universität Münster (Germany); C. Gorzelany, Ruprecht-Karls-Universität Heidelberg (Germany); K. Thomas, Westfälische Wilhelms-Universität Münster (Germany); M. Schwarz, I. Riemann, Fraunhofer-Institut für Biomedizinische Technik (Germany); C. Mess, V. Niemeyer, Ruprecht-Karls-Universität Heidelberg (Germany); T. A. Luger, Westfälische Wilhelms-Universität Münster (Germany); K. Koenig, JenLab GmbH (Germany); S. W. Schneider, Ruprecht-Karls-Universität Heidelberg (Germany)

Increasing incidence of inflammatory skin diseases such as Atopic Dermatitis (AD) has been noted in the past years. According to recent estimations around 15% of newborn subjects are affected with a disease severity that requires medical treatment. Although its pathogenesis is multifactorial, recent reports indicate that an impaired physical skin barrier predispose for the development of AD. The major part of this barrier is formed by the stratum corneum (SC) wherein cornocytes are embedded in a complex matrix of proteins and lipids. Its components were synthesized in the stratum granulosum (SG) and secreted via lamellar bodies at the SC/SG interface.

Within a clinical in vivo study we focused on the skin metabolism at the SC/SG interface in AD affected patients in comparison to healthy subjects. Measurement of fluorescence life-time of NADH provides access to the metabolic state of skin. Due to the application of a 5D intravital tomographic skin analysis we facilitate the non-invasive investigation of human epidermis in the longitudinal course of AD therapy. We could ascertain by blinded analysis of 40 skin areas of 20 patients in a three month follow-up that the metabolic status at the SC/SG interface was altered in AD compromised skin even in non-lesional, apparent healthy skin regions. This illustrates an impaired skin barrier formation even at non-affected skin of AD subjects appearing promotive for the development of acute skin inflammation. Therefore, our findings allow a deeper understanding of the individual disease development and the improved management of the therapeutic intervention in clinical application.

In-vivo investigation of the evolution of skin barrier repair after mechanical injury

S. Walston, M. Chu, I. Seo, P. R. Bargo, N. Kollias, Johnson & Johnson CPPW (United States)

The outmost layer of skin, the stratum corneum (SC), serves a primary function of skin barrier which is vital for the existence of terrestrial life. Disruption of the SC stimulates a repair response in the underlying viable epidermis which has been shown to be regulated by ions (i.e. Calcium), cytokines, proteinase-activated receptor 2 (PAR2) and nitric oxide (NO). Signaling pathways for SC formation and the composition of the lipid-enriched lamellar membranes that compose the SC have been extensively studied in the few studies that have been performed on evaluating human SC repair in vivo, non-invasively. In the present work tape stripping was used as a model for mechanical injury of the SC and the injuries were followed for several days. Seven healthy volunteers were given informed consent form and recruited for the study. Tape stripping was performed on the arms and legs of the volunteers until all the SC was removed. The injured site and a control adjacent site were measured before, immediately after, 3 hours, 1, 2, 3, 7 and 10 days after the injury. Transepidermal water loss (TEWL), tryptophan fluorescence and reflectance confocal microscopy were used to determine permeability of the skin barrier, cell turnover and epidermis morphology, respectively. The results show an exponential rate of recovery for the skin permeability (TEWL) which contrasted with a linear increase in the thickness of the SC as determined by confocal microscopy. This may indicate that the quality of the SC after re-formation may not only be determined by its thickness but also it morphology. Cell turnover increased rapidly immediately after the injury to 2.5 times the levels of the control site, attaining a maximum of 3.5-4 times greater levels after three days and slowly returned to baseline levels after the ten days. Correlation of the cell turnover to the thickness of the viable epidermis was observed and further studies are under way to interpret these results.

DNA/RNA, DNA-DNA, and DNA-protein interactions in diagnosis of skin cancers by IR microspectroscopy

N. Skrebova Eikje, MC Professional OÜ (Estonia)

New diagnostic and complimentary tools in dermatology are desired to improve clinical diagnoses. Skin tissue infrared (IR) microspectroscopy may work as an optical diagnostic method for detection of skin cancer and its progression, because of the great advantage of IR spectra to provide an enormous amount of information about the structure of proteins and nucleic acids that might characterize skin carcinogenesis and certain skin cancers. So, IR absorption spectra of BCC, SCC and MM skin samples revealed appearance of DNA peaks at about 965, 1071, 1084, 1095 and 1245 cm⁻¹, the intensities of which strongly correlated with the intensity of the multiplet around 1055 cm⁻¹. Moreover, the latter DNA/RNA peak was significant in all cancerous samples. The sensitivity of IR microspectroscopy to study DNA-DNA and DNA-protein interactions was very high, and showed as common, so as specific features for BCC, SCC and MM. In conclusion, skin IR tissue changes in the 900-1300 cm⁻¹ region due to DNA and DNA/RNA intensity variations are true indicators of cancerous disease and its activity, and need further investigations. This may lead the development of skin tissue IR microspectroscopy technique as a tool in diagnosing skin cancer and its progression in dermatological environment.

Postoperative assessment in a cutaneous flap model using spatial frequency domain imaging

A. Yafi, S. Patel, R. B. Saager, Beckman Laser Institute and Medical Clinic (United States); D. J. Cuccia, Modulated Imaging, Inc. (United States); G. Evans, Univ. of California, Irvine (United States); A. J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

The purpose of this study is to investigate the capabilities of a new optical wide-field imaging technology known as Spatial Frequency Domain Imaging (SFDI) to quantitatively assess reconstructive tissue status. Twenty two cutaneous pedicle flaps were created on eleven rats based on the inferior epigastric vessels. After baseline measurement, all flaps underwent vascular ischemia, induced by clamping the supporting vessels for two hours (either arterio-venous or selective vascular occlusion). A normal saline solution was injected to the control flap, and hypertonic hyperoncotic saline solution to the experimental flap. The flaps were monitored another two hours after reperfusion and then once every 24 hrs for a total of 96 hrs. SFDI measurements were made to record flap status over the duration of the experiment, including the ischemia and reperfusion phase. After reperfusion and saline solution was administered, oxyhemoglobin (HbO2) and tissue oxygen saturation (StO2) values gradually returned to baseline levels in those flaps that ultimately survived. However, flaps for which HbO2 and StO2 remained at the same ischemic level appeared to be compromised and eventually became necrotic after 24-48 hours in both occlusion groups. As a point of reference, the chromophore concentrations for intact skin...
demonstrated minimal fluctuations over the course of data acquisition. This study demonstrates the potential of this optical technology to assess tissue metabolic status in a very precise and quantitative way, enabling wide-field visualization of chromophore concentration. The results of this study suggest that SFDI may provide a means for prospectively identifying dysfunctional flaps well in advance of failure.

7883A-31, Session 6

In-vivo multiphoton imaging of collagen remodeling after micro-ablative fractional rejuvenation

R. Cicchi, D. Kapsokalyvas, M. Troiano, P. Campolmi, C. Morini, A. Cosci, D. Massi, T. Lotti, F. S. Pavone, Univ. degli Studi di Firenze (Italy)

The potential of multiphoton microscopy in providing in-vivo early diagnosis of skin lesions has already been demonstrated, while its capability in therapy or follow-up has not been deeply explored so far. Two-photon excited fluorescence and second-harmonic generation microscopy were used in combination to follow-up collagen remodeling after laser micro ablative rejuvenation. Treated regions of volunteers were imaged with multiphoton microscopy before and after treatment, and we found a strong age-dependence of the treatment effectiveness. In particular, the photo-rejuvenating effect was negligible in young subjects (< 30 years), whereas a significant production of new collagen was observed in aged subjects (> 70 years). Quantification of the amount of newly produced collagen and its organization were performed by means of two image-analysis methods, respectively based on second-harmonic to autofluorescence ageing index of dermis and grey-level co-occurrence matrix. The obtained results demonstrate the performance of laser fractional micro ablative rejuvenation without the need of an invasive biopsy as well as the wide range of applications for multiphoton microscopy in clinical dermatology.

7883A-32, Session 6

Dual-effect laser handpiece for modification of tissue permeability

K. McMillan, gRadiant Research, LLC (United States)

Many opportunities exist for the combination of light with drugs, and these may be particularly advantageous when the drug can be applied topically. The challenge with topical application is obtaining therapeutic dosages. The premise of this work is that it is advantageous to modify the permeability of skin to improve both amount of drug reaching the target tissue and the time that the drug resides in that tissue. Topically applied agents that reach the dermis are removed by diffusion and uptake by blood vessels. Using the techniques of photothermal vascular targeting, it may be possible to decrease vascular uptake in the dermal layer. In this work, the dermal distribution of an exemplary topically applied drug, mitomycin C, is calculated, considering both diffusion and vascular uptake in the skin, according to a distributed model. Monte Carlo calculations are used to characterize the depth and extent of damage to the vascular supply of skin using parameters consistent with an exemplary 585 nm pulsed dye laser, and those results are used to modify the vascular uptake parameters in the distributed model. Then, the clinical significance of the modifications of skin permeability is evaluated by comparing intradermal drug exposures predicted by the model, with exposures of MMC known to be effective in killing squamous cell carcinoma cells. Results indicate that vascular targeting has the potential to improve the effectiveness of topical drugs, for treatment of skin cancer and for other applications. Lastly, a newly developed laser handpiece for modification of tissue permeability is described.

7883A-33, Session 6

In-vivo optical investigation of psoriasis

D. Kapsokalyvas, R. Cicchi, N. Bruscinio, A. Cosci, D. Massi, T. Lotti, F. S. Pavone, Univ. degli Studi di Firenze (Italy)

Psoriasis is an autoimmune disease of the skin characterized by hyperkeratosis, hyperproliferation of the epidermis, inflammatory cell accumulation and increased dilatation of dermal papillary blood vessels. Cases of psoriasis were investigated in vivo with optical means in order to evaluate the potential of in vivo optical biopsy. A Polarization Multispectral Dermoscope was employed for the macroscopic observation. Features such as the ‘dotted’ blood vessels pattern was observed and illustrated with high contrast. The density of blood vessels and thickness were found to be significantly higher compared to normal skin. On the other hand, microscopic observation was performed with a custom made multiphoton microscope. Imaging extended from the surface of the lesion down to the papillary dermis, at a depth of 200 µm. The morphological observation of psoriatic lesions, compared to healthy skin, revealed an abnormal and thick stratum corneum, an increased epidermal thickness and deep epidermal proliferation. Furthermore, dermal papillae appeared larger in diameter and in length. Additionally, spectral and fluorescence lifetime imaging measurements revealed higher fluorescence in psoriasis due to hyperkeratosis. These in vivo observations are consistent with the ex vivo histopathological observations, supporting both the applicability and potentiality of multispectral dermoscopy and multiphoton microscopy in the field of in vivo optical investigation and biopsy of skin.

7883A-34, Poster Session

Improvement of in vivo rat skin optical clearing with chemical penetration enhancers

J. Wang, D. Zhu, Britton Chance Ctr. for Biomedical Photonics (China)

Optical method plays an important role in clinical diagnosis and treatment, but suffers from limited penetration depth of light in turbid tissue. The optical clearing technique can improve the light delivery significantly through immersion of tissues into Optical Clearing Agents (OCAs). However, the barrier function of stratum corneum makes it difficult for optical clearing of skin by topical application of OCAs. Addition of penetration enhancers to OCAs can improve the skin clearing efficacy, but most investigations were performed on in vitro skin. Here, to evaluate the efficacy of this method on in vivo skin, direct observation and measurement of diffuse reflectance spectra were performed after topical application of different mixtures. One OCA, PEG-400, and three penetration enhancers (PEs), Thiazone, Azone and Propylene Glycol (PG), were used. The results indicated that the addition of penetration enhancers could improve the optical clearing efficacy of rat skin in vivo significantly, the dermal blood vessels could be observed directly with PEs. Among the three penetration enhancers, Thiazone induced the largest enhancement of clearing efficacy, and the enhancement induced by PG is the least. This study is very helpful for in vivo application of OCAs to enhance skin optical clearing non-invasively.
Skin optical properties control by delivery of molecules and particles through a fractionally ablated skin

E. A. Genina, L. E. Dolotov, A. N. Bashkatov, V. V. Tuchin, N.G. Chernyshyevsky Saratov State Univ. (Russian Federation); G. B. Altshuler, I. V. Yaroslavsky, D. Tabatadze, Palomar Medical Technologies, Inc. (United States); A. V. Belikov, A. V. Skrypnik, Saint-Petersburg State Univ. of Information Technologies, Mechanics and Optics (Russian Federation); C. C. Dierickx, Skin and Laser Ctr. (Belgium)

We are presenting a variety of technologies for effective delivery of dye molecules in solutions and micro- and nanoparticles in suspensions as well as skin optical properties control.

Fractional ablation was provided by a modified StarLux/Lux2940 system (Palomar Medical Technologies Inc.) emitting 250 µs pulses. In vitro human and porcine skin and in vivo human and mini-pig skin were investigated. Clinical photography and quantitative analysis of ablated and treated skin sites using OCT and reflectance spectroscopy were used. To enhance particle redistribution within the treated skin, an ultrasound device Dynatron-125 was employed. For in vitro study 10 pig skin sites were investigated. For in vivo study, total of 24 skin sites including control of one mini-pig and 20 of one human subject were investigated. Suspensions of PEGylated TiO2 nanoparticles (100 nm), ZrO2 (5 nm), and Al2O3 microparticles (27 mm) with concentrations from 5 to 500 mg/ml and hydrocortisone were delivered into the skin. Biopsy and histology was done for in vivo mini-pig treated skin sites.

Monte Carlo computer modeling of human skin spectral reflectance at delivery of differently-sized nanoparticles with a variety of refractive indices and concentration was done.

It was found that particles and hydrocortisone molecules can be delivered into epidermis and dermis with fractional Er:YAG laser, application of ultrasound allows for more effective particle delivery within the skin, particles may stay within the skin in vivo during a few weeks. Computer modeling well describes the main features of the skin back reflectance spectra with the inserted nanoparticles.

Raman spectra and optical coherent tomography images of skin

A. E. Villanueva-Luna, Instituto Nacional de Astrofisica, Optica y Electronica (Mexico); J. Castro-Ramos, S. Vazquez-Montiel, J. A. Delgado Atencio, Instituto Nacional de Astrofisica, Optica y Electronica (Mexico); A. Flores-Gil, Univ. Autonoma del Carmen (Mexico); A. Vazquez-Villa, Instituto Nacional de Astrofisica, Optica y Electronica (Mexico)

The optical coherence tomography images are useful to see the internal profile and structure of sample. In this work, OCT images were recorded in 10 volunteers with different skin tone which were related to Raman spectra. The areas where we obtained OCT images and Raman spectra were in the middle of the fingers, between the forefinger and thumb, fingernails and the tips of the middle finger and thumb areas measured were for the purpose of finding the extracellular fluids with contain uric acid, cholesterol and glucose that are reported in the literature. The excitation wavelength used for this work is 785 nm, a spectrometer of 6 cm-1 resolution. The spectral region used ranges from 300 to 1800 cm-1. We use an OCT with 930nm of Central Wavelength, 1.6nm of Image Depth, 6 mm of image width and 6.2 um of axial resolution. As a main objective to find variations that are keratin in nails that were taken as sample.
7883B-39, Session 1

Fourier-domain versus time-domain optical coherence tomography of the prostate nerves

S. Chitchian, The Univ. of North Carolina at Charlotte (United States); G. A. Lagoda, A. L. Burnett, The Johns Hopkins Univ. (United States); N. M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: The main advantage of Fourier domain (FD) optical coherence tomography (OCT) over time domain (TD) OCT is faster image acquisition rates for potential 3D image reconstruction. However, FD-OCT suffers from poor image depth in opaque tissues (e.g., prostate). In this study, we compare the two different OCT techniques for imaging the rat prostate and cavernous nerves, in vivo.

Methods: A TD-OCT system acquiring images at 200 x 200 pixels, 11 micron axial and 25 micron lateral resolutions in tissue and an acquisition rate of 0.7 frames per second (fps) was used. A swept-source FD-OCT system with 256 x 2000 pixels, 9 micron axial and 15 micron lateral resolutions in tissue, and an acquisition rate of 25 fps, was also used. Signal-to-noise ratio (SNR), contrast-to-noise ratio (CNR) and equivalent number of looks (ENL) were measured to assess OCT performance. Image denoising was also performed during post-processing.

Results: Limitations of FD-OCT included increased multiple scattering, reduced penetration depth, low frequency noisy images, loss of resolution with increasing depth, central wavelength saturation, and complex conjugate ambiguity. SNR's were higher for TD-OCT compared to FD-OCT (41 vs. 33 dB). TD-OCT also performs higher ENL values (2467 vs. 1016), corresponding to smoother imaging. However, FD-OCT is superior for differentiating the cavernous nerves from the prostate, with a higher CNR (15 vs. 11 dB).

Conclusion: TD-OCT performs better for imaging opaque tissues, such as the prostate, where image depth is critical. Denoising is also more effective for TD-OCT compared to FD-OCT with respect to SNR.

7883B-41, Session 1

Study on extremity oxygenation assessing of hemodialysis patients based on near-infrared spectroscopy

C. Wang, National Chiao Tung Univ. (Taiwan); C. Chuang, National Taiwan Univ. (Taiwan); C. Lin, Taipei Veterans General Hospital (Taiwan); Y. Hsieh, National Chiao Tung Univ. (Taiwan); C. Sun, National Yang-Ming Univ. (Taiwan)

Hemodialysis utilizes counter current flow, where the dialysate is flowing in the opposite direction to blood flow in the extracorporeal circuit. Several side effects, including low blood pressure, fatigue, chest pains, nausea, headaches and muscle-cramps, are originated by removing too much fluid rapidly. Meanwhile, it leads to reduce oxygen concentration, especially with the ipsilateral of the arteriovenous fistula. Near-infrared spectroscopy (NIRS) has been shown to be an effective tool for measuring local changes of tissue in hemodynamics. Diffuse photon can penetrate several centimeters through the tissue to measure the difference in the concentrations of oxy-hemoglobin (HbO2) and deoxy-hemoglobin (Hb). Thus, the aim of this study is to investigate the influence of hemodynamic analysis on hemodialysis patients. The NIRS measurements were carried out with an OxiPlex instrument (Noninvasive tissue oximeter; ISS Inc., Champaign, USA). This instrument features eight intensity-modulated laser diodes, eight emitting at a wavelength of 692 nm and eight at 834 nm, and one gain-modulated photomultiplier tube detectors. The data from all four illumination-collection distances are analyzed by using of a frequency-domain multistance method to calculate the oxygen saturation of hemoglobin (%StO2) in the tissue. Extremity oxygenation with three conditions during the hemodialysis, i.e., pre-dialysis, in-dialysis and after-dialysis, are all measured for physiological analysis. The experimental results indicate the muscle pain in-dialysis is caused by reduction of oxygen saturation. Thus, NIRS provides an assessment tool for helping the treatment of hemodialysis patient with muscle pain in-dialysis.

7883B-42, Session 1

Optical biopsy using light-reflectance spectroscopy for prostate cancer diagnosis

V. Sharma, N. L. Patel, H. Liu, The Univ. of Texas at Arlington (United States)

Ultrasound guided biopsy procedure for prostate cancer diagnosis is the current gold standard but suffers from the inherent inability of ultrasound to identify small tumor volumes. The procedure is only anatomically guided, with lack of information on the location of cancerous lesions, thus requiring systematic random sampling of prostate tissue. Various sampling techniques have been developed, including saturation biopsies, but the detection rate of cancer still remains low. Broad-band (visible to near-infrared range) light reflectance spectroscopy (LRS) has the ability to differentiate tissue types, and thus can be highly beneficial if it can be used to differentiate prostate cancer from normal tissue in-vivo. In this study, we propose LRS as a technique to augment the detection of prostate cancer, which can be coupled with the current needle biopsy set-up. LRS with a needle-like, bifurcated, fiber optic probe (100-µm source-detector separation) measures light absorption and light scattering of underlying tissue, which are indicators of structural (i.e., cell size and density) and functional properties (i.e., vascular hemodynamics) of the tissue. A rat prostate tumor model was developed, involving subcutaneous injection of cancer cells in the foreback of the animals. In-vivo measurements were made using the LRS probe on tumor tissue as well as on normal surrounding tissue after one week of injection. Our preliminary results (n=3) are encouraging, and reveal a consistently significant increase in total blood concentration in tumor tissue as compared to the normal tissue. Scattering, on the other hand, was significantly reduced in tumor as compared to normal tissue.

7883B-189, Session 1

The regression of a transmissible venereal tumor in a canine prostate was detected by triple-wavelength trans-rectal optical tomography under trans-rectal ultrasound guidance

J. Zhen, K. E. Bartels, G. R. Holyoak, J. W. Ritchey, C. F. Bunting, Oklahoma State Univ. (United States); G. Slodobod, The Univ. of Oklahoma Health Sciences Ctr. (United States); D. Piao, Oklahoma State Univ. (United States)

Canine transmissible venereal tumor (TVT) has been reported to spontaneously regress due to the host cell-mediated immune response. In this study, we report the first non-invasive optical observation of TVT regression in a canine prostate using a triple-wavelength trans-rectal near-infrared (NIR) optical tomography imager guided by trans-rectal ultrasound (TRUS) visualization of the prostate. The triple-wavelength NIR imager acquiring at 705nm, 785nm and 808 nm was able to reliably quantify both the total hemoglobin concentration (HbT) and oxygen...
saturation (StO2) in prostate. The TVT tumor in the canine prostate as a model of prostate cancer was induced in a 7-year-old, 27 kg dog. A 2mL (2.5x10^6 cells/mL) suspension of homogenized TVT cells recovered from an in vivo subcutaneously propagated TVT tumor in an NOD/SCID mouse were injected in the cranial aspect of the right lobe of the canine prostate. After injection, the dog was monitored weekly over a 9-week period using trans-rectal NIR and TRUS in grey-scale and Doppler. A TVT nodular mass in the right lobe expanded to peak volume at approximately week-7, and then regressed during weeks 8 and 9. Regression of the TVT mass observed with trans-rectal optical tomography was characterized as having a gradually decreasing StO2 level and a gradually increasing region of reduced StO2 in the inner area of the tumor foci, coupled as having a gradually decreasing StO2 level in the periphery of the mass. Gross necropsy and histopathology confirmed a necrotic core in the TVT mass imaged.

7883B-43, Session 2

Holmium:YAG lithotripsy: effects of laser power parameters and backstop on lithotripsy

J. Qiu, The Univ. of Texas at Austin (United States); J. M. Teichman, The Univ. of British Columbia (Canada); T. E. Milner, The Univ. of Texas at Austin (United States)

The ideal dosimetry parameters of Ho:YAG lithotripsy are poorly defined. Prior studies showed minor preference for a modest pulse energy (0.5 J) at a high repetition rate versus high pulse energy (1.5 J) settings at lower repetition rates. However, larger ablation craters and faster lithotripsy may be achieved with higher pulse energies but at a cost of greater stone retropulsion. The use of stabilization techniques can reduce stone retropulsion. Pulsed Ho:YAG laser radiation is capable of destroying all metal anti-retropulsion materials. BackStop is a novel polymer-based anti-retropulsion material. Response of BackStop to pulsed Ho:YAG radiation is unknown, but predicted to withstand energy better than metal based anti-retropulsion devices since BackStop retains a solid form at increased temperature. Further, a solid shape, form, and function should not be compromised even if an ablation crater may be created. The results of this study compare stone ablation characteristics with and without BackStop polymer, and test the ability of BackStop polymer to withstand shape and function in response to Ho:YAG laser energy.

7883B-44, Session 2

Comparison of stone retropulsion for holmium:YAG laser lithotripsy at high-pulse energies versus thulium fiber laser lithotripsy at high-pulse rates

R. Blackmon, The Univ. of North Carolina at Charlotte (United States); P. Irby, Carolinas Medical Ctr. (United States); N. M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: Holmium:YAG laser is capable of operating at high pulse energies, but is limited to operation at low pulse repetition rates (~10 Hz) during lithotripsy. Thulium fiber laser is limited to low pulse energies (~35 mJ), but can operate at high pulse rates (~1000 Hz). Thulium fiber laser has previously been shown to vaporize stones 5-10 times more efficiently than Holmium laser at low pulse energies. This study compares stone retropulsion for two different operation modes of Holmium and Thulium fiber lasers.

Methods: Thulium fiber laser (wavelength=1908 nm) was operated with 35-mJ pulse energy, 500-microsecond pulse duration, and 10-400 Hz pulse rates. Holmium laser (wavelength=2120 nm) was operated with 35-540 mJ pulse energy, 350-microsecond pulse duration, and 10 Hz pulse rate. Laser energy was delivered through 270-micron optical fibers in contact mode with 6-mm-diameter plaster-of-Paris stone phantoms, submerged in saline bath. Stone retropulsion distance was measured for each set of laser parameters (n=10 samples), for fixed total energy (42 kJ) delivered and fixed number (1200) of laser pulses.

Results: Retropulsion with Holmium laser increases linearly with pulse energy. Retropulsion with Thulium fiber laser is minimal at pulse rates less than 150 Hz, then rapidly increases.

Conclusions: Holmium:YAG laser operation at low pulse energies provides minimal stone retropulsion at expense of lower stone vaporization rates. Thulium fiber laser has significantly lower threshold energy for stone vaporization than Holmium laser, making operation at low pulse energies and high pulse rates a more attractive balance between minimal stone retropulsion and rapid stone vaporization.

7883B-45, Session 2

Anticancer magnetic nanoparticles with magnetically induced hyperthermia

Y. Song, J. Koo, Y. Arum, J. Yoon, J. Oh, Pukyong National Univ. (Korea, Republic of)

We demonstrated the magnetic hyperthermia system for noninvasive urology treatment to analyze the anticancer activity of cisplatin-loaded magnetic nanoparticles (Fe3O4-APTS-cisplatin). The heating capability by applying an AC magnetic field depends on the properties of core size in magnetic nanoparticles, and RF frequency.

In this study, we synthesized three different magnetic nanoparticles, Fe3O4, Fe3O4-APTS, and Fe3O4-APTS-cisplatin by thermal decomposition methods, and two different radio-frequencies magnetic inputs (140 kHz and 163 kHz) were used to this study. The core size of magnetic nanoparticles were ~14nm measured by transmission electron microscopy (TEM) and average magnetization of Fe3O4, Fe3O4-APTS, and Fe3O4-APTS-cisplatin were 60, 52, and 40 emuFe/g by superconducting quantum interference device (SQUID), respectively. The amount of targeted magnetic nanoparticles into the cell was evaluated in vitro to human cervical carcinoma tumor with Prussian blue stain and cytotoxic effects on cell viability were analyzed by MTT Assay. Hyperthermia induced by the application of an AC magnetic field and anticancer drug in the presence of the suspension caused Hela cell death, which was found to be proportional to the quantity of the drug loaded magnetic nanoparticles and the application time of the AC magnetic field.

It has been successfully demonstrated that magnetic hyperthermia combining anticancer drug nanoparticles is useful for site specific anti-tumor treatment. Moreover, the AC magnetic activation significantly increased the diffusion of the anticancer drug by easily disruption of the actin and microtubule cytoskeletons of cells.

7883B-46, Session 2

Continuous-wave optical stimulation of the prostate cavernous nerves

S. Tozburun, C. Cilip, Univ. of North Carolina at Charlotte (United States); G. A. Lagoda, A. L. Burnett, The Johns Hopkins Univ. (United States); N. M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: Successful optical stimulation of the rat prostate cavernous nerves (CN) has been previously demonstrated using pulsed near-infrared laser radiation. However, intracavernous pressure (ICP) response times were slow. This study explores continuous-wave (CW) ONS of the rat CN, in vivo, as a faster method than pulsed ONS for potential use in identification and preservation of CN’s during prostate cancer surgery.

Methods: A Thulium fiber laser (wavelength=1870 nm) and diode laser (wavelength=1455 nm) were used because both wavelengths produce an optical penetration depth (400 microns) closely matching the rat CN diameter. ICP responses were obtained with the Thulium fiber laser
operated in CW mode and, for comparison, in pulsed mode with 5-ms pulses at 10-100 Hz. Successful ONS was then also demonstrated using the 1455 nm wavelength in CW mode.

Results: A threshold total optical energy (450-600 mJ) was required to create the desired temperature rise in the nerve for initiating optical stimulation. A stimulation threshold temperature (41-43 °C) and a nerve damage temperature (47-48 °C) was observed, defining a therapeutic window for safe and reproducible ONS. ICP response times gradually decreased as the Thulium fiber laser pulse rate was increased from 10-100 Hz with the fastest ICP response observed in CW mode.

Conclusions: Continuous-wave irradiation of CN results in faster deposition of thermal energy, corresponding to a faster temperature rise, and consequently a faster ICP response time than pulsed irradiation. In addition, the 1455 nm diode laser may provide an inexpensive, compact alternative laser for ONS.

7883B-47, Session 3

Comparison of three near-infrared laser wavelengths for non-invasive laser coagulation of the canine vas deferens

C. Cilip, N. M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: Successful noninvasive laser coagulation of the canine vas deferens, in vivo, has been previously reported. However, the therapeutic window for treatment is relatively narrow. This study determines the dependence of vas thermal coagulation on laser wavelength for development of a noninvasive laser vasectomy procedure.

Methods: Noninvasive laser coagulation of canine vas tissue, ex vivo, was performed using three commonly available near-infrared laser wavelengths: 808, 980, and 1075 nm. Each laser delivered an average power of 9.2 W, 500-ms pulse duration, pulse rate of 1.0 Hz, and 3.2-mm-diameter laser spot, synchronized with cryogen spray cooling of the scrotal skin surface for a total treatment time of 60 s. Vas burst pressures were measured to determine strength of vas closure and compared to previously reported ejaculation pressures. Gross inspection of vas and scrotal skin was also performed immediately after the procedure as an indicator of thermal coagulation and skin burns.

Results: The 1075 nm laser produced the highest vas burst pressures (288 ± 28 mmHg), significantly greater than previously reported ejaculation pressures (136 ± 29 mmHg). The 808 nm wavelength produced vas burst pressures of 141 ± 61 mmHg, however, minor scrotal skin burns were sometimes observed. The 980 nm wavelength was unable to produce thermal coagulation of the vas, with low burst pressures (89 ± 58 mmHg) and severe scrotal skin burns.

Conclusions: The 1075 nm wavelength was the only near-IR wavelength that consistently thermally coagulated the vas with a strong degree of closure and without scrotal skin burns.

7883B-48, Session 3

Computer simulations of light and heat transport in tissue for non-invasive laser coagulation of the human vas deferens

G. Schweinsberger, C. Cilip, S. Trammell, H. Cherukuri, N. M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: Noninvasive laser coagulation of the canine vas, in vivo, has been previously reported. However, there is a significant difference between the optical properties of canine and human skin. In this study, Monte Carlo simulations of light transport through tissue and heat transfer simulations are performed to determine the feasibility of noninvasive laser vasectomy in humans.

Methods: A laser wavelength of 1064 nm was chosen for deep optical penetration in tissue. Monte Carlo simulations determined spatial distribution of absorbed photons inside tissue layers (epidermis, dermis, vas). The results were convolved for a 3-mm-diameter laser beam, and then used as the spatial heat source for the heat transfer model. Laser pulses of 500-ms duration, cycling at 1 Hz for 60 s, and cryogen spray cooling were incident on the tissue. Average laser power (5-9 W), cryogen pulse duration (60-100 ms), cryogen cooling rate (0.5-1.0 Hz), and increase in transmission due to optical clearing (0-50 %) were varied to determine optimal treatment parameters.

Results: After application of an optical clearing agent to increase skin transmission by 50%, an average laser power of 6 W, cryogen pulse of 60-ms, and cooling rate of 1.0 Hz resulted in vas temperatures up to 63°C, sufficient for thermal coagulation, while 1 mm of skin surface (epidermis and dermis) remained safely below 45 °C.

Conclusions: Monte Carlo and heat transfer simulations indicate that it is possible to noninvasively thermally coagulate the human vas if an optical clearing agent is applied to the scrotal skin prior to the procedure.

7883B-49, Session 3

Active cooling fiber delivery device for bovine prostate vaporization with high-power 180W 532-nm laser

S. Peng, H. W. Kang, H. Pirzadeh, D. G. Stinson, American Medical Systems, Inc. (United States)

A novel fiber delivery device with Active Cooling Cap (ACCTM) is designed to transmit up to 180W of 532 nm laser light to treat benign prostatic hyperplasia. Under such high power tissue ablation, an effective cooling plays an important key to maintain fiber power transmission and ensure the reliability of the fiber delivery device. To handle high power and reduce fiber degradation, ACC fiber features a larger fiber size (750 micrometer) and an internal fluid channel to ensure better cooling of fiber tip to prevent the cap from burning, detaching, or even shattering during treatment, which could lead to injury to patients. The internal cooling channel was created with a metal cap and an outer flow tubing that surrounds the optical fiber and inlet flow tubing. The ACC fibers were utilized to investigate the effect of various power levels from 120 to 200 W on in vitro bovine prostate vaporization using a 532 nm laser system. The ACC fiber with 180W doubled the tissue removal rate of the current HPS fiber at 120W. The fiber maintained a constant tissue vaporization rate during the whole tissue ablation process. The coagulation for 180W was about 20% thicker than that for 120W. The ACC fibers delivered 180W of 532-nm laser to ablate bovine prostate tissue efficiently. In conclusion, the new ACC fibers at 180W doubled the tissue removal rate, maintained the vaporization efficiency during 400kJ energy delivery, and induced similar coagulation in comparison with the existing HPS fiber at 120W.

7883B-50, Session 3

Interaction between high-power 532-nm laser and prostatic tissue: in-vitro evaluation for laser prostatectomy

H. W. Kang, S. Peng, D. G. Stinson, American Medical Systems Holdings, Inc. (United States)

Photoselective vaporization of the prostate (PVP) has been developed for effective treatment of obstructive benign prostatic hyperplasia. To maximize tissue vaporization for large prostate glands, identifying the optimal power level for PVP is still necessary. We investigated the effect of various power levels on in vitro bovine prostate vaporization with a 532-nm laser system. PVP was performed on 114 bovine prostatic tissue specimens. A custom-made 532-nm laser was employed to provide power levels from 120 to 200W, delivered through a newly designed 750-µm side-firing fiber. Tissue vaporization efficiency was evaluated in terms of power (P; 120–200W), treatment speed of fiber (TS; 2–8 mm/s), and working distance between fiber and tissue surface (WD; 1–5 mm).
Coagulation depth was also estimated macroscopically and histologically (H&E) at various Ps. Both 180 and 200W yielded comparable vaporized volume (104.3±24.7 vs. 104.2±23.9 mm3 for WD=1 mm; 83.8±20.9 vs. 81.2±20.7 mm3 for WD=2 mm; p=0.99); thus, 180W was identified as the optimal power to maximize tissue vaporization, by removing tissue up to 80% faster than 120W (41.7±9.9 vs. 23.2±3.4 mm3/s at TS=4 mm/s and WD=2 mm; p<0.005). Tissue vaporization was maximized at TS=4 mm/s and vaporized equally efficiently at up to 3 mm WD (104.5±16.7 mm3 for WD=1 mm vs. 93.4±7.4 mm3 for WD=3 mm at 180W; p=0.33). The mean thickness of coagulation zone for 180W was 20% thicker than that for 120W (1.31±0.17 vs. 1.09±0.16 mm; p<0.005). In vitro the 532-nm LBO laser demonstrated that 180W was the optimal power to maximize tissue vaporization efficiency with enhanced coagulation characteristics.

**Interactions between high-power 532-nm laser and prostatic tissue: in-vivo evaluation for laser prostatectomy**

R. S. Malek, Mayo Clinic (United States); H. W. Kang, S. Peng, D. G. Stinson, M. T. Beck, E. Koulick, American Medical Systems Holdings, Inc. (United States)

Our in vitro study demonstrated that 180W was the optimal power to reduce photoselective vaporization of the prostate (PVP) time for larger prostate glands. In this study, we investigated anatomic and histologic outcomes and vaporization parameters of 180W laser performed with a new 750-μm side-firing fiber in a survival study of living canines. Eight male canines underwent antegrade PVP with the 180W 532-nm laser. Four each animals were euthanized 3 hours or 8 weeks postoperatively. Prostates were measured and histologically analyzed after hematoxylin and eosin (H&E), triphenyltetrazolium chloride (TTC), or Gomori trichrome (GT) staining. Compared to the previous 120W laser, PVP with the 180W laser bloodlessly created a 76% larger cavity (mean 11.8 vs. 6.7 cm3; p=0.014) and vaporized tissue at a 77% higher rate (mean 2.3 vs. 1.3 cm3/min; p=0.03) while H&E- and TTC-staining demonstrated its 33% thicker mean coagulation zone (2.0±0.4 vs. 1.5±0.3 mm). H&E-stained cross-sectional prostatic tissue specimens from the 3-hour (acute) group showed histologic evolution of concentric non-viable coagulation zone, partially viable hyperemic transition zone of repair, and viable non-treated zone. H&E- and GT-stained specimens from the 8-week (chronic) group revealed healed circumferentially epithelialized, non-edematous, prostatic urethral channels with no increase in collagen in the subjacent prostatic tissue vis-à-vis the normal control. Our canine study demonstrates 180W 532-nm laser PVP with its new fiber has a significantly higher vaporization rate with a more hemostatic coagulation zone, but equally favorable tissue interaction and healing, compared with our previous 120W canine study.

**Ablation of uterine tissues with 522-nm GreenLight laser**

E. Koulick, M. T. Beck, American Medical Systems Holdings, Inc. (United States)

Tissue removal using 532nm laser found application in the treatment of Benign Prostate Hyperplasia. We investigated suitability of the 532nm laser for effective ablation of the uterus tissue and, therefore, for treatment of Abnormal Uterine Bleeding. Special diffuser fiber has been developed to allow quick and uniform coagulation of the large area of tissue. The fiber was tested using ex-vivo and in-vivo models. Using GreenLight™ laser capable of delivering 120W of power, we have achieved uniform and deep ablation of tissue area compatible with human size uterus in 90 second ablation procedure.

**Ablative efficiency of lithium triborate laser vapourisation and conventional transurethral resection of the prostate: a comparison using trans-rectal three-dimensional ultrasound volumetry**

O. Gross, T. Sulser, L. J. Hefermehl, D. D. Strebel, R. Largo, A. Mortezavi, C. Poyet, D. Eberli, M. Zimmermann, A. Müller, Univ. Hospital Zürich (Switzerland); M. S. Michel, Univ. Mannheim (Germany); M. Müntener, H. Seifert, T. Hermanns, Univ. Hospital Zürich (Switzerland)

INTRODUCTION AND OBJECTIVES: Long-term results after 120W lithium triborate (LBO) laser vapourisation of the prostate (LV) are still lacking. A sufficiently extensive de-obstruction is a prerequisite for a long-lasting improvement of voiding dysfunction. To investigate the efficiency of tissue ablation following 120W LBO LV and transurethral resection of the prostate (TURP) transrectal threedimensional (3D) ultrasound volumetry was performed.

METHODS: Between 03/2008 and 03/2010 110 patients underwent routine LBO LV (n=61) or TURP (n=49). Transrectal 3D ultrasound with planimetric volumetry of the prostate was performed preoperatively, after catheter removal, 6 weeks and 6 months.

RESULTS: Median prostate volume was 52.5ml (LV) and 46.9ml (TURP) (p=0.19). After catheter removal, median absolute volume reduction (LV 7.05ml, TURP 15.8ml) as well as relative reduction of the initial volume (15.9% vs. 34.2%) were significantly lower in the LV group (p<0.001).

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After 6w / 6m, absolute volume reduction was still lower in the LV group but not significantly anymore (6w: LV 15.3ml vs. TURP 17.9ml; 6m: LV 18.4ml vs. TURP 21ml). The relative volume reduction was still significantly lower in the LV group at 6w (31.5% vs. 39.6%, p<0.001) and 6m (37% vs. 46.9%, p=0.001). Subjective and objective outcome parameters improved significantly in both groups.

CONCLUSIONS: LBO-LV is an efficient procedure for prostatic de-obstruction evidenced by an absolute tissue ablation not significantly different to that after TURP. However, TURP seems to be superior due to a higher ablation in relation to the initial volume. The investigated outcome parameters show that up to 6 months this difference has no clinical impact.

7883B-55, Session 4

Proper laser-fiber sweeping angle for the effective tissue vaporization using XPS™ with MoXy™ fiber

W. J. Ko, Columbia Univ. Medical Ctr. (United States) and National Health Insurance Corp. Ilsan (Korea, Republic of); H. W. Kang, American Medical Systems Holdings, Inc. (United States); D. Rajabhandharaks, D. G. Stinson, American Medical Systems, Inc. (United States); B. B. Choi, Weill-Cornell Univ. Medical Ctr. (United States) and Metropolitan Urology (United States)

Introduction: We identify the optimal fiber sweeping angle to maximize tissue vaporization efficiency using XPS™ with MoXy™ fiber.

Materials and Methods: Porcine kidney was used for tissue sample which was prepared to have size of 2x2cm. Ten tissue specimens were used for each angle (n=70). XPS™ (Xcelerated Performance System) was used for Laser system and MoXy™ liquid cooled fiber was used for laser fiber. The power was fixed at 120W. The fiber sweeping speed and treatment speed were 0.5 sweep/sec and 2mm/sec. The sweeping angles were 0, 15, 30, 45, 60, 90, 120 degree. The efficiency was tested in a vaporization chamber equipped with motorized laser-fiber movement. Vaporized cavity size was measured by liquid paraffin molding.

Results: The ablation rates were 1.17, 1.46, 1.45, 1.31, 1.12, 0.95, 0.62 ml/min at 0, 15, 30, 45, 60, 90, 120 degree. The maximum Ablation rate according to the sweeping angle was shown at 15 and 30 degrees. The results of the comparison of % difference between other degrees and 60 degree showed that 0 vs 60 (0.7%, p=0.73), 15 vs 60 (29.5%, p<0.05), 30 vs 60 (28.6%, p<0.05), 45 vs 60 (16.9%, p=0.05), 90 vs 60 (15.2%, p<0.05), 120 vs 60 (44.8%, p<0.05). The sweeping angle greater than 60 is less effective on tissue vaporization. The results at 0 and 60 degree were not statistically significant.

Conclusions: The result at 0 degree is worse than 15, 30 degree. The optimal SA for tissue vaporization is 15, 30 degree.

7883B-56, Session 4

The optimized laser fiber sweeping speed(SS) for the effective tissue vaporization at various power levels using XPS™ with MoXy™ fiber

W. J. Ko, Columbia Univ. Medical Ctr. (United States) and National Health Insurance Corp. Ilsan (Korea, Republic of); H. W. Kang, American Medical Systems Holdings, Inc. (United States); D. Rajabhandharaks, D. G. Stinson, American Medical Systems, Inc. (United States); B. B. Choi, Weill-Cornell Univ. Medical Ctr. (United States)

Introduction: The ablation volume is different according to the laser fiber SS and laser power. Yet the data on what the proper SS and the difference of SS results at different power levels are lacking. We investigated the laser fiber SS for the effective tissue vaporization and the difference of the ablation volume at different power levels.

Materials and Methods: Porcine kidney was used for tissue sample which was prepared to have size of 2x2cm. Ten tissue specimens were used for each speed and power level (n=80). XPS™ and MoXy™ fiber was used. The fiber sweeping speeds were 0.5, 1, 1.5, 2 sweep/sec. The treatment speed was 2mm/sec and the sweeping angle was 60°. The investigated power levels were at 120W and 180W. The efficiency was tested in a vaporization chamber equipped with motorized laser-fiber movement. Vaporized cavity size was measured by liquid paraffin molding.

Results: The ablation rates at 0.5, 1, 1.5, 2 sweep/sec were 1.12, 1.00, 0.73, 0.69 ml/min in 120W and 1.97, 1.64, 1.25, 1.24 in 180W. The maximum ablation rate according to SS was shown at 0.5 sweep/sec in both power levels. The results of % difference (other SS vs 1 sweep/sec) showed no statistical significance between 0.5 and 1.

Conclusions: The effective SS for tissue vaporization is 0.5 and 1 sweep/sec. The result showed the same trend at the two power levels and SS at 0.5 sweep/sec was almost twice effective for tissue vaporization at 180W compared to 120W.

7883B-57, Poster Session

A reagent-free method for diagnosing urinary tract infection: the potential of urine autofluorescence

S. Menon Perinchery, U. Kuzhiumpambalib, S. Vemulpad, E. M. Goldsys, Macquarie Univ. (Australia)

Urinary tract infections (UTIs) are known to alter the normal urine composition which, in principle, can lead to changes in urine autofluorescence. This paper describes the study of human urine (normal and UTI) by using UV fluorescence excitation/emission matrices and synchronous spectra and proposes a method of diagnosing UTI without any sample preparation. The method is based on excitation in the shorter UV region (250-350 nm) which shows good discrimination between the normal urine and UTI samples. The synchronous scans with an offset of Δλ = 90 nm were also able to differentiate between normal urines and UTI samples. These differences were observed even though the two known major urine fluorophores, tryptophan and indoxyl sulfate were present in the normal urine and UTI samples in similar concentration as established by HPLC analysis. Although the identity of substances responsible for the altered autofluorescence in UTI is not established, our study shows that autofluorescence has the potential to differentiate between normal human urines and those with UTI.
High-frequency endobronchial ultrasonography identifies airway wall structures in patients with laryngotracheal stenosis

S. D. Murgu, H. G. Colt, Univ. of California, Irvine (United States)

Background and Objective: While the cause of nonmalignant laryngotracheal stenosis (LTS) is not always apparent clinically, histopathologic findings of resected strictures distinguish idiopathic (ITS) from post intubation or tracheostomy related stenoses (PITS). High frequency endobronchial ultrasound (EBUS) accurately identifies normal airway wall structures and cartilage destruction from malacia or tumor. The purpose of this study was to demonstrate how EBUS can be applied to reveal characteristic findings distinctive for ITS and PITS in real-time.

Materials and Methods: High frequency EBUS using a 20 MHz radial probe was used to characterize airway wall microstructures in the area of hypertrophic tissue formation in two patients with subglottic ITS and two patients with PITS (one post tracheostomy; one post endotracheal intubation).

Results: In PITS after tracheostomy, EBUS showed hypertrophic tissues overlying a disrupted tracheal cartilage. The cartilaginous ring had brighter echogenicity compared to normal airway cartilage suggesting calcification, consistent with histological examination of resected specimens. EBUS could not distinguish between ITS and PITS after endotracheal intubation, however, revealing a homogeneously iso-echoic layer consistent with hypertrophic tissue overlying an intact hyper-echoic layer corresponding to the tracheal cartilage in both instances.

Conclusion: In-vivo real time high frequency EBUS reveals characteristic changes but does not differentiate PITS after tracheostomy from ITS and PITS from after endotracheal intubation. Studies are warranted to determine whether the addition of other optical or acoustic bronchoscopic technologies could further differentiate among various etiologies of nonmalignant laryngotracheal stenosis. This information could have invaluable management and medico-legal implications.

Reflectance confocal microscopy of human head-and-neck tissues in vivo and ex vivo

F. Palmer, I. Nixon, A. Moreira, S. Patel, M. Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

The feasibility of imaging human head-and-neck tissues was investigated with a bench-top reflectance confocal microscope, for potential clinical applications. The optical sectioning of 1-3 um and lateral resolution of 0.7-1.0 um compares well to that of standard pathology. The image contrast is due to endogenous reflectance. The imaging is in real-time at 20 frames per second. Confocal imaging in buccal mucosa in vivo shows nuclear and cellular detail in the epithelium and epithelial junction, and connective tissue and blood flow in the underlying lamina propria, to depth of 400 um. Similar detail, including filiform and fungiform papillae, is seen on the dorsal and ventral tongue in vivo, albeit with reduced resolution contrast and a reduced depth of 250 um, due to keratinization. Imaging of cellular detail and blood flow is demonstrated in oral precancers (leukoplakia, erythroplakia) in vivo. Confocal mosaicing microscopy allows rapid imaging of large areas of fresh tissue ex vivo and may enable pathology at-the bedside. Images are acquired on surgical excisions and stitched together into mosaics. Mosaics display tissue with 2X-30X magnifications. Mosaics of thyroid tissue from head-and-neck surgery show capsule, follicles, and follicular cells, to depth of 150 um, with good visual correlation of both benign and malignant tumors to pathology. Normal parathyroid tissue is distinguished from diseased (adenoma) on the basis of stromal fat content. Imaging of cellular detail is shown in excised bone, muscle, nerve and parotid tissues. Current work is focused on designing confocal endoscopes for intra-oral and intra-operative imaging.
7883C-187, Session 1

**Wide-field optical imaging and spectroscopy of premalignant lesions in the oral cavity**

M. C. Pierce, R. A. Schwarz, Rice Univ. (United States); M. D. Williams, A. K. El-Naggar, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); N. Vigneswaran, Univ. of Texas Dental Branch (United States); A. M. Gillenwater, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); R. R. Richards-Kortum, Rice Univ. (United States)

No abstract available

7883C-69, Session 2

**Spatio-temporal processing of massive glottic images from high speed laryngostroboscopy**

K. Izdebski, Pacific Voice and Speech Foundation (United States); Y. Yan, Santa Clara Univ. (United States)

No abstract available

7883C-165, Session 2

**Optical coherence tomography imaging of the human airway with a piezoelectric scanning catheter**

S. Moon, M. Rubinstein, G. Liu, A. Saidi, B. J. Wong, Z. Chen, Beckman Laser Institute and Medical Clinic (United States)

No abstract available

7883C-177, Session 2

**Optical coherence tomography in the field of laryngology: further perspectives**

T. Just, H. W. Pau, Univ. Rostock (Germany); E. Lankenau, G. Hüttmann, Univ. zu Lübeck (Germany)

No abstract available

7883C-188, Session 2

**Imaging vibrating vocal cord with high speed 1um swept source OCT and ODT**

G. Liu, M. Rubinstein, W. Qi, Beckman Laser Institute and Medical Clinic (United States); A. I. Foulad, Univ. of California, Irvine (United States); B. J. Wong, Z. Chen, Beckman Laser Institute and Medical Clinic (United States)

No abstract available

7883C-190, Session 2

**Multispectral imaging of the oral cavity in HIV patients**

A. F. Zuluaga, Remicalm LLC (United States); C. M. Flaht, The Univ. of Texas Health Science Ctr. at Houston (USA); M. Nichols,

Autofluorescence imaging in soft tissues has shown great potential in detecting early stage premalignant lesions. Common benign confounders associated with loss of normal autofluorescence are often associated with vascular conditions. Narrowband reflectance illumination in a band coinciding with high preferential hemoglobin absorbance provides an independent view of tissue that is complimentary to the information provided by autofluorescence. Representative cases and trends are presented, highlighting the benefits of this approach when visualizing premalignant and malignant lesions in HIV-positive patients.

7883C-62, Session 3

**Comparison of lasers used in stapedotomy using specialized visualization techniques for mechanical and thermal effects in an inner ear model**

R. M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); D. Kamalski, T. de Boorder, W. Grolman, Univ. Medical Ctr. Utrecht (Netherlands)

The outcome of stapedotomy depends on several surgical steps. Using laser light, the ossicular chain can be handled and the oval window can be punctured with a non-touch method. Various lasers are being used or considered, however, it is not clear which settings and characteristics will contribute to optimal or adverse effects (vestibule damage and loss hearing frequencies).

Using a unique high speed thermal imaging setup based on Schlieren techniques, the mechanical and thermal effects during laser stapedotomy were studied in an inner ear model consisting of human, fresh frozen stapes positioned on a liquid filled cavity in a gel cast. The cw KTP (532 nm), cw and pulsed CO2 (10.6 um), cw Thulium (1.9 um), pulsed Er:YSGG (2.78 um) coupled to special fiber delivery systems were applied at typical clinical settings for comparison.

The imaging techniques provided a good insight in the extent of heat conduction beneath the footplate and (explosive) vapour formation on both sides. For the pulsed laser modes, explosive vapour expansion can to be controlled with optimized pulse energies while for continuous wave lasers the thermal effects can be controlled with the pulse length and repetition rate. The fluence at the tip of the delivery system and the distance to the footplate has a major impact on the ablation effect. The pulsed IR lasers with fiber delivery show to be most promising for a controlled stapedotomy.

7883C-68, Session 3

**The effect of low-level laser therapy (LLLT) on noise-induced hearing loss**

C. Rhee, C. W. Bahk, J. Ahn, M. Suh, Dankook Univ. Hospital (Korea, Republic of)

Aim: The effects of the LLLT and its usage in neuronal tissues have been well established. The aim of this study was to see the effect of the LLLT in rescuing the hair cells of the cochlea after a noise-induced hearing loss. Methods: Bilateral ears of 11 adult male SD rats (200 g) with 22 ears were exposed to noise (narrow band noise, 120 dB, 16 kHz, 6 h) and starting the following day, the left ears of the rats were irradiated at an laser output of 165 mW/cm² for 60 minutes per day with an 830 nm diode laser
for 12 days in a row. Right ears were used as control ears. The hearing levels were measured at each frequency of 4, 8, 12, 16 and 32 kHz before the noise exposure and also after 1st, 5th, 10th and 12th irradiations to observe the recovery of hearing thresholds. Each threshold measurement was confirmed by a second investigator for prevention of predisposed statements.

Results: The initial hearing levels in all frequencies are 26.5±4.7, 24.5±5.0, 24.0±5.2, 24.0±3.2 and 24.5±5.5 dB SPL in 22 ears. After the noise exposure, the thresholds increased markedly (63.5±15.1, 64±16.8, 71.5±11.3, 73.5±15.6 and 67.5±14.4 dB SPL in 4, 8, 12, 16 and 32 kHz, respectively) and after three to five days of irradiation into left ears, recovery in hearing levels was recorded. After 12th irradiation, the thresholds of the treated left ears recovered significantly (24±5.5, 24±3.5, 24±11.9, 24±12.9 and 21±2.2 dB SPL, p<0.05) comparing to that of the untreated right ears measured 36.3±22.9, 45±15.8, 66.3±22.9, 50±16.8 and 43.8±21.4 dB SPL.

Conclusion: The results of this study suggest that the LLLT may promote the recovery of hearing thresholds after a noise-induced hearing loss.

7883C-150, Session 3
Interstitial PDT for vascular anomalies

W. K. Jerjes, Univ. College London Hospitals NHS Foundation Trust (United Kingdom); T. Upile, Univ. College Hospital (United Kingdom); C. A. Mosse, Univ. College London (United Kingdom); S. Morley, Univ. College Hospital (United Kingdom); C. Hopper, Univ. College London Hospitals NHS Foundation Trust (United Kingdom)

Introduction: Photodynamic therapy has proved its successfulness in the management of variety of pathologies involving the human body. Our aim in this prospective clinical study is to assess the outcome following interstitial photodynamic therapy for patients with vascular anomalies. Patients’ reports on quality of life with clinical and radiological evaluation were the main end point parameters used to assess the outcome.

Materials and methods: Forty three patients were referred to the UCLH Head and Neck Centre for treatment of vascular anomalies of the head and neck, including: infantile and congenital haemangiomas, venous, lymphatic and arteriovenous malformations. After multidisciplinary discussion, all patients underwent interstitial photodynamic therapy under general anaesthesia, using 0.15mg/kg mTHPC as the photosensitising agent. Following treatment, patients were followed-up for a mean of 21 months.

Results: Fifteen out of nineteen patients who presented with long-term pain reported improvement after treatment. Also, 7/8 reported significant reduction of bleeding related to their vascular anomaly. Improvement of swelling was reported by 28/35 patients; while reduction of infection episodes was evident in 8/11 patients and 31/36 reported reduction in the disfigurement caused by their pathology. Significant reduction of swelling problems was reported in 9/12 patients, and breathing problems in 7/9 patients.

Clinical assessment showed that half of the patients had “good response” to the treatment. Moderate clinical response was reported by 13 (30.2%) patients. Radiological assessment comparing imaging 6-week post-PDT to the baseline showed moderate response in 11 (25.6%) patients and significant response in 15 (34.9%) patients.

Conclusion: This study on 43 patients with vascular anomalies undergoing interstitial photodynamic therapy provided evidence that PDT is a successful modality in the management of these pathologies that are resistant to conventional modalities, with minimal side effects.

7883C-166, Session 3
Identifying dosimetry parameters for percutaneous laser blepharoplasty using the Ho:Yag (2100 nm) laser

A. J. Zemek, Univ. of California, Irvine (United States)

No abstract available

7883C-167, Session 3
Cellular-level imaging of the functional mammalian inner ear

S. Battis, Stanford Univ. School of Medicine (United States); E. L. Cheung, N. H. Blevins, G. R. Popelka, Stanford Univ. (United States); M. J. Schnitzer, Stanford Univ. School of Medicine (United States)

No abstract available

7883C-168, Session 3
Three-dimensional reconstruction of a cochlea with x-ray tomography

M. Hwang, Northwestern Univ. (United States); C. Rau, Diamond Light Source Ltd. (United Kingdom); A. J. Fishman, S. Shintani-Smith, Northwestern Univ. (United States); W. Lee, Argonne National Lab. (United States); K. Richter, Univ. of California, Berkeley (United States)

No abstract available

7883C-178, Session 3
In vivo and in vitro studies on PEG-coated, biofunctionalized titanium PORP middle ear prosthesis

J. F. R. Ilgner, Univ. Hospital Aachen (Germany); S. Biedron, Stanford Univ. School of Medicine (United States); E. Fadeeva, Laser Zentrum Hannover e.V. (Germany); M. Loebler, Univ. of California, Irvine (United States); E. Huynh, A. L. Nuttall, Oregon Health & Science Univ. (United States)

No abstract available

7883C-186, Session 3
Optical microangiography provides in vivo 3D images of intracochlear microstructures and microvascular perfusion in mice


No abstract available
Diode laser cartilage reshaping
A. M. El Kharbotty, T. E. Tayeb, Y. Mostafa, National Institute of Laser Enhanced Sciences (Egypt); H. Ibrahim, Ministry of Health (Egypt)

Abstract: Loss of facial or ear cartilage due to trauma or surgery is a major challenge to the otolaryngologist and plastic surgeons as the complicated geometric contours are difficult to be animated. Diode laser (980 nm) has been proven effective in reshaping and maintaining the new geometric shape achieved by laser. This study focused on determining the optimum laser parameters needed for cartilage reshaping with a controlled water cooling system. Harvested apocrine cartilages were angulated with different degrees and irradiated with different diode laser powers (980nm, 4x8mm spot size). The cartilage specimens were maintained in a deformation angle for two hours after irradiation and serially examined and photographed.

High-power Diode laser irradiation with water cooling is a cheap and effective method for reshaping the cartilage needed for reconstruction of difficult situations in otorhinolaryngologic surgery.

Triggered optical coherence tomography for dynamic imaging of vocal folds during phonation
J. B. Kobler, Massachusetts General Hospital (United States); E. W. Chang, Boston Univ. (United States) and Wellman Ctr. for Photomedicine (United States); S. M. Zeitels, Massachusetts General Hospital (United States); S. Yun, Wellman Ctr. for Photomedicine (United States)

Optical coherence tomography (OCT) has previously proven useful for anatomic imaging of the superficial layers of the vocal fold. These layers are the site of vocal fold oscillation, so there would be obvious benefit to imaging this tissue dynamically during phonation for better understanding vocal biomechanics and the impact of pathology on voice. To this end we developed a voice-triggered Fourier-domain OCT system and tested it using excised calf larynges that were phonated using airflow, while mounted in a chamber for simultaneous OCT imaging. The method is analogous to videostroboscopy, where multiple cycles of periodic vocal fold motion are used to synthesize oscillations that can be viewed in slow motion. In the case of OCT, however, the imaging is cross-sectional rather than from a surface view, thus capturing novel information about vocal fold deformation and the mucosal traveling wave. This information includes observations and quantifiable measurements of shape, amplitude and velocity of the surface of the mucosal wave and of strain in underlying layers to depths of 1 - 2mm. The imaging process can be rapidly repeated to capture multiple planes and thus yield 4D volume data sets (x,y,z voxels across different phases of the vibratory cycle). Image quality is improving, and the long term goal is to incorporate this capability into a clinical endoscopy system. Another application, with possible future clinical value, is measuring the deformation of implanted biomaterials during phonation to test for their biomechanical compatibility with native tissue.

Finite element approach for optimal electrode configuration in electro-mechanically reshaping cartilage
C. Manuel, Univ. of California, Irvine (United States)

No abstract available

Optical imaging, therapeutics, and advanced technology in otolaryngology and head and neck surgery
M. A. Biel, Univ. of Minnesota, Twin Cities (United States)
No abstract available

Light-activated composite filler for soft tissue restoration
No abstract available

Developing endoscopic ultrafast laser microsurgery of scarred vocal folds
C. L. Hoy, The Univ. of Texas at Austin (United States); M. Yildirim, W. N. Everett, The Univ. of Texas at Austin (United States); J. B. Kobler, Massachusetts General Hospital (United States); A. Ben-Yakar, The Univ. of Texas at Austin (United States)
No abstract available

Phase I Amphinex-Bleomycin clinical photochemical internalization trial
W. K. Jerjes, Univ. College London Hospitals NHS Foundation Trust (United Kingdom); K. Berg, The Norwegian Radium Hospital (Norway); Z. Hamdoun, Univ. College Hospital (United Kingdom); C. A. Mosse, Univ. College London (United Kingdom); A. Høgset, PCI Biotech AS (Norway); S. G. Bown, Univ. College London (United Kingdom); C. A. Mosse, Univ. College London (United Kingdom); A. Høgset, PCI Biotech AS (Norway); S. G. Bown, Univ. College London (United Kingdom); D. Carnell, Univ. College Hospital (United Kingdom); C. Hopper, Univ. College London Hospitals NHS Foundation Trust (United Kingdom)

Introduction/Aims: Photochemical internalization (PCI) is a novel technology that facilitates the delivery of macromolecules into cytoplasm. The initial mechanism and practical application was described by Berg et al. in 1999.

This, first in human trial, is an open, phase I dose escalating study to evaluate the safety and tolerance of the photosensitizer (amphinex) that is used to initiate the photochemical internalization process with bleomycin as the chemotherapeutic agent. We present our final report following the management of 14 patients with various malignancies.

Material/Methods: Patients monitoring and follow-up start from Day -14 and continue to Day 28. The drug safety and tolerance are assessed by measuring the concentration (PK) of amphinex in plasma and urine after centrifugation and samples freezing under -20°C. Assessment of amphinex accumulation in skin is performed by fluorescence spectroscopy. Skin sensitivity testing is conducted using white light.
Results: The 14 patients in this trial received 0.25-1.5mg/kg amphinex (Day 0) approximately 93hrs prior to a slow bleomycin infusion (15000u/m2) and subsequent illumination (Day 4) with 652nm diode laser with 60J/cm2 to initiate PCI. No immediate clinical symptoms were reported prior to amphinex administration and no immediate drug adverse events were identified.

Conclusions: The most striking finding is the dramatic tumour responses. Complete tumour response of the target lesions of 13/14 patients was achieved. The starting dose of Amphinex for the study was set at a level not expected to trigger a PCI response, however there appeared to be a localized synergistic effect with photo-activation.

7883C-156, Session 5
Hyperthermia enhanced ICG mediated laser therapy for head and neck tumors
G. Shafirstein, K. Barnes, N. Koonce, J. Weber, R. Griffin, Univ. of Arkansas for Medical Sciences (United States)
No abstract available

7883C-157, Session 5
Localized drug delivery using laser activated liposomes for head and neck cancer
G. Shafirstein, L. Bernock, K. Barnes, Univ. of Arkansas for Medical Sciences (United States); J. Moran, E. Hamilton, M. Borrelli, Univ. of Arkansas for Medical Sciences (United States)
No abstract available

7883C-164, Session 5
Foscan mediated interstitial photodynamic therapy for head and neck cancer
R. L. P. van Veen, H. J. C. M. Sterenborg, J. B. Aans, D. J. Robinson, Erasmus MC (Netherlands); B. Karakullukçu, O. Hamming-Vrieze, F. Hoebers, Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis (Netherlands); M. Witjes, Univ. Medical Ctr. Groningen (Netherlands); P. C. Levendag, Erasmus MC (Netherlands); I. B. Tan, Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis (Netherlands)
No abstract available

7883C-173, Session 5
Enhanced transfection of tumor suppressor genes by photochemical internalization
H. Hirschberg, Beckman Laser Institute and Medical Clinic (United States)
No abstract available

7883C-179, Session 6
Histopathology of oral leukoplakia premalignancy and mimics: overlapping features of optical diagnostic implications
A. K. El-Naggar, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States)
No abstract available

7883C-180, Session 6
Oral cancer screening approach based on labeling exfoliated oral cells with molecularly-targeted optical contrast agents
V. Leautaud, C. R. Horres, Rice Univ. (United States); V. S. Bhattar, The Univ. of Texas M. D. Anderson Cancer Ctr. (United States); M. D. Williams, A. M. Gillenwater, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); R. R. Richards-Kortum, Rice Univ. (United States)
No abstract available

7883C-181, Session 6
 Autofluorescence guided diagnostic evaluation of suspicious oral mucosal lesions: opportunities, limitations, and pitfalls
N. Vigneswaran, The Univ. of Texas Dental Branch at Houston (United States)
No abstract available

7883C-182, Session 6
Application of high resolution microendoscopy to real-time surgical margin detection and robotic surgery
A. Sikora, Mount Sinai School of Medicine (United States)
No abstract available

7883C-183, Session 6
Real-time spectroscopic evaluation of oral lesions and comparisons with histopathology
R. A. Schwarz, W. Gao, Rice Univ. (United States); J. H. Nguyen, The Univ. of Texas Health Science Ctr. at Houston (United States); N. Vigneswaran, Alliance for NanoHealth (United States); K. Adler-Storthz, The Univ. of Texas Health Science Ctr. at Houston (United States); V. S. Bhattar, M. D. Williams, A. M. Gillenwater, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); R. R. Richards-Kortum, Rice Univ. (United States)
No abstract available
Clinical diagnosis of oral precancer and cancer with optical coherence tomography

M. Tsai, Chang Gung Univ. (Taiwan); C. Lee, T. Chi, K. Yang, C. Chiang, C. Yang, National Taiwan Univ. (Taiwan)

Oral lesions in different oral carcinogenesis stages, including normal control, mild dysplasia (MiD), moderate dysplasia (MoD), early-stage squamous cell carcinoma (ES-SCC), and well-developed SCC (WD-SCC), are clinically scanned for diagnosis with an optical coherence tomography (OCT) system. Based on the analyses of the OCT images, the stages of dysplasia (MiD and MoD) and SCC (ES-SCC and WD-SCC) can be differentiated from normal control by evaluating the depth-dependent standard deviation (SD) values of lateral variations. Due to the higher density of connective tissue papillae in the ES-SCC stage, the SD values of the slowly-varying lateral scan profiles in the ES-SCC samples are significantly larger than those in the WD-SCC sample. Also, ES-SCC can be differentiated from WD-SCC by comparing the exponential decay constants of averaged A-mode scan profiles. Also, a computer analysis procedure for clinical OCT images of healthy or precancerous mucosa is demonstrated to reasonably plot the boundary between epithelium (EP) and lamina propria layers, determine the EP thickness, and estimate the dysplastic cell distribution range based on SD mapping. SD of histology image intensity shows quite consistent mapping with dysplastic cell distribution due to its more random structures. Based on the SD mapping in an OCT image, the laterally-average depth-range percentages of 70 % SD maximum level can be a reasonably good threshold for the diagnoses of mild dysplasia and moderate dysplasia. With all these diagnosis golden rules based on OCT scanning, biopsy-free, real-time diagnosis of oral cancer and precancer becomes possible.

Polarization-sensitive optical coherence tomography imaging of benign and malignant laryngeal lesions: an in vivo study

J. A. Burns, Massachusetts General Hospital (United States); K. H. Kim, Pohang Univ. of Science and Technology (Korea, Republic of); J. F. de Boer, Vrije Univ. Amsterdam (Netherlands); R. R. Anderson, S. M. Zeitels, Massachusetts General Hospital (United States)

Objectives/Hypothesis: Optical coherence tomography (OCT), an imaging technology that provides cross-sectional subsurface tissue structure images using backscattered light, is promising non-invasive, cross-sectional imaging modality for in-vivo assessment of vocal fold layered microstructure. Polarization-sensitive OCT (PS-OCT) augments conventional OCT by providing images corresponding to changes in the polarization state of reflected light. This study imaged various benign and malignant laryngeal pathologies in patients undergoing direct laryngoscopy under general anesthesia to determine whether PS-OCT would provide useful additional information about vocal fold microstructure and glottic surface pathology.

Study Design: Prospective clinical trial.

Methods: Twenty-three patients who were undergoing microlaryngoscopy under general anesthesia for benign (N=18) and malignant (N=5) glottic disease were imaged bilaterally with OCT and PS-OCT (N=46 vocal folds). Intraoperative microphotography guided placement of the imaging probe. Normal-appearing glottic tissue was also imaged if present. When clinically indicated, biopsy or complete removal of the lesion established histologic confirmation.

Results: PS-OCT provided high quality images that complemented information obtained with conventional OCT for specific vocal fold pathologies. Scar tissue was characterized by a birefringence pattern that was more intense than the signal obtained from normal glottic tissue. Conclusions: Combining PS-OCT with OCT during human vocal cord imaging provides useful information in characterizing vocal cord lesions.

In vivo detection of oral cancer based on OCT-derived morphological and FLIM-derived biochemical biomarkers of the oral mucosa

P. Pande, S. Shrestha, J. Park, B. E. Applegate, J. A. Jo, Texas A&M Univ. (United States)

Early cancer detection in the oral cavity holds great promise for improving survival rates and quality of life of survivors. Both biochemical and morphological changes accompany the transition from normal to neoplastic tissue. Optical coherence tomography (OCT) provides structural description of the tissue with micron resolution. Fluorescence lifetime imaging microscopy (FLIM) provides information about the tissue biochemical composition (in particular collagen, NADH and FAD). We therefore hypothesize that their synergy will increase the accuracy for early detection of oral cancer. In this study, we report on the discriminatory power of intrinsic morphological and biochemical biomarkers of oral neoplasia derived from co-registered OCT and multispectral FLIM images recorded in vivo from benign and malignant lesions in a hamster cheek pouch model of oral cancer. The co-registered OCT and FLIM images of the oral mucosa, acquired simultaneously using a recently developed multimodal system, were correlated with the tissue histopathology. Morphological features derived from the OCT images include thickness of different tissue layers, presence or absence of the basement membrane, and texture features. Biochemical features derived from the FLIM images include: relative intensity and lifetime values of the tissue autofluorescence at three emission bands, and surrogates of the relative concentration of collagen, NADH and FAD in the oral mucosa. The combination of these features within a statistical classification algorithm will have the potential for non-invasive automated early detection of oral cancer. Future efforts will be devoted to demonstrating the accuracy of this multimodal approach to detect malignant oral lesions in human patients.

Optical coherence tomography in the assessment of oral squamous cell carcinoma resection margins

Z. Hamdoon, Univ. College Hospital (United Kingdom); W. K. Jerjes, Univ. College London Hospitals NHS Foundation Trust (United Kingdom); G. P. McKenzie, Michelson Diagnostics Ltd. (United Kingdom); A. Jay, Univ. College Hospital (United Kingdom); C. Hopper, Univ. College London Hospitals NHS Foundation Trust (United Kingdom)

Background and Objectives: Incomplete surgical removal of cancer is believed to be the main cause of local recurrence and high mortality. This study assessed the use of optical coherence tomography (OCT) to examine oral squamous cell carcinoma resection margins to see if this modality could aide the surgeon during surgical resection.

Material and Methods: Twenty-eight T1-T2 N0 oral squamous cell carcinoma (OSCC) patients took part in this study. Following tumour resection, the specimen resection margins were scanned in the immediate ex vivo phase. Two independent assessors (surgeons trained to interpret optical tomographic images) commented on the four resection margins of each specimen (tumour-free or tumour-involved). The findings were compared to the corresponding gold standard histopathology. The average epithelial thickness for both tumour-free and tumour-involved margins were calculated.

Results: The pathology report of 112 margins revealed 90 tumor-free margins and 22 tumor-involved margins. The mean epithelial thickness at the tumour-free resection margins was 360µm; While, it was 567µm for the tumour-involved margins and by comparing both values the relation was significant (P<0.001).
Working upon the data from both surgeons, the overall sensitivity and specificity were 86% and 88%, respectively. Whilst the positive predictive value was 66% and the negative predictive value was 96%. OCT accuracy for the first assessor was 88% and for the second assessor 84%.

The assessors’ inter-observer agreement was very good for superior, inferior and medial margins (0.806, 0.713 and 0.718, respectively); while agreement on the lateral surgical margin status was good (0.644).

Conclusion: Optical coherence tomography is a valuable tool in the assessment of surgical margins. Tumour-involved margins can be identified by architectural changes and increase in epithelial layer thickness on the OCT image. Further studies are required to assess tumour margins in vivo, eliminating the bias of specimen shrinkage post resection.

7883C-152, Session 7

Optical coherence tomography in the assessment of suspicious oral lesions: prospective study
Z. Hamdooon, Univ. College Hospital (United Kingdom); W. K. Jerjes, Univ. College London Hospitals NHS Foundation Trust (United Kingdom); G. P. McKenzie, Michelson Diagnostics Ltd. (United Kingdom); A. Jay, Univ. College Hospital (United Kingdom); C. Hopper, Univ. College London Hospitals NHS Foundation Trust (United Kingdom)

Background and Objectives: Optical coherence tomography (OCT) is an evolving optical technology that is capable of delivering real-time, high-resolution signatures of tissue. The purpose of this prospective clinical study was: (1) to assess the sensitivity and specificity of OCT in identifying potentially malignant and malignant oral conditions, (2) to determine the inter-observer agreement in the analysis of specific image parameters, and (3) to find out the oral epithelial thickness for different pathology groups.

Materials and Methods: This prospective study involved 125 suspicious oral lesions from 119 patients. The lesions were surgically biopsied and subjected to OCT in the immediate ex vivo phase. Two independent readers (surgeon and pathologist) examined the OCT images and assessed several cellular features including keratin layer (KL), epithelial layer (EP), basement membrane (BM) and lamina propria (LP), and recorded their findings using special OCT reading score. The sensitivity, specificity and accuracy of OCT to predict “the need for surgical biopsy” were calculated. The epithelial thickness was also measured.

Results: Optical coherence tomography achieved a sensitivity of 85% and a specificity of 78% in the assessment of potentially malignant and malignant diseases. The positive and negative predictive values were 86.5% and 77.5%, respectively. The accuracy was 82% and the kappa coefficient of inter-observer agreement was 0.72 on “the need for biopsy”. OCT imaging of oral lesions provided valuable information on the oral epithelial thickness. The mean epithelial thickness of benign lesions was 338µm, dysplasia 455µm and for invasive carcinoma was 650µm.

Conclusion: This study proposes that OCT can accurately identify wide spectrum of tissue pathology. Further studies can assess the role of OCT in evaluating and guiding surgical biopsies and monitoring disease.

7883C-159, Session 7

Developing applications for OCT in endocrine surgery and upper airway
M. Rubinstein, J. Boyd, S. Moon, J. Zhang, A. Saidi, Z. Chen, J. Kim, B. J. Wong, Beckman Laser Institute and Medical Clinic (United States)

No abstract available

7883C-169, Session 7

pH-dependent mechanisms of electromechanical reshaping
E. C. Wu, Univ. of California, Irvine (United States)
No abstract available

7883C-170, Session 7

Evolution of electric field during electromechanical reshaping of septal cartilage
D. E. Protosenko, Beckman Laser Institute and Medical Clinic (United States)
No abstract available

7883C-176, Session 7

Progress in medical applications of terahertz technology in the head and neck
V. P. Wallace, The Univ. of Western Australia (Australia)
No abstract available

7883C-184, Session 7

In vivo application of OCT for tissue inspection with artifact reduction and automated segmentation
A. Heisterkamp, S. Donner, Laser Zentrum Hannover e.V. (Germany); F. Witte, I. Bartsch, Medizinische Hochschule Hannover (Germany); B. Rosenhahn, Leibniz Univ. Hannover (Germany); A. Krüger, Laser Zentrum Hannover e.V. (Germany)
No abstract available

7883C-185, Session 7

Optical coherence tomography of the middle ear: clinical applications
J. F. R. Ilgner, Univ. Hospital Aachen (Germany); C. Farkas, European Laser Institute (Germany); M. Westhofen, RWTH Aachen (Germany)
No abstract available

7883C-186, Session 8

Fluorescence-guided surgical resection of oral cancer reduces recurrence
P. M. Lane, C. F. Poh, The BC Cancer Agency Research Ctr. (Canada); L. Zhang, The Univ. of British Columbia (Canada); M. Rosin, C. E. MacAulay, The BC Cancer Agency Research Ctr. (Canada)

Approximately 36,000 people in the US will be newly diagnosed with oral cancer in 2010 and it will cause 8,000 new deaths. The death rate
Photodynamic therapy outcome for oral squamous cell carcinoma

W. K. Jerjes, Univ. College London Hospitals NHS Foundation Trust (United Kingdom); Z. Hamdoon, T. Upile, Univ. College Hospital (United Kingdom); C. A. Mosse, Univ. College London (United Kingdom); C. Hopper, Univ. College London Hospitals NHS Foundation Trust (United Kingdom)

Introduction: This prospective clinical study assessed the oncological outcomes following surface illumination mTHPC-photodynamic therapy of T1/T2 N0 oral squamous cell carcinoma (OSCC) patients.

Material/Methods: Thirty-eight patients took part in this study. Their mean age at the first diagnosis of OSCC was 58.0 years. Common clinical presentation was an ulcer mainly identified in the tongue, floor of mouth or buccal mucosa. Current and ex-smokers represented 89.5% of the cohort; while current and ex-drinkers were 86.8%. Clinically 29 patients had T1 disease while 9 had T2 disease.

Results: Pathological analysis revealed that 12 patients had well differentiated SCC, 16 moderately-differentiated and 10 had poorly-differentiated cancer. All patients underwent mTHPC-PDT and were followed-up postoperatively. At last clinic review post-PDT, 26/38 patients showed complete normal clinical appearance in the primary tumour site. Nine patients showed complete response after 1 round of PDT, 22 patients underwent 2 rounds and 7 patients had 3 rounds. Recent biopsies from the study cohort showed that 15 had normal mucosa, 5 with hyperkeratinisation, 10 with dysplastic changes, 2 with carcinoma in situ and 6 showed recurrent SCC. Overall recurrence was 15.8% and the 5-year survival was 84.2%. Death from loco-regional and distant disease spread was identified in 3 patients.

The recurrence group comprised 6 patients. The mean age of 1st diagnosis of the recurrence group was 59.3 years. Most common presentation was an ulcer involving the buccal mucosa or retromolar area, identified in current or ex-smokers or current drinkers. The surgical margins in this group were also evaluated following laser or surgical excision, neck dissection and reconstruction.

Conclusions: mTHPC-photodynamic therapy is a comparable modality to other traditional interventions in the management of low-risk tumours of the oral cavity, with less morbidity.

Photodynamic therapy outcome for oral dysplasia

W. K. Jerjes, Univ. College London Hospitals NHS Foundation Trust (United Kingdom); Z. Hamdoon, T. Upile, Univ. College Hospital (United Kingdom); C. A. Mosse, Univ. College London (United Kingdom); C. Hopper, Univ. College London Hospitals NHS Foundation Trust (United Kingdom)

Introduction: Photodynamic therapy (PDT) is a minimally invasive surgical intervention used in the management of tissue disorders. It can be applied before, or after, any of the conventional modalities, without compromising these treatments or being compromised itself.

Materials and Methods: In this prospective study, a total of 147 consecutive patients with oral potentially malignant disorders were treated with surface illumination PDT, using 5-ALA or mTHPC as the photosensitiser. The average age was 53±8.9 years. The patients' recovery was uneventful and no complications reported. Comparisons with the clinical and histopathological features and rate of recurrence as well as malignant transformation were made. The patients were followed-up for a mean of 7.3 years.

Analysis and Results: Homogenous leukoplakias were identified in 55 patients, non-homogenous leukoplakias in 73 patients, whereas 19 patients had erythroplakias. Ex- and current lifelong smokers formed 84.4% of the recruited patients. While people who currently smoke and drink formed 38.1% (56 patients) of the cohort. Erythroplakias were mainly identified in heavy lifelong smokers. The most common identified primary anatomical locations were the lateral border of tongue, floor of mouth and retromolar area. Moderate dysplasia was identified in 33 patients while 63 patients had severe dysplasias; and 32 patients had a histopathological diagnosis of carcinoma in situ.

The rate of recurrence post-PDT was approximately 11.6%. Malignant transformation was observed in 11 patients (7.5%), in the tongue, floor of mouth and retromolar area. Recurrence and malignant transformation was mainly identified in erythroplakias and non-homogenous leukoplakias. The final outcome of the cohort showed that 11 (7.5%) suffered from progressive disease, 5 (3.4%) had stable disease, 12 (8.2%) were considered partially responsive to the therapy. Complete response was identified in 119/147 patients (81%).

Conclusion: 5-ALA-PDT and/or mTHPC-PDT offer an effective alternative treatment for oral potentially malignant disorders.
Selective sinoatrial node optical mapping to investigate the mechanism of sinus rate acceleration

S. Lin, T. Shinhara, B. Jong, P. Chen, Kranernt Institute of Cardiology (United States)

Studies using isolated sinoatrial node (SAN) cells indicate that rhythmic spontaneous sarcoplasmic reticulum Ca release (Ca clock) plays an important role in SAN automaticity. However, it is difficult to translate these findings into intact SAN because the SAN is embedded in the right atrium (RA). Cross contamination of the optical signals between SAN and RA prevented the definitive testing of Ca clock hypothesis in intact SAN. The objective of this study was to use a novel approach to selectively map intact SAN to examine the Ca clock mechanism. We simultaneously mapped intracellular Ca (Ca(i)) and membrane potential (Vm) in 10 isolated, Langendorff-perfused normal canine RAs. Electrical conduction from the SAN to RA was inhibited with high potassium (10 mmol/L) Tyrode’s solution, allowing selective optical mapping of Vm and Ca(i) of the SAN. Isoproterenol (ISO, 0.03 µmol/L) decreased cycle length of the sinus beats from 586±17 ms at baseline to 366±32 ms, and shifted the leading pacemaker site from the middle or inferior SAN to the superior SAN in all RAs. The Ca(i) upstream preceded the Vm in the leading pacemaker site by up to 18±2 ms. ISO-induced changes to SAN were inhibited by ryandine (3 µmol/L), but not ZD7288 (3 µmol/L), a selective If blocker. We conclude that high extracellular potassium concentration (10 mmol/L) can cause SAN-RA conduction block, allowing selective optical mapping of the intact SAN. Acceleration of Ca cycling in the superior SAN underlies the mechanism of sinus tachycardia during sympathetic stimulation.

Diagnostic imaging for interrogating atherosclerotic plaque burden using multimodal CARS

A. C. T. Ko, L. B. Mostaço-Guidolin, A. Ridsdale, M. S. D. Smith, M. Hewko, A. F. Pegoraro, E. M. Kohlenberg, B. J. Schattka, National Research Council Canada (Canada); M. Shiomi, Kobe Univ. School of Medicine (Japan); A. Stolow, M. G. Sowa, National Research Council Canada (Canada)

Due to lower spatial resolution, radiation toxicity or lower specificity and sensitivity towards early lesions, differentiating atherosclerotic plaque burden in arteries using conventional clinical imaging modalities have proved to be challenging. Recent advancement reported in MDCT, IVUS and OCT towards useful methodologies to differentiate different plaque types emphasizes the importance of a multi-prong approach in obtaining both morphological and compositional information in understanding plaque development, plaque burden and predicting the risk of plaque rupture. Recently nonlinear optical (NLO) imaging has demonstrated potential in atherosclerosis imaging by providing a minimally invasive, label-free method for fast biochemical imaging at sub-cellular resolution. [1-4] NLO imaging interrogates biochemical morphologies at or near the intact tissue surfaces with unprecedented depth resolution. In this study, a multi-modal, single-laser CARS based nonlinear optical microscope was used for ex vivo co-localized imaging of extra-cellular proteins (elastic lamina, collagen fibrils and etc.) and lipid-rich structures (such as foam cells) within intact aortic tissue obtained from myocardial infarction-prone rabbits. Based on the extra-cellular differences observed in the biochemical morphology between healthy arterial lumen and regions dominated by atherosclerotic lesions, a diagnostic parameter for differentiating atherosclerotic plaque burden within the vessel was developed. This parameter is calculated from the individual CARS, SHG and TPEF signal intensities and intensity differences between these imaging channels. Using this parameter we were able to distinguish plaques burden relative to the age of the rabbit. Texture analysis of collagen fibrils visualized by SHG on atherosclerotic lumen in relation with plaque burden will also be discussed.

Fluorescence imaging of macrophages in atherosclerotic plaques using plasmonic gold nanorose

T. Wang, The Univ. of Texas at Austin (United States); V. V. Sapozhnikova, J. J. Mancuso, X. Li, The Univ. of Texas Health Science Ctr. at San Antonio (United States); B. Willsey, K. P. Johnston, The Univ. of Texas at Austin (United States); M. D. Feldman, The Univ. of Texas Health Science Ctr. at San Antonio (United States); T. E. Milner, The Univ. of Texas at Austin (United States)

Macrophage is one of the most important cell types involving in the progression of atherosclerosis which leads to myocardial infarction. We report fluorescence imaging to visualize macrophages in atherosclerotic plaques using plasmionic gold nanorose as a nontoxic biocompatible fluorescence dye. Atherosclerotic lesions were created in the aorta of a New Zealand white rabbit subjected to a high cholesterol diet and double balloon injury. The rabbit was injected with 30 nm gold nanoroses coated with dextran. The macrophages with endocytosed nanoroses in ex vivo atherosclerotic tissues were imaged by a laboratory fluorescence imaging system. A HeNe laser at 633 nm was used as an excitation light source and a long-pass filter was utilized to collect fluorescence emission at wavelengths longer than 650 nm. Fluorescence images showed the presence and location of nanoroses, which was further confirmed by TEM images of the tissue cross-sections showing fluorescence. Results of our study suggest that macrophages in atherosclerotic plaques can be identified by fluorescence imaging using plasmionic gold nanorose.

Visualizing the subcellular structure of human coronary atherosclerosis using µOCT

L. Liu, J. A. Gardecki, S. K. Nadkarni, J. D. Toussaint, Y. Yagi, B. E. Bouma, G. J.  Tearney, Massachusetts General Hospital (United States)

Progress in understanding, diagnosis, and treatment of coronary artery disease (CAD) has been hindered by our inability to visualize cells and extracellular components associated with human coronary atherosclerosis in situ. Current tools for microstructure investigation at sub-cellular level, histology and SEM, are destructive, sample only a small portion of the specimen, and are prone to artifact. Intracoronary optical coherence tomography (OCT) provides spatial resolution on the order of 10 µm, which is too coarse for visualizing cells and sub-cellular features.

Here we present a new class of OCT imaging technologies, which we term µOCT, that have an order of magnitude improved resolution...
compared with conventional intracoronary OCT systems. We used µOCT to image human coronary arteries procured from explant hearts, endothelial cell cultures and swine coronary arteries. Two µOCT systems were employed, a SD-OCT system that achieves 2 µm x 2 µm x 1 µm (x, y, z) resolution (in tissue) at 8 fps and a full-field optical coherence microscopy (FFOCM) device that is capable of obtaining images with 1 µm x 1 µm x 1 µm (x, y, z) resolution (in tissue) at a rate of 0.5 fps.

Images obtained with these technologies uniquely show many cellular and subcellular features associated with atherosclerosis, thrombosis, and response to interventional therapy. These results suggest that µOCT technologies may become powerful new tools for investigating human coronary disease. Future development of a µOCT intracoronary catheter could allow subcellular characterization of the coronary wall in living patients.

7883D-74, Session 2
Simultaneous co-registered morphological and biochemical imaging of coronary atherosclerotic plaques using a dual-modal optical system combining OCT and FLIM

J. Park, P. Pande, S. Shrestha, B. E. Applegate, J. A. Jo, Texas A&M Univ. (United States)

Atherosclerotic plaque rupture causes acute cardiovascular events, and may lead to sudden death. Characterization of plaque morphology and composition are key factors to diagnose plaque vulnerability. Optical coherence tomography (OCT) provides structural characterization of plaques with micron resolution. Fluorescence lifetime imaging microscopy (FLIM) provides information about the plaque biochemical composition (in particular collagen and lipids). We have recently developed a bench-top dual-modality optical imaging system for simultaneous co-registered OCT and multispectral FLIM imaging of biological tissue. The system is capable of a-line rate of 59 kHz for OCT (with 7.6 µm and 10 µm axial and lateral resolutions, respectively) and pixel-rate of at least 30 kHz for FLIM (with 100 µm lateral resolution). The system was used to acquire OCT/FLIM images of postmortem human coronary atherosclerotic plaques. The co-registered OCT/FLIM images were then correlated with the plaque histopathology. Morphological features derived from the OCT images include: plaque and cap thickness, area of necrotic core and calcifications. Biochemical features derived from the FLIM images include: relative intensity and lifetime values of the tissue autofluorescence at three emission bands, and surrogates of the relative concentration of elastin, collagen and lipids within the plaque. Based on these features, it was possible to characterize different types of plaque, including: fibrotic, fibrolipid, fibrocalsified, thick and thin cap fibroatheromas. The combination of these features within a statistical classification algorithm will have the potential for automated detection of vulnerable plaques. Current efforts are devoted to developing a catheter imaging system for intracoronary OCT/FLIM imaging of atherosclerotic plaques.

7883D-75, Session 2
Combination of Raman spectroscopy and optical frequency domain imaging for coronary atherosclerosis

H. Wang, Massachusetts General Hospital (United States); J. A. Gardecki, Massachusetts General Hospital (United States); C. P. Fleming, B. E. Bouma, G. J. Tearney, Massachusetts General Hospital (United States)

The simultaneous acquisition of biochemical composition and morphological structures will provide complementary information that will likely expand our definition of plaque vulnerability based on structural and chemical information. Raman spectroscopy is an optical technique that can detect classes of compounds such as lipids (cholesterol, cholesterol esters, triglycerides), structural proteins (collagen, elastin, actin) and calcifications, the predominant molecules present in coronary artery disease. Since Raman spectroscopy operates without knowledge of the underlying microstructure, it is difficult to place spectroscopic signatures in their appropriate morphological context. Optical frequency domain imaging (OFDI) detects the 3D tissue microstructure with high spatial resolution and has demonstrated the ability to identify important morphological features relevant to coronary artery disease. Their combination could result in high resolution images rendered with molecular signatures, revealing the intrinsic features of different types of atherosclerotic lesions.

We will discuss the design and fabrication of an integrated Raman and OFDI intracoronary catheter that will acquire Raman spectra and OFDI images simultaneously. Performance of the catheter will be assessed with phantoms and coronary arteries ex vivo. Diagnostic potential will be compared to histology.

7883D-77, Session 2
Dual-modality catheter for optical frequency domain imaging and near-infrared fluorescence imaging

H. Yoo, J. W. Kim, M. S. Shishkov, E. namati, Massachusetts General Hospital (United States); T. F. Morse, The Boston Univ. Photonics Ctr. (United States); R. L. Shubochkin, Boston Univ. (United States); J. R. McCarthy, B. E. Bouma, F. A. Jaffer, G. J. Tearney, Massachusetts General Hospital (United States)

Understanding coronary artery disease requires methods that can be used in human patients for investigating the microstructure, composition, and molecular mechanisms underlying this disease. Optical frequency domain imaging (OFDI) and near-infrared fluorescence (NIRF) imaging are
complementary techniques for obtaining microstructural and molecular information from artery walls, respectively. In this study, we report a dual-modality intra-arterial imaging in vivo that simultaneously obtains microstructural and molecular information from the artery. The catheter is comprised of a double-clad fiber (DCF) and a ball-lens, contained within a rotating cable and a transparent sheath (2.4Fr). The single-mode inner core of the DCF transmits the OFDI light, whereas the multi-mode inner cladding is utilized to excite and detect NIR fluorescence. A custom-made rotary junction scans the optics along the artery wall in a helical pattern to obtain co-registered 3-D structural OFDI and 2-D molecular NIRF images. Comprehensive 3D datasets were acquired from a coronary stent that was covered by fluorescently labeled thrombus then deployed in an iliac artery of a living New Zealand White rabbit. The results clearly delineate the microstructures of the luminal arterial wall, metallic stent struts, and thrombus as well as NIRF emission. The NIRF signals, fused on the luminal surfaces in the OFDI cross-sectional images, show strong correspondence with the thrombus identified by the OFDI. This novel catheter could open up new opportunities for improving our understanding of coronary atherosclerosis, including characterizing coronary inflammation found in plaques at risk for causing heart attacks.

7883D-78, Session 2

Design, construction, and validation of a multimodal intravascular diagnostic catheter combining IVUS and fluorescence lifetime spectroscopy detection channels

J. Bec, H. Xie, D. R. Yankelevich, F. Zhou, Y. Sun, N. Ghata, R. C. Aldredge, L. Marcu, Univ. of California, Davis (United States)

We report the development and validation of an intravascular rotary catheter that enables bi-modal interrogation of arterial pathologies based on fast-frame time-resolved fluorescence spectroscopy (TRFS) and intravascular ultrasound (IVUS). The catheter is based on a parallel design that allows for independent rotation of the ultrasonic and optical channels within an 8 Fr catheter sheath and integrates a low volume flushing channel for blood removal in the optical pathways. In current configuration, the two channels consist of a standard IVUS catheter with single element transducer (40 MHz) and a side-viewing UV-grade silica/ silica fiber optic (400 µm core) with collimating optics. The catheter is terminated by a small (1.25x3mm) metal housing to keep the fiber centered within the sheath. To clear the field of view from blood, a saline solution can be flushed in the sheath channel containing the fiber optic and deflected to the vessel wall by a cavity in the housing. The grooves design was optimized with a computational fluid dynamics (CFD) model pursued in a parallel study. The ability of the catheter to operate in intraluminal setting in blood flow, the effect of probe-to-tissue distance on optical signal and ability to generate co-registered TRFS and IVUS data were demonstrated in blood vessel phantoms. Current results demonstrate the feasibility of the described catheter for parallel interrogation of vessel walls based on TRFS and IVUS and to generate robust TRFS data that can be co-registered with IVUS images. These results facilitate further development of a bi-modal TRFS/IVUS technique for intravascular diagnosis of atherosclerotic cardiovascular diseases including vulnerable plaques.

7883D-79, Session 3

Optical coherence tomography image artifacts in native coronary arteries: effect on plaque characterization ex vivo and prevalence in vivo

G. van Soest, T. P. M. Goderie, E. Regar, G. Nieves, S. Koljenovic, A. G. J. L. H. van Leenders, R. W. Serruyts, Erasmus MC (Netherlands); A. F. W. van der Steen, Erasmus MC (Netherlands) and Interuniversity Cardiology Institute of the Netherlands (Netherlands)

Background: Intravascular OCT is used for procedure guidance and artery wall diagnostics in catheterization laboratories worldwide. Like any imaging technique, intravascular OCT can produce artifacts. These artifacts have not been systematically studied to date. The purpose of this study was to identify the causes of classification errors in coronary atherosclerotic plaque characterization by OCT, with special interest in image artifacts.

Methods: Regions of interest were selected and imaged with OCT in 50 cross-sections, from 14 human coronary arteries, sectioned from 14 hearts at autopsy. Plaques were classified by interpretation of OCT images. Histology was the benchmark. Typical classification errors were identified. To assess the impact on clinical imaging, 37 randomly selected OCT pullbacks from the Erasmus MC database were inspected and scored for occurrence of image artifacts that were observed to cause classification errors in the ex vivo study.

Results: OCT was able to correctly classify 32 out of 50 cross-sections. Systematic classification errors in OCT were intimal thickening classified as fibro-atheroma in 10 cross-sections. Based on image analysis, we identified five distinct image artifacts confounding plaque classification. Their occurrence and appearance depended on catheter optics, superficial macrophage infiltration, and catheter position. 34 out of 37 in vivo pullbacks were found to exhibit these artifacts to varying degrees.

Conclusions: Typical image artifacts were observed ex vivo and in vivo. Some of these were found to affect the interpretation of OCT data. We categorized the artifacts and explain their occurrence based on the physics of OCT image formation, providing elements of a new guideline for image analysis.

7883D-80, Session 3

Tools for experimental characterization of the non-uniform rotational distortion in intravascular OCT probes

M. L. Dufour, C. Bisaillon, G. Lamouche, S. Vergnonle, M. Hewko, F. D’Amours, M. G. Sowa, National Research Council Canada (Canada)

The Industrial Material Institute (IMI) together with the Institute for Biodiagnostic (IBD) has developed its own optical catheters for cardiovascular imaging applications. Those catheters have been used experimentally in the in vitro coronary artery model of the Langendorff beating heart and in a percutaneous coronary intervention procedure in a porcine model. For some catheter designs, non-uniform rotational distortion (NURD) can be observed as expected from past experience with intra-vascular ultrasound (IVUS) catheters.

A two-dimensional (2D) coronary artery test bench that simulates the path that gives access to the coronary arteries has been developed. The presence or absence of NURD can be assessed in the test bench using custom-built cardiovascular Optical Coherence Tomography (OCT) imaging system. A square geometry instead of the circular shape of an artery is used to simulate the coronary arteries. Thereby, it is easier to visualize NURD when it is present. The cumulated torsion induced by the friction on the catheter is measured along the artery path.

NURD is induced by the cumulated torsion force that is balanced by the varying friction force. Thus pullback force was measured and is correlated with NURD observed in the 2D test bench. Finally, a model is presented to help understanding the mechanical constraint that leads to the friction force variations.
Improved phantoms of coronary arteries for optical coherence tomography

C. Bisaillon, M. L. Dufour, G. Lamouche, National Research Council Canada (Canada)

We report significant improvements to a previously reported method to fabricate coronary artery phantoms for Optical Coherence Tomography (OCT). The method consists in the deposition of multiple layers on a rotating tubular structure. Each layer replicates the optical properties of the corresponding layer measured from a porcine coronary artery, and has elasticity similar to arteries for low deformations. The method also allows including various features in the phantoms to represent arteries affected by atherosclerosis. We have previously presented phantoms with inclusions that have the distinct optical signature of intima thickening, calcification and lipid pool in OCT images.

The improvements presented in the current paper include: more representative optical properties, phantoms with more realistic calcified and lipid plaques, and phantoms simulating more complex diseased structures.

First, we will present results of the characterization of a larger number of arteries, that lead to phantoms that are more representative of the average optical properties found in arteries. Second, we will present improvements to our method to fabricate phantoms that have hard calcifications and liquid lipid pools with shapes that are more similar to those found in clinical situations. Third, we will present phantoms of arteries affected by other conditions such as Thin Cap Fibro-Atheroma, restenosis after stenting, etc.

Accurate reconstruction of longitudinal views by single Doppler beam tracking in intracoronary optical frequency domain imaging

J. Ha, Massachusetts General Hospital (United States); M. S. Shishkov, Wellman Ctr. for Photomedicine (United States); H. Yoo, Massachusetts General Hospital (United States); G. J. Tearney, B. E. Bouma, Wellman Ctr. for Photomedicine (United States)

To create a two- or three-dimensional image of vessels, a rotary junction motor spins the fiber probe in its outer transparent sheath at 100 revolutions per second, while a linear motor pulls it back within the sheath at speeds of 5 - 20 mm/s, causing the imaging beam to trace a helix along the inside of the vessel wall. Although the motor controls the velocity of the fiber probe relative to its protective sheath precisely, the sheath itself can move significantly within the coronary artery during the cardiac cycle. As a result, a derivation of the imaging beam from its helix track distorts the image created and thus introduces inaccuracies in the shapes and sizes of atherosclerotic plaques. Although radial distortions arising from displacements of the sheath perpendicular to its axis can be minimized through lumen surface-aligning algorithms, accurate compensation for longitudinal displacements based on image characteristics is even more challenging. As a solution to this problem, we propose a single beam based heterodyne Doppler interferometer. In contrast to conventional ways which require two beams to identify one of two motions in Doppler tracking, tracking relative longitudinal velocities of a catheter using a single beam is used for compensating distortions in catheter-based imaging. This is mainly because the summation of relative radial motion velocities over the single frame acquisition time is zero. To evaluate the feasibility of the tracking scheme, in vivo imaging of porcine coronary artery with a stent is performed.

Characterization of atherosclerotic plaques using combined time-resolved fluorescence spectroscopy and ultrasonic backscatter microscopy

Y. Sun, J. E. Phipps, M. Lam, H. Xie, Univ. of California, Davis (United States); M. C. Fishbein, Univ. of California, Los Angeles (United States); J. M. Cannata, K. K. Shung, The Univ. of Southern California (United States); L. Marcu, Univ. of California, Davis (United States)

This study investigated the use of a combined time-resolved fluorescence spectroscopy (TRFS) and ultrasonic backscatter microscopy (UBM) technique as a tool for detection and characterization of atherosclerotic plaques. Experiments were conducted ex vivo in endarterectomy carotid plaque samples (10 patients). High resolution ultrasound image (50 microns) was first acquired for each sample to reconstruct microanatomical features of the plaques and the region of interests were determined from these UBM images for fluorescence interrogation. Tissue autofluorescence induced with a nitrogen pulse laser (337 nm, 700 ps) from 95 distinct areas was used to retrieve the spectroscopic spectrum and time-resolved parameters. Ultrasonic spectrum analysis was applied on radiofrequency signals and spectrum similarity was used to differentiate distinct compositions. Lesions were evaluated histopathologically and quantified as to the percentage of different components. We determined that the spectroscopic parameters at discrete emission wavelengths enhanced by the ultrasonic spectral parameters (i) allowed for discrimination (sensitivity 85%, specificity 90%) of various compositional and pathological features associated with plaque vulnerability, and (ii) showed a high correlation between ultrasound tissue characterization, vessel wall fluorescence quantification, and plaque biochemical content: elastin, collagen, inflammatory cells, and necrosis (P < 0.05). Our results suggest that UBM and TRFS technique can be used to provide complementary information for identification of inflammatory cells and calcified lesions important in plaque formation and rupture. Current findings enable future development of combined ultrasound guided fluorescence spectroscopy clinical devices for rapid investigation of atherosclerotic plaques and detection of vulnerable plaques with improved sensitivity and specificity.
such as blood oxygenation, volume and flow. As a matter of fact, their utility has been demonstrated in a variety of applications for functional imaging of the brain, optical mammography and monitoring of muscle metabolism. However, due to technological and practical difficulties, their potential for cardiac monitoring has not yet been exploited. In this work we show the feasibility of the in-vivo determination of absorption and scattering spectra of the cardiac muscle in the 600-1100 nm range, and of monitoring myocardial tissue hemodynamics by time domain near-infrared spectroscopy (NIRS) at 690 nm and 830 nm. Both measurements have been performed on the exposed beating heart during open chest surgery in pigs, an experimental model closely mimicking the clinical cardio-surgical setting, by adopting two dedicated and optimized instrumentations for time-resolved diffuse NIRS.

7883D-85, Session 4

Toward development of an intravascular diagnostic catheter based on fluorescence lifetime spectroscopy: study of an optimized blood flushing system

N. Ghata, Univ. of California, Davis (United States) and ANSYS, Inc. (United States); R. C. Aldredge, J. Bec, L. Marcu, Univ. of California, Davis (United States)

Fluorescence lifetime spectroscopy techniques have demonstrated potential for characterization and diagnosis of arterial vessels pathologies. However, the intravascular application of such techniques is hampered by the presence of blood hemoglobin that affects both the delivery of the excitation light to and the collection of the fluorescence light from the vessel wall. We report here a computational fluid dynamics model that allows for the optimization of blood flushing parameters in a manner that minimizes the amount of saline needed to clear the optical field of view. A 3D turbulence (k - epsilon) model was employed to simulate the flow inside and around a side-viewing fiberoptic rotating catheter. The influence of uniform angular rotation of the catheter on the mixing and delivery of flushing fluids (e.g. saline) is studied and results are compared among cases involving catheters with various infusion geometries both with and without rotational motion.

Current results suggest better mixing for lower blood speed or flow rate (Re < 400) as compared to higher blood flow rate (Re > 700) for the same catheter geometry and the mixing can also be improved by changing the angle of the tip. Rotational motion of the catheter tip shows further improvement in the mixing process. Thus, depending on the range of blood flow conditions (Re), the catheter tip can be designed for very effective delivery of the flushing fluids.

7883D-86, Session 4

Correlations between matrix metalloproteinase expression and fluorescence lifetime spectroscopy measurements in human atherosclerotic plaque

J. E. Phipps, N. Hatami, Univ. of California, Davis (United States); M. C. Fishbein, Univ. of California, Los Angeles (United States); L. Marcu, Univ. of California, Davis (United States)

Matrix metalloproteinases (MMPs) are enzymes that play an important role in the pathogenesis of atherosclerosis. MMP-2 and -9 significantly affect the stability of atherosclerotic plaques through their roles in degrading extracellular matrix components and promoting the migration and proliferation of smooth muscle cells. In this study we tested: 1) whether MMP-2 and -9 expression varied between clinically relevant plaque phenotypes (e.g. intimal thickening, thick fibrotic cap, thin cap overlying a necrotic core) and 2) whether a time-resolved laser-induced fluorescence spectroscopy (TR-LIFS) system could differentiate varying MMP levels. This technique is sensitive to collagen, elastin, and lipid fluorescence, all of which are influenced by MMPs, thus we expect to find correlations between TR-LIFS measurements and MMP expression. Measurements were acquired from plaques retrieved from carotid endarterectomies (29 specimens, 77 locations) and correlated with histopathology and MMP expression. It was found that MMP-2 and -9 expression increased with markers of plaque vulnerability (R > 0.9, p<.001) and that multiple TR-LIFS parameters were capable of identifying MMP expression levels. For example, average fluorescence lifetime (1) increased with MMP-9 expression at 450 nm (R = -.94, p<.001), correlating to a decrease in collagen (R = 0.88, p = 0.05) and 2) decreased with MMP-2 expression at 540 nm (R = -.91, p<.001) correlating to an increase in necrosis (R = -.97, p<.001), both markers of increased risk of plaque rupture. These results indicate that TR-LIFS can discriminate varying levels of MMP-2 and -9, improving the sensitivity of this system to markers of plaque vulnerability.

7883D-87, Session 5

Intravascular OCT catheterization: from in-vitro coronary testing to percutaneous coronary intervention

M. Hewko, M. L. Dufour, S. Vergnole, M. S. D. Smith, F. D’Amours, National Research Council Canada (Canada); F. Hussain, St. Boniface General Hospital (Canada); G. Lamouche, M. G. Sowa, National Research Council Canada (Canada)

During the past several years, we have developed a swept source optical coherence tomography (SS-OCT) system for intravascular coronary imaging. Our previous studies have demonstrated the capability of our custom built SS-OCT system, fiber optic imaging catheter, and catheter rotational and pullback system to acquire high resolution 3-D imaging of coronary arteries. Those earlier in vitro studies utilized a modified Langendorff porcine beating heart model as the testing environment for system development. We have moved to an in vivo model of a porcine percutaneous coronary intervention model to further evaluate and advance the intravascular SS-OCT system towards a near clinical procedure environment. The evolution of the catheter design, reduction of imaging artifacts and integration with standard cardiac catheter lab tools and procedures will be discussed. Blood displacement techniques of through-balloon imaging and bolus flushing will be compared and discussed. The transition of measurements from the in vitro coronary artery model of the Langendorff heart to a percutaneous coronary intervention procedure in a porcine model will be discussed. Imaging results from both models and blood displacements techniques will be compared.

7883D-88, Session 5

Feasibility of optical frequency domain imaging assessment for micro thrombus on stent struts: a comparison study with scanning electron microscopy

A. Tanaka, Wellman Ctr. for Photomedicine (United States); D. Winsor-Hines, Boston Scientific Corp. (United States); M. J. Suter, K. A. Gallagher, Massachusetts General Hospital (United States); D. Allocco, Boston Scientific Corp. (United States); G. J. Tearney, B. E. Bourn, Massachusetts General Hospital (United States)

Background: The rare but serious complication of late stent thrombosis remains an important issue in interventional cardiology. Accurate assessment of thrombus overlying stent struts may allow better management of patients receiving drug eluting stent (DES). Aim: The aim of this study was to investigate the accuracy of Optical
Frequency Domain Imaging (OFDI) for assessing microscopic thrombus (microthrombus) on stent struts compared with scanning electron microscopy (SEM).

Methods: We implanted 10 stents (5 BMS and 5 DES) in 10 denuded coronary arteries in 4 Yorkshire swine. Animals were sacrificed 7 days after stent implantation and each coronary artery was bisected along its longitudinal aspect to create half-cylinders that were subsequently imaged by 3D OFDI and SEM ex vivo. OFDI datasets were volume rendered and displayed using a longitudinal cutaway view. Microthrombus was defined in the 3D OFDI images as protruding mass on stent struts or mass attached stent struts with irregular surface.

Results: We analyzed 360 stent struts from the 10 co-registered SEM and OFDI images. Microthrombus could be seen on 15 (4.2%) of all struts in SEM. There was no difference in the prevalence of microthrombosis on struts between BMS and DES (BMS 3.8% vs DES 4.4%, p=0.79). The sensitivity and specificity of OFDI for detecting microthrombus were 93% and 99%, respectively.

Conclusion: In this limited pilot study, OFDI was found to be capable of accurately detecting microthrombus on stent struts ex vivo. These results suggest that OFDI could be useful to assess microthrombus after stent implantation.

Non-thermal myocardial electrical conduction block by photosensitization reaction with catheterization in right atrium isthmus of porcine heart in vivo

A. Ito, T. Kajihara, T. Suenari, M. Takahashi, Keio Univ. (Japan); T. Kimura, K. Fukumoto, S. Takatsuki, S. Miyoshi, Keio Univ. School of Medicine (Japan); T. Arai, Keio Univ. (Japan)

We have studied a new type of myocardial catheter ablation with photosensitization reaction to realize non-thermal therapy for atrial arrhythmia, such as atrial fibrillation. Photochemically-generated reactive oxygen species may induce myocardial electrophysiological damage without heat generation. In this study, to demonstrate photosensitization reaction-induced myocardial electrical conduction block, the inferior vena cava to tricuspid annulus (IVC-TA) isthmus linear ablation was conducted with photosensitization reaction in porcine heart in vivo, using a newly developed laser catheter (7 Fr.). The end point of the procedure was the production of IVC-TA isthmus block under the electrophysiological analysis by diagnostic catheter with 10-bipole electrodes placed in right atrium along the isthmus. Talioporin sodium (NP68) as a photosensitizer was injected intravenously to pigs at 2.5–7.5 mg/kg. About 15 min after the injection, the laser light at the wavelength of 663 nm with a catheter output power density of 40–60 W/cm² in about 2 mm spot size was irradiated through the laser catheter point by point in line crossing the isthmus under the fluoroscopic guidance. Before the photosensitization procedure, electrical signal generated at the distal electrodes of the diagnostic catheter, propagated through the isthmus in order. During the irradiation, electrical potential at the irradiated area was diminished. After the completion of the irradiation line, the bidirectional conduction block on the IVC-TA isthmus was validated by pacing from the distal and proximal bipole. These results indicated that photosensitization reaction could achieve the electrical conduction block of myocardial tissue immediately after the irradiation. We think that photosensitization reaction could be a novel therapy for atrial fibrillation therapy.

Basic study of effects on the smooth muscle cells’ proliferation with novel short-term thermal angioplasty in vitro and in vivo

We investigated the effect on smooth muscle cells’ proliferation with stretch-fixing in both in vitro and in vivo porcine study to determine the optimum heat condition of novel short-term angioplasty called Photo-thermo Dynamic Balloon Angioplasty (PTDBA). We have proposed PTDBA to improve vascular dilatation performances. We obtained the sufficient arterial dilatation by short-term heating (< 15 s, <70 °C) and low dilatation pressure (< 0.4 MPa) without excessive intimal hyperplasia on chronic phase. The smooth muscle cells were found to be fixed with stretched shape in vascular wall after PTDBA in vivo. The deformation rate of smooth muscle cells’ nuclei was 1.6 ± 0.1 after PTDBA (15 s, 65 °C, 0.35 MPa). The smooth muscle cells, which were extracted from porcine arteries, were cultured on the specially designed equipment to give stretch-fixing stimulus in vitro. It was observed that the proliferation rate of stretch-fixed smooth muscle cells was changed by the stretch-fixing rate of the cells. The cell proliferation was inhibited at 20 % stretching compared to 15 % stretching significantly (p < 0.05). The immunostaining specimens of basic Fibroblast Growth Factor (bFGF), which is one of the smooth muscle cells’ growth factor, and its receptor FGFR-1 were made from the porcine arteries in vivo. We found that the distributed locations of bFGF and FGFR-1 were different after PTDBA. We think that the combination of these in vitro and in vivo results suggested the possibility for the inhibition of the excessive cell proliferation after PTDBA.

7883D-93, Session 6

In-vivo experimental study on laser welded ICG-loaded chitosan patches for vessel repair

F. Rossi, P. Matteiini, Istituto di Fisica Applicata Nello Carrara (Italy); G. Esposito, A. Albanese, A. Puca, G. Maira, Univ. Cattolica del Sacro Cuore (Italy); R. Pini, Istituto di Fisica Applicata Nello Carrara (Italy); G. Rossi, Univ. degli Studi di Camerino (Italy)

Laser welding of microvessels provides several advantages over conventional suturing techniques: surgical times reduction, vascular healing process improvement, tissue damage reduction. We present the first application of biopolymeric patches in an in vivo laser assisted procedure for vessel repair. The study was performed in 10 New Zealand rabbits. After anesthesia, a 3-cm segment of the right common carotid artery was exposed and clamped proximally and distally. A linear lesion 3 mm in length was carried out. We used a diode laser emitting at 810 nm and equipped with a 300 µm diameter optical fiber. To close the cut, ICG-loaded chitosan films were prepared: chitosan is characterized by biodegradability, biocompatibility, antimicrobial, haemostatic and wound healing-promoting activity. ICG is commonly used in the laser welding procedures and it was included in the biopolymeric matrices. The membranes were used to wrap the whole length of the cut, and then they were laser welded in the correct position by delivering single laser spots to induce local patch/tissue adhesion by photothermal effect. The result is an immediate closure of the wound, with no bleeding at clamps release. The animals were observed during follow-up and sacrificed after 2, 30 and 90 days. All the repaired vessels were patent, no bleeding signs were documented. The carotid samples underwent histological examinations. The advantages of the proposed technique are: simplification of the surgical procedure and shortening of the operative time; good strength of the vessel repair; decreased foreign-body reaction, reduced inflammatory response and improved vascular healing process.

7883D-94, Session 6

Optical pacing

M. W. Jenkins, Case Western Reserve Univ. (United States); A. R. Duke, Vanderbilt Univ. (United States); S. Gu, Y. Doughman, H. J. Chiel, H. Fujioka, M. Watanabe, Case Western Reserve Univ. (United States); E. D. Jansen, Vanderbilt Univ. (United States); A. M. Rollins, Case Western Reserve Univ. (United States)

The extrinsic control achievable using electrical pacing has been critical to advancing our understanding of cardiac electrophysiology. Unfortunately, electrical pacing of the embryonic heart is invasive and difficult to achieve consistently and without tissue damage. A simple noninvasive technique to control heart rate would allow one to manipulate forces applied to cells in early developing embryos, enabling a new class of experiments exploring the roles of mechanotransduction and electrical activation. Recently, we have demonstrated the ability of pulsed infrared light to pace embryonic quail hearts. A pulsed infrared laser operating at 1.875 µm noninvasively locked the heart rate of quail embryos to the pulse frequency of the laser. A laser Doppler velocimetry (LDV) signal was used to verify the pacing. At low radiant exposures, embryonic quail hearts were reliably paced in vivo without detectable damage to the tissue. Here, we describe thresholds for stimulation, which regions of the heart can be stimulated and the optical parameters necessary for successful pacing. Optical pacing (OP) has great potential as a tool to study embryonic cardiac dynamics and development. OP does not require contact, has high spatial precision and avoids stimulation artifacts in electrode recordings. OP will not only enable a new class of experiments in developmental cardiology, but also may become a useful tool for investigating cardiac electrophysiology, single-cell (cardiomyocyte) dynamics, and cardiac tissue engineering. Furthermore, OP may potentially be capable of pacing the adult heart, which could lead to clinical applications.

7883D-95, Poster Session

The usefulness of optical analyses for detecting vulnerable plaques using rabbit models

K. Nakai, M. Ishihara, S. Kawauchi, National Defense Medical College (Japan); M. Shiomi, Kobe Univ. School of Medicine (Japan); M. Kikuchi, T. Kaji, National Defense Medical College (Japan)

Purpose: Carotid artery stenting (CAS) has become a widely used option for treatment of carotid stenosis. Although technical improvements have led to a decrease in complications related to CAS, distal embolism continues to be a problem. The purpose of this research was to investigate the usefulness of optical methods (Time-Resolved Laser-Induced Fluorescence Spectroscopy [TR-LIFS] and reflection spectroscopy [RS]) as diagnostic tools for assessment of vulnerable atherosclerotic lesions, using rabbit models of vulnerable plaque.

Materials & Methods: Male Japanese white rabbits were divided into a high cholesterol diet group and a normal diet group. In addition, we used a Watanabe heritable hyperlipidemic (WHHL) rabbit, because we confirmed the reliability of our animal model for this study. Experiment 1: TR-LIFS. Fluorescence was induced using the third harmonic wave of a Q switch Nd:YAG laser. The TR-LIFS was performed using a photonic multi-channel analyzer with ICCD (wavelength range, 200 - 860 nm). Experiment 2: RS. Reflection spectra in the wavelength range of 900 to 1700 nm were acquired using a spectrometer.

Results: In the TR-LIFS, the wavelength at the peak was longer by plaque formation. The TR-LIFS method revealed a difference in peak levels between a normal aorta and a lipid-rich aorta. The RS method showed increased absorption from 1450 to 1500 nm for lipid-rich plaques. We observed absorption around 1200 nm due to lipid only in the WHHL group.

Conclusion: These methods using optical analysis might be useful for diagnosis of vulnerable plaques.
Conf. 7883E-97, Session 1

Ex-vivo fluorometry of intracranial tumor biopsies excised during fluorescence-guided resection and implications for intra-operative instrument sensitivity

A. Kim, Ontario Cancer Institute (Canada); M. Brantsch, C. Niu, Univ. of Toronto (Canada); E. Lebovitz, K. Kolste, F. Leblond, P. A. Valdes, K. D. Paulsen, Dartmouth College (United States); D. W. Roberts, Dartmouth Hitchcock Medical Ctr. (United States); B. C. Wilson, Ontario Cancer Institute (Canada)

The surgical treatment of intracranial tumors remains a challenge. The complete tumor resection is hampered by the difficulty of visualizing residual tumor cells in the resection bed near the end of the surgical procedure. Fluorescence-guided surgery has shown promise in improving detection of residual tumor cells. The most clinically advanced form of fluorescence-guided surgery uses 5-aminolevulinic acid (5-ALA)-induced protoporphyrin IX (PpIX) fluorescence-guided resection. The first step is to characterize the pattern of blood interference in the fluorescence spectrum. In this study, a fiber-optical based fluorescence spectroscopy system with a laser excitation light of 405 nm was used during fluorescence guided brain tumor resection using 5-aminolevulinic acid (5-ALA). The blood interference pattern in the fluorescence spectrum collected from the brain was studied in 15 patients. The operation situation was modeled in the laboratory by using blood drops from the finger tip on a stable fluorescent plastic as a tissue mimic and the data was compared to the data from the in vivo measurements on brain. The blood, when not blocking the optical signal totally, weakens the collected fluorescence intensity and leaves traces of oxy and de-oxy hemoglobin in addition to some other chromophores in the brain fluorescence spectrum.
Astrogliotic tissue displays markedly increased levels of ALA-induced PpIX fluorescence, making it useful for fluorescence-guided resection in glioma surgery. In patients with temporal lobe epilepsy (TLE) and corresponding animal models, there are areas of astrogliosis that often co-localize with the epileptic focus, which can be resected to eliminate seizures in the majority of treated patients. We tested the hypothesis that ALA-induced PpIX fluorescence could visually accentuate epileptogenic tissue, which could potentially identify margins in epilepsy surgery. First it must be determined whether the astrogliosis seen in epilepsy exhibits PpIX fluorescence that is sufficiently localized. We used pilocarpine to induce chronic seizure activity in rats, an established model of chronic TLE. Weeks later, subcutaneous EEG was recorded to verify epileptiform abnormalities, and video monitoring was used to quantify overt seizures. The rats were given ALA, euthanized, and brains examined post-mortem for PpIX fluorescence and neuropathological analysis. Preliminary evidence indicates increased PpIX fluorescence in areas associated with chronic epileptic changes and seizure generation in TLE, including the hippocampus and parahippocampal areas. In addition, strong PpIX fluorescence was clearly observed in layer II of the piriform cortex, a region known for its epileptic reorganization and involvement in the generation of seizures in animal studies. We are further investigating ALA-induced PpIX fluorescence to verify whether it can consistently identify epileptogenic zones, which could warrant its extension to clinical studies as an adjuvant guidance technology for the resection of epileptic tissue.

**7883E-100, Session 2**

**Evaluation of the use of antibody conjugated plasmonic nanoparticles for brain tumor delineation**

K. C. Seekell, C. Wilson, G. Grant, A. P. Wax, Duke Univ. (United States)

Complete resection of a pediatric brain tumor is a major factor in the survival rates of patients following surgery. This can be difficult as the tumor often resides near vital regions of the brain. Delineation of the brain tumor is an essential tool in helping the surgeon to remove the entire tumor while retaining normal tissues. Imaging techniques such as preoperative MRI and intraoperative fluorescence measurements have been used to detect brain tumor margins. In this study, the use of plasmonic gold nanoparticles as intraoperative contrast agents for tumor delineation was tested. Plasmonic nanoparticles have high scattering cross sections due to surface plasmon resonance. Antibody conjugated nanoparticles target and bind to specific cell receptors, thereby creating a biomarker for the molecule in question. Gold nanoparticles are non-cytotoxic and do not photobleach, which are significant advantages over fluorescent agents. The use of anti-EGFR conjugated gold nanorods for brain tumor delineation is evaluated by comparing this method to bioluminescence imaging, the gold standard in imaging tumor models in mice. GBM270 cancer cells with high EGFR expression were transfected with the luciferase gene and implanted into nude mice. Brain slices from these mice were incubated with anti-EGFR conjugated nanorods to allow for binding. A modified microscope was designed to separately image the signals from both the bioluminescence and the nanorod scattering. Analysis of the images showed that both delineation methods identified the same areas on the samples with high specificity. Gold nanoparticles can be an effective and safe method for intraoperative tumor delineation.

**7883E-102, Session 2**

**Enhanced transfection of a brain tumor suppressor gene by photochemical internalization**

C. H. Chou, Beckman Laser Institute and Medical Clinic (United States); Y. Zhou, Univ. of California, Irvine (United States); C. Sun, Beckman Laser Institute and Medical Clinic (United States); S. J. Madsen, Univ. of Nevada, Las Vegas (United States); H. Hirschberg, Beckman Laser Institute and Medical Clinic (United States)

Introduction: Photochemical internalization (PCI) is a photodynamic therapy-based approach for improving the delivery of macromolecules into the cell cytosol. The utility of PCI for the delivery of a tumor suppressor gene (PAX-6) was investigated in monolayers and spheroids consisting of F98 rat glioma cells. Materials and Methods: F98 monolayers or spheroids were incubated in AlPcS2a for 18 h followed by: (1) viral vector (1 h incubation) and light treatment, (2) non-viral vector (3-4 h incubation) and light treatment. In all cases, light treatment was performed with a diode laser at a wavelength of 670 nm. Replication deficient adenovirus expressing PAX6 (Ad-PAX6) and green florescent protein (Ad-GFP) was used for all viral transfection experiments. The non-viral vectors, Fugene HD or jetPEI, were used with a different plasmid construct (GFP-Luciferase-PAX-6) in the non-viral transfection studies. Results: Viral transfection in conjunction with PCI resulted in a significant increase in GFP expression in cell monolayers and throughout the spheroids. However, no PAX-6 expression was observed using real-time PCR under these conditions. In contrast, significant PAX-6 gene expression was observed following non-viral transfection of F98 glioma cells. PCI resulted in a 2-5 fold increase in PAX-6 expression compared to controls. Conclusions: Collectively, the results suggest that AlPcS2a-mediated PCI can be used to enhance transfection of a tumor suppressor gene such as PAX-6, in malignant glioma cells. Enhancement of gene transfection by PCI treatment though was more pronounced using viral vectors than that observed with Fugene HD or jetPEI.
Nonlinear optical imaging: toward chemical imaging during neurosurgery

B. Dietzek, Friedrich-Schiller-Univ. Jena (Germany); T. Meyer, Institut für Photonische Technologien e.V. (Germany); B. F. M. Romeike, R. Reichart, R. Kaff, Friedrich-Schiller-Univ. Jena (Germany); J. Popp, Institut für Photonische Technologien e.V. (Germany)

Tumor recognition and precise tumor border detection presents a central challenge during neurosurgery. In this contribution we present our recent all-optical approach to tackle this problem. We introduce various nonlinear optical techniques, such as coherent anti-Stokes Raman scattering (CARS), second-harmonic generation (SHG) and two-photon fluorescence (TPF), to study the morphology and chemical composition of (ex vivo) brain tissue. As the experimental techniques presented are contact-free all-optical techniques, which do not rely on the administration of external (fluorescence) labels, we anticipate that their implementation into surgical microscopes will provide significant advantages in online tumor border detection.

In this contribution an introduction to the different coherent optical spectroscopic methods will be presented and their implementation into a multimodal microscopic setup will be discussed. Furthermore, we will exemplify their application to brain tissue, i.e. both pork brain as a model for healthy human brain tissue and human brain samples taken from surgical procedures. The data to be discussed show the capability of a joint CARS/SHG/TPF multimodal imaging approach in highlighting various aspects of tissue morphochemistry. The consequences of this microspectroscopic potential, when combined with the powerful technology of existing surgical microscopes, will be discussed.

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Developing in-vivo micropathology for molecular image-guided brain tumor resection

J. T. C. Liu, Stony Brook Univ. (United States); M. J. Mandella, Stanford Univ. School of Medicine (United States); N. O. Loewke, Stanford Univ. (United States); D. Wang, Stony Brook Univ. (United States); F. V. Cochran, G. S. Kino, Stanford Univ. School of Medicine (United States); O. Solgaard, Stanford Univ. (United States); C. H. Contag, Stanford Univ. School of Medicine (United States)

The outcomes of brain tumor patients correlate with the degree of surgical resection. However, cautious resection is necessary to avoid neurological damage, especially in pediatric patients. Real-time image guidance will allow for improved resection in a larger proportion of patients, while reduce the debilitating effects of over-aggressive resections. Confocal microscopy, if modified for deep tissue imaging, enables visualization of sub-surface cells that are in their natural undisturbed tissue microenvironment, where cell-surface proteins may accurately be labeled with exogenous contrast agents. Furthermore, cellular and glandular morphologies may be resolved in real time, providing additional diagnostic information.

We have developed a surgical confocal microscope with a 2-mm diameter GRIN relay lens at the distal tip for in vivo micropathological guidance during resection of medulloblastoma, the most common form of pediatric cancers. The microscope we have developed utilizes a dual-axis confocal architecture to efficiently reject out-of-focus light for high-contrast optical sectioning within intact tissues. A biaxial MEMS scanning mirror is implemented to achieve a large field of view. Imaging studies have been performed with tissues from a transgenic mouse (Ptc1-/-; p53-/-; Math1-GFP) that spontaneously develops medulloblastoma with colocalized GFP expression. We are also developing topical-applied fluorescent contrast agents for delineating tumor margins with molecular specificity. These techniques will allow surgeons to unambiguously distinguish between normal and cancerous tissues for chemically-specific and spatially-precise tumor debulking.

Real-time intra-operative full-range complex FD-OCT guided cerebral blood vessel identification and brain tumor resection in neurosurgery

K. Zhang, Y. Huang, The Johns Hopkins Univ. (United States); G. Pradilla, B. Tyler, The Johns Hopkins Hospital (United States); J. U. Kang, The Johns Hopkins Univ. (United States)

Neurosurgery requires very precise and delicate manipulation of surgical tools through tight spaces surrounded by critical tissues. Therefore, it is extremely helpful to provide surgeons with real-time intraoperative imaging capabilities that are able to locate critical anatomical structures such as brain-tumor interface and cerebral blood vessels. In this work, we demonstrated an ultra-high-speed complex Fourier domain optical coherence tomography (FD-OCT) system used for real-time intraoperative imaging to guide two common neurosurgical procedures: cerebral blood vessel identification and brain tumor resection.

The complex FD-OCT system uses an 800nm-band superluminescent light source and a CMOS line-scan camera based spectral engine. The complex-conjugate artifact exists in standard FD-OCT is removed through graphics processing unit (GPU) accelerated online signal processing and a maximum real-time imaging speed of 244,000 A-scan/s is realized. A 3mm full-range imaging depth and 3D resolutions of 5µm/20µm in axial/lateral direction are achieved in current configuration.

The cerebral blood vessel identification experiment is conducted ex vivo on human cadaver specimen. Specific cerebral arteries and veins in different positions of the specimen are visualized and the spatial relations between adjacent vessels are indentified through real-time 3D visualization.

The brain tumor resection experiment is conducted in vivo on 9L and F98 gliomas established in rat brains. The brain-tumor boundary can be clearly identified in depth using sagittal, coronal and axial slices of the intraoperatively acquired 3D data set. Computer assisted real-time image processing is implemented to help segment the target tumor.

Multimodal confocal imaging for delineating brain cancer: the feasibility study

D. J. Wirth, Univ. of Massachusetts Lowell (United States); M. Snuderl, S. Sheth, W. Curry, Massachusetts General Hospital (United States); A. N. Yaroslavsky, Univ. of Massachusetts Lowell (United States) and Massachusetts General Hospital (United States)

Background and Significance: Complete resection of brain tumors may result in improved patient survival and better quality of life. A noninvasive method for accurate delineation of brain cancers would be valuable. The goal of this study was to establish the feasibility of using dye enhanced multimodal optical imaging for intraoperative discrimination of cancer tissue. Materials and Methods: Brain specimens were obtained from the surgeries performed at the Department of Neurosurgery at
Massachusetts General Hospital. Normal and cancer brain tissues were investigated. Cancer samples included high and low grade gliomas, meningiomas, astrocytomas, and glioblastomas. The tissues were briefly stained in 0.2 mg/ml aqueous solution of methylene blue (MB) or 1 mg/ml aqueous solution of demeclocycline (DMN). Excess dye was rinsed off and the tissues were imaged. Multimodal confocal images were acquired using an in house build system. A 60X Olympus LUCPlan FL N objective with 0.70 NA was employed. Reflectance and fluorescence signals of MB and DMN were excited at 658 nm and 405 nm, respectively. Fluorescence emission of MB and DMN was registered from 670 nm to 710 nm and from 430 nm and 600 nm, respectively. The system provided lateral resolution of 0.6 µm and axial resolution of 7 µm. H&E histopathology was processed from each imaged sample. The resulting optical images were correlated with histopathology.

Results and Conclusions: The analysis of normal and cancerous tissues indicated clear differences in appearance. The investigated cancer types exhibited distinctive characteristics in both the reflectance and fluorescence responses. These results suggest the feasibility of discriminating cancer tissue using multimodal confocal imaging.

Fluorescence and reflectance spectroscopy for protoporphyrin IX quantification in tissue-like media

G. Palte, H. Stepp, G. Hennig, A. Johansson, Ludwig-Maximilians-Univ. München (Germany)

PpIX induced by administration of ALA is being successfully employed for tissue diagnosis and photodynamic therapy (PDT) of, for example, brain malignancies. To guide tissue biopsy by fluorescence during stereotaxy, correct quantification of the PpIX accumulation is required. However, the detected fluorescence intensity and spectral shape are influenced and distorted by the varying optical properties of the surrounding tissue. Quantitative PpIX measurements thus need to disentangle these effects in order to provide the undistorted, intrinsic fluorescence. Numerous methods for obtaining the intrinsic fluorescence have been developed and optimized for certain fluorochromes. PpIX poses a particular case where excitation and fluorescence are spectrally well separated. Furthermore, the fluorescence appears within the red wavelength region where absorption in tissue is relatively weak.

Here, four experimental approaches towards assessing the intrinsic fluorescence for PpIX in four sets of homogeneous phantom materials at tissue-like conditions covering in total, µ= 0.1 - 200 cm-1, µs= 6 - 68 cm-1, were tested and compared; 1) single fiber with diameter 200-800 µm, multilabor fiber probe for combined fluorescence and reflectance measurements with evaluation based on 2) a theoretical model of light propagation or 3) based on an empirical ratio between fluorescence and reflectance signals, or 4) a multilabor fiber probe for differential path length measurements. All methods could be realized with an outer probe diameter of less than 1.5 mm. Method 3 could quantify the PpIX concentration for each subset with an accuracy of ±5-10%, whereas a calculation based on a plane wave geometry resulted in deviations greater than 56%.

Cortical blood flow imaging of mouse stroke model by high-speed spectral OCT

I. Grulkowski, Nicolaus Copernicus Univ. (Poland); G. Wilczynski, Nencki Institute of Experimental Biology (Poland); D. Bukowska, M. Szkulmowski, Nicolaus Copernicus Univ. (Poland); J. Wlodarczyk, Nencki Institute of Experimental Biology (Poland); K. Karnowski, D. Ruminski, A. A. Kowalczyk, M. Wojtkowski, Nicolaus Copernicus Univ. (Poland)

The advancements in neuroimaging during the last few decades have opened a large number of new possibilities in neuroscience, for example in monitoring brain injury due to the stroke. Non-invasive optical methods of brain imaging provide with the unprecedented insight not only in the structure of the living tissue but also the functionality of the tissue. Among other optical modalities Optical Coherence Tomography (OCT) appears to be a useful tool which enables to obtain two- and three-dimensional depth-resolved cross-sectional images with micrometer resolution.

We have developed a spectral OCT system for small animal imaging. Utilization of a high-speed CMOS detector enabled us to perform in vivo OCT imaging with high temporal and spatial resolution which is necessary for in vivo studies. Apart from revealing brain structural architecture, the system allows for qualitative and quantitative measurements of axial velocity component of blood flow. In order to visualize the flow, we used joint Spectral and Time domain OCT (STDOCT) analysis.

Here, we imaged the brain vascular network of an anesthetized mouse stroke model. Implementation of non-standard scanning protocols made it possible to visualize a broad range of velocity values. We have demonstrated the impact of induced stroke on the brain vasculature. The preliminary studies have revealed local ischemia in the areas of the stroke.

Neuro-endovascular optical coherence tomography imaging: clinical feasibility and applications

M. S. Mathews, Univ. of California, Irvine (United States); J. Su, E. Heidari, Beckman Laser Institute and Medical Clinic (United States); M. E. Linskey, Univ. of California, Irvine (United States); Z. Chen, Beckman Laser Institute and Medical Clinic (United States)

Optical Coherence Tomography (OCT) is a high resolution in vivo imaging modality that has found wide clinical application for retinal imaging. More recent work has found it to be feasible and useful for luminal imaging (such as gut and respiratory) as well as for coronary vascular imaging. However there has been very little work demonstrating its safety, feasibility, and applicability for intracranial use. The authors report on a clinical series of intracranial OCT imaging using neuroendovascular techniques. OCT imaging was carried out in patients using groin access under fluoroscopic visualization, with approval from the Institutional Review Board. Clinical imaging findings were correlated with cadaveric specimen imaged with OCT and then visualized histologically using a trichrome stain. Safety was evaluated using pre- and post-procedure clinical examination and post-procedure Magnetic Resonance Imaging in some cases. There was no clinical or radiographic evidence of post-procedure complications. OCT findings correlated well with histology. The study demonstrated that clinical neuroendovascular OCT imaging is safe and feasible. Potential applicability for visualization of intracranial pathology such as intracranial aneurysms, arteriovenous malformations, plaques, and dissections will be discussed.
vibratome tissue slicer under the sample arm. A coronal section was imaged by the multi-functional OCT, and then a slice of the brain was removed by the vibratome allowing for imaging the deeper regions in the brain. The procedure was repeated multiple times. Each session had 250 cross-sectional images acquired at 20 frames per second with an integration time of 50 µs for a depth profile (A-line). The axial resolution is about 5.5 µm in tissue. The reflectivity images show that the white matter in the brain exhibits higher backscattering and faster signal decay in depth compared to the grey matter. The results also show that birefringence, which is visualized by the phase retardance images, can be used to discern structures in the brain as the myelinated nerve tracts are highly birefringent. The axis orientation images present additional information related to the alignment of the fiber bundles. Moreover, the system can make use of the Doppler effect to simultaneously identify cerebral blood vessels in living brain. The technique, with successful development of a fiber-optic probe, has clinical potential to aid neurosurgical interventions.

7883E-113, Session 5
Seeing the focus of epilepsy through a hyperspectral camera during neurosurgery

In epilepsy surgery the focus of epilepsy should be delineated as accurately as possible to minimize damage to critical brain structures. Conventional focus localization techniques include scalp EEG, MEG, SPECT, and PET. These are all indirect methods, i.e. the focus has to be estimated, co-registered to MRI and relocated to the cortex during surgery. In this study we explored the use of a hyper-spectral camera in directly seeing oxygenation changes in the cortex.

An epileptic patient had recurring seizures every five minutes with tonic spasms in one hand, continuing for 24 hours a day. MR images and intracranial EEG recordings demonstrated seizure onset in the primary motor hand area. After removing the intracranial electrode grid, the exposed cortex was imaged using a hyper-spectral camera mounted to the surgical microscope. During a 7 minute scan, a group of 4 wavelength images was captured every second. By calculating the oxy-hemoglobin concentration, a local depletion of oxygen was seen in the motor cortex of the hand, corresponding to the intracranial EEG findings. After multiple subpial transections in this motor area, clinical seizures abated.

Thus, we were able to monitor seizure activity in the cortex directly observing local oxygen consumption. Although this is a special case with localized and predictable cortical changes, it is proof-of-principle that we can visualize local brain oxygen consumption at high resolution in real-time. This opens prospects of intra-operative function localization of sensory evoked stimuli, or, in the awake patient, of voluntary motor or speech activity.

7883E-114, Session 5
Study on the early diagnosis of Alzheimer's disease with near-infrared spectroscopy based on three-dimensional Monte Carlo modeling
C. Chuang, National Taiwan Univ. (Taiwan); C. Sun, National Yang-Ming Univ. (Taiwan); C. Chen, National Taiwan Univ. (Taiwan); C. Wang, Y. Hsieh, National Chiao Tung Univ. (Taiwan)

The techniques of CT and MRI are the most commonly used instrumental aids in the diagnosis of dementia and Alzheimer's disease. Besides, optical imaging of the brain is a rapidly growing field because it has attracted considerable interest recently due to a number of theoretical advantages in comparison with other brain imaging modalities. The near-infrared light is moderately absorbed by water, hemoglobin, and other significant body substances and thus can penetrate several centimeters inside the human brain. Brain atrophy is concomitant with Alzheimer's disease and other degenerative dementias, therefore, the difference of optical properties between normal subjects and patients, who with brain atrophy such as dementia and Alzheimer's disease, can indicate the status of brain structure. In our study, the photon migration in human brain of the normal, elderly and Alzheimer's subjects with various source-detector separations are analyzed based on three-dimensional Monte Carlo simulation. The whole brain MRI structure image is introduced as modeling in simulation. The three-dimensional brain model consists of scalp, skull, CSF layer, gray matter, and white matter. The backscattered
diffuse photons from each layer in brain are recorded by marking the deepest layer for brain status analysis. The expanded CSF layer affects the behavior of photon migration as waveguide effect thus the detected light distribution on brain surface implies the significant difference between normal and Alzheimer's subjects.

7883E-115, Session 5

Joint attention studies in normal and autistic children using NIRS

U. Chaudhary, M. Hall, Florida International Univ. (United States); A. Gutierrez, D. Messinger, Univ. of Miami (United States); G. Rey, Miami Children's Hospital (United States); A. Godavarty, Florida International Univ. (United States)

Autism is a socio-communication brain development disorder. It is marked by degeneration in the ability to respond to joint attention skill task, from as early as 12 to 18 months of age. This trait is used to distinguish autistic from non-autistic. In this study Near infrared spectroscopy (NIRS) is being applied for the first time to study the difference in activation and connectivity in the frontal cortex of typically developing (TD) and autistic children between 4-8 years of age in response to joint attention task. The optical measurements are acquired in real time from frontal cortex using Imagent (ISS Inc.) - a frequency domain based NIRS system in response to video clips which engenders a feeling of joint attention experience in the subjects. A block design consisting of 5 blocks of following sequence 30 sec joint attention clip (J), 30 sec non-joint attention clip (NJ) and 30 sec rest condition is used. Preliminary results from TD child shows difference in brain activation (in terms of oxy-hemoglobin, HbO) during joint attention interaction compared to the non-joint interaction and rest. Similar activation study did not reveal significant differences in HbO across the stimuli in, unlike in an autistic child. Extensive studies are carried out to validate the initial observations from both brain activation as well as connectivity analysis. The result has significant implication for research in neural pathways associated with autism that can be mapped using NIRS.

7883E-116, Session 5

Resting-state functional connectivity in newborn infants using DOT

S. L. Ferradal, B. R. White, S. M. Liao, J. P. Culver, Washington Univ. in St. Louis (United States)

Resting-state functional connectivity is a powerful method that can help to reveal the functional organization of the brain. It has been extensively demonstrated that low-frequency spontaneous correlations of BOLD-fMRI signals collected at rest define different functional resting-state networks (RSNs) in the human brain. Recent functional connectivity MRI (fc-MRI) studies have shown that changes in the functional connectivity of RSNs can provide new insights for the study of neurological disorders such as Alzheimer’s, schizophrenia and depression. Since fc-MRI enables brain function assessment in the absence of specific tasks, it also represents a very attractive tool for studying premature infants and intensive care patients. However, the clinical use of fc-MRI in these particular populations is mainly limited by the lack of portability. Alternatively, diffuse optical tomography (DOT) has the advantage of being able to evaluate the same neurovascular physiology as BOLD-fMRI using portable and wearable systems. We have previously developed techniques for spatially mapping functional connectivity with DOT (fc-DOT) in adults (White et al. Neuroimaging 2009). In this work, we examine resting-state functional connectivity in sleeping neonates, showing fc-DOT maps obtained for both, healthy term and premature infants, as well as a premature infant with an occipital stroke. We expect that in the future fc-DOT can provide bedside continuous brain function assessment for the early detection of neurological disorders associated with prematurity.

7883E-117, Session 6

Synthetic reconstruction of dynamic blood flow using optical coherence tomography of cortical microvasculature

E. Baraghis, Ecole Polytechnique de Montréal (Canada); M. Gillis, Montréal Heart Institute (Canada); V. J. Srinivasan, Massachusetts General Hospital (United States); É. Thorin, Montréal Heart Institute (Canada); C. Boudoux, F. Lesage, Ecole Polytechnique de Montréal (Canada)

Optical Coherence Tomography (OCT) has recently been used to produce 3D angiography of microvasculature in the rodent brain in-vivo and blood flow maps of large vessels. Key enabling developments were novel algorithms for detecting Doppler shifts produced by moving scatterers and new scanning protocols tailored to increase sensitivity to slow flow. These progresses were pushed by the need for a non invasive imaging modality to monitor quantitative blood flow at a higher resolution and at a greater depth than could be achieved by other means. The rationale for this work originates from new hypotheses regarding the role of blood regulation in neurodegenerative diseases and from our current investigation of animal models of vascular degeneration. In this work we demonstrate the synthetic reconstruction of dynamic blood flow over the course of a cardiac cycle in an 800µm wide by ~500µm deep B-Frame slice with a lateral resolution of 10µm and a depth resolution of 7µm. Images were taken using a cranial window over the exposed cortex of mice. Electrocardiography recordings were co registered to the OCT A lines at high temporal resolution. QRS peak detection was then used to locate the position of each A-line in the cardiac cycle and to reconstruct a synthetic temporal frame over one cardiac cycle. A movement correction algorithm was used to compensate for respiratory motion. Doppler images were used to measure temporal variations of flow and diameters of small arterial vessels. This measure was further correlated with pressure and the cardiac cycle which may provide a new avenue to measure vessel compliance.

7883E-118, Session 6

Identification of prefrontal cortex activation while performing Stroop test using diffuse optical tomography

S. Khadka, S. R. Chityala, F. Tian, H. Liu, The Univ. of Texas at Arlington (United States)

Stroop test is commonly used as a diagnostic tool for psychological problems that are related to attention and cognitive control of the human brain. Studies have shown prefrontal cortex (PFC) activation during attention and cognitive process which are validated by a few Stroop test studies as well. The use of diffuse optical tomography (DOT) for human brain mapping is becoming more prevalent. The DOT systems are comparatively inexpensive, compact, and portable and have a good temporal resolution which might be useful for clinical diagnosis. This study is to find potential biomarkers (temporal and spatial profile of DOT) in PFC that can correlate with the attention and cognitive control in the human brain. Such biomarkers may be helpful in diagnosis of diseases such as Alzheimer’s, Schizophrenia along with other cognition-related problems. Our initial study was conducted using a multi-channel DOT system with 8 sources and 16 detectors that were placed on the PFC area. The protocol used in our study was adapted from the traditional Stroop test and based on a blocked-design (Color word name matching versus Color word color matching). Preliminary data analysis indicates that while performing the task, there is an increase in oxy-hemoglobin concentration whereas deoxy-hemoglobin does not show any significant change. We will present the results from a greater pool of subjects with more detailed spatial and temporal analysis. We expect consistent temporal as well as spatial pattern among subjects and find quantifiable parameters that can be useful in diagnosis of attention- and cognitive control-related diseases.
7883E-119, Session 6

Optimizing statistical analysis with a general linear model for diffuse optical tomography to image rapid brain function events

M. Hassanpour, B. R. White, A. T. Eggebrecht, J. P. Culver, Washington Univ. in St. Louis (United States)

Near infrared spectroscopy (NIRS) has advantages as a functional neuroimaging tool. Compared with functional magnetic resonance imaging (fMRI), NIRS is portable and wearable, providing improved access to a wider variety of clinical settings and a more naturalistic setting for studies of brain development in children. Recently, new high-density diffuse optical tomography (HD-DOT) methods have demonstrated significant improvements in image quality. However, most HD-DOT studies have used slow block-averaging behavioral paradigms which require approximately 20 seconds per neurobehavioral stimulus. Both clinical studies and brain development studies in children would benefit from faster (~2 seconds between stimuli) event-related paradigms as used in the majority of fMRI studies. These event-related designs can also provide improved contrast to noise. Some NIRS studies with sparse imaging arrays have explored the use of event-related designs to evaluate the hemodynamic response function to neural stimuli. However, a critical component of the statistical analysis is estimation of the spatial correlation in the imaging data which differs significantly in HD-DOT compared to sparse-NIRS. Thus, the statistical methods need to be optimized and evaluated specifically for HD-DOT. In this work, we have implemented a general linear model (GLM) to estimate the hemodynamic response to event-related stimuli. We have also estimated spatial parameters required for statistical analysis and then generated statistical parametric maps (SPM) of activation in left and right visual cortex using voxel-wise t-tests. This approach provides us with an analytical tool to study more complex brain functions such as language development and learning processes in children.

7883E-120, Poster Session

Odor-induced hemodynamic response changes observed using near-infrared spectroscopy (NIRS) on the rat olfactory bulb

S. Lee, D. Koh, Y. Seo, Korea Univ. (Korea, Republic of); H. J. Lee, C. Im, J. Koh, H. Shin, Hallym Univ. (Korea, Republic of); B. Kim, Korea Univ. (Korea, Republic of)

Different smells induce odor-specific spatial patterns of olfactory bulb neural activity.

Previously, we reported different neural activity changes in rat olfactory nervous system to various odor stimulations and confirmed accurate discrimination of odor chemicals by decoding simultaneously recorded many neuron activities.

NIRS is a useful tool for study of brain function non-invasively that is detecting change in the concentrations in the brain, especially. When local neural activity in the brain is increased, hemoglobin concentration is changed due to oxygen consumption called neurovascular coupling.

In this study, we tried to draw the possibility of discriminating different kinds of odor stimulations by measuring hemodynamic responses in olfactory bulb. We applied 5 different odor stimulations such as Plane air (PA), Isoamyl acetate (IAA), Isopropl-benzene (IB), 1,7-Octadiene, 98% (OT) and 2-Heptanone, 99% (HEP). PA did not cause any changes in hemodynamic responses, but IAA, OT, IB and HEP showed different patterns of changes in hemodynamic responses. A similar trend of odor-induced hemodynamic responses was observed from all five rats.

Our results showed that the temporal change of hemoglobin oxygenation after odor presentation appears to be specific for each chemical odor smell. This result seems that hemodynamic responses to specific odor stimulations (in this case, 5 different chemicals) could be decoded and applied to casual connectivity of specific odor pathways between olfactory bulb and cerebrum.

7883E-121, Poster Session

Temporal mapping and connectivity using NIRS for language-related tasks

M. Hall, U. Chaudhary, Florida International Univ. (United States); G. Rey, Miami Children's Hospital (United States); A. Godavarty, Florida International Univ. (United States)

Near Infrared Spectroscopy (NIRS) offers an invaluable tool to monitor the functionality of the brain. The present study is aimed at using NIRS to understand the functionality of the temporal cortex in response to language-related tasks. A block-design based Word Expression and Word Reception tasks were independently presented to the participants (15 normal subjects) during the imaging study. Herein, the activation, connectivity, and lateralization in the temporal cortex are correlated. In the future, the work is focused to target the pediatric epileptic populations, where understanding the temporal brain functionality in response to language is essential in pre-surgical clinical environment.
7883F-122, Session 1

Optical coherence tomography as a guiding tool for minimally invasive surgery of the spine

K. Beaudette, M. Strupler, M. Driscoll, Ecole Polytechnique de Montréal (Canada); S. Parent, CHU Sainte-Justine (Canada); R. Maciejko, C. Aubin, C. Boudoux, Ecole Polytechnique de Montréal (Canada)

Scoliosis is a complex tridimensional deformation of the spine which, for severe cases, requires correction with surgical instrumentation. As these traditional surgeries involve invasive procedures resulting in the fusion of instrumented vertebrae, alternative minimally invasive procedures are currently under investigation. Amongst these techniques, one relies on inserting a small staple at the junction between the intervertebral disc and the growth plate in order to locally induce corrective growth modulation. To ensure the efficiency of the intervention, precise identification and localization of spinal structures are critical for a proper installation of the staple. This study aims at assessing the potential of optical coherence tomography (OCT) as a guiding system for minimally invasive procedures on the spine.

Ex vivo OCT imaging performed on an animal model allowed identification of microstructures such as vertebral bodies, intervertebral discs and growth plates. To assess in vivo performances of OCT, we designed and built a handheld probe having an axial resolution of 22µm and a lateral resolution of 10µm. Images are acquired and displayed at 20 fps over a field of view of 5mm using a commercial wavelength-swept OCT system. Images of musculoskeletal structures acquired during an open surgery on a porcine model will be presented.

7883F-123, Session 1

Efficacy of near-infrared spectroscopy in monitoring and detection of skeletal muscle ischemia

R. L. Harris, Univ. of Northern British Columbia Prince George Campus (Canada); B. Shadgan, UBC Muscle Biophysics Lab. (Canada); W. D. Reid, UBC Muscle Biophysics Lab. (Canada) and The Univ. of British Columbia (Canada); P. J. O’Brien, The Univ. of British Columbia (Canada)

The purpose of this study was to assess the efficacy and reliability of continuous wave near infrared spectroscopy (NIRS) for continuous monitoring and detection of changes in muscle oxygenation and hemodynamics during tourniquet-induced muscle ischemia throughout orthopedic trauma surgery. Changes in oxygenated (O2Hb), deoxygenated (HHb), and total hemoglobin (tHb) levels in leg muscles distal to the tourniquet of 21 patients (aged 19-69 yrs) with ankle fractures were monitored at 10 Hz before, during and after surgery. Tourniquet time (ischemia interval) varied among subjects, from 21 to 74 min (Mean±SD: 44±14). NIRS measured a progressive increase in HHb (2.6±2 µM) during the first minute of tourniquet inflation. Following tourniquet inflation a progressive increase in HHb (24.9±10.6 µM) with a concomitant decrease in O2Hb (23.3±8.1 µM) in the tourniquet-affected muscle was consistent across subjects. These changes in ΔHHb and ΔO2Hb began to reverse immediately after tourniquet release as reflected by a sharp increase in O2Hb (23.3±12 µM) during the first minute of leg muscle reperfusion in all subjects. Our findings confirmed that NIRS is an efficient method for non-invasive monitoring and detection of skeletal muscle ischemia and may have a diagnostic value for early detection of muscle ischemia in critical clinical conditions such as compartment syndrome.

7883F-124, Session 1

Steroid-induced osteoporosis monitored by Raman spectroscopy

J. R. Maher, M. Takahata, H. A. Awad, A. J. Berger, Univ. of Rochester (United States)

Glucocorticoids, a class of steroid hormone, are frequently used to treat inflammatory disorders such as rheumatoid arthritis. Unfortunately, extended exposure to glucocorticoids is the leading cause of physician-induced osteoporosis, leaving patients susceptible to fractures at reported rates of 30-50%. Although other drugs are being tested to reverse this effect, ~10% of treated patients still suffer subsequent fractures. In clinical trials of these drugs, the standard metric for bone quality (bone mineral density [BMD], measured via X-ray) has been unable to predict the likelihood of fracture.

We hypothesize that Raman spectroscopy-derived parameters might provide better correlations with fracture risk and thus help to evaluate drug efficacy. A variety of bone diseases and disorders (including osteoporosis) lead to characteristic changes in the Raman spectrum of bone. Typically, compromised bone strength and quality is evidenced by a reduction in the mineral-to-matrix ratio (MTMR), as quantified by the ratio of a phosphate to a protein peak.

In this presentation, we report correlations between Raman spectra and biomechanical strength tests on the bones of placebo- and glucocorticoid-treated mice. Both wild-type mice and a transgenic model of rheumatoid arthritis (TNF-Tg) have been studied. A two-way ANOVA model reveals statistically significant differences in the MTMR as influenced by glucocorticoid treatment and mouse type (wild-type vs. TNF-Tg). In addition, we discuss strategies to extract key Raman parameters of bone through overlying soft tissue, including the use of multiple source-detector separations and multivariate spectral fitting.

7883F-125, Session 1

Transcutaneous Raman spectroscopy for assessing progress of bone-graft incorporation in bone reconstruction and repair

P. I. Okaybare, F. W. Esmonde-White, Univ. of Michigan (United States); S. A. Goldstein, Univ. of Michigan Medical School (United States); M. D. Morris, Univ. of Michigan (United States)

Bone grafts are frequently used for a variety of reconstructive approaches in orthopedic surgery. Allografts are used in repair of segmental or significant bone loss and reconstruction following tumor resection. However, successful allograft incorporation remains uncertain. Consequently, there is significant need for methods to monitor the fate of these constructs. Few noninvasive methods can fully assess the progress of graft incorporation and to provide information on the metabolic status of the graft, such as the mineral and matrix composition of the regenerated tissue that may provide early indications of graft success or failure. For example, Computed tomography and MRI provide information on the morphology of the graft/host interface. Limited information is also available from DXA. To address this challenge, we present here the implementation of a noninvasive Raman spectroscopy technique for in-vivo assessment of allograft incorporation in an animal model.
Critical sized-defect is created in rat’s tibia and stabilized with an internal fixation plate. The defect is reconstructed using an auto or allograft and Raman spectra of bone specimens at several time points during healing using an array of optical-fibers in contact with the skin of the rat over the tibia while the rat is anaesthetized. The array allows excitation and collection of Raman spectra through the skin at various positions around the tibia. Raman parameters such as mineral/matrix, carbonate/phosphate and cross-linking are recovered and monitored. The system is calibrated against highly-constructed phantoms that mimic the morphology, optics and spectroscopy of the rat. This new technology provides a non-invasive method for in-vivo assessment of bone graft incorporation in animal models and can be adapted for similar study in human subjects.

7883F-127, Session 2

Experimental evaluation of bone drilling using short-pulsed laser ablation

B. Emigh, E. Hsu, E. Sorensen, R. An, H. K. Haugen, J. E. Hayward, G. Wohl, B. Dunlop, D. R. Williams, M. Anvari, Q. Fang, McMaster Univ. (Canada)

Pedicle screw spinal fixation is a procedure used specifically to achieve solid bone fusion in orthopedic patients. Pedicle screw insertion is challenging as the pedicle itself consists of only a narrow passage of bone into which screws need to be inserted. Pilot holes are commonly used to reduce the incidence of ill-placed screws and are typi-cally drilled using mechanical burr drills. Laser ablation has several potential advantages over mechanical drills used in orthopedics such as: (i) no mechanical vibration, (ii) non-contact interaction, and (iii) hemostatic and aseptic effects. We used a Ti:Sapphire laser and porcine bone specimens to evaluate the ablation efficiency and potential for drilling pedicle screw pilot holes using short-pulsed laser radiation. A number of laser drilling parameters have been evaluated including focus spot size, pulse duration (between 100 fs and 50 ps), pulse energy, irradiance, and wavelength. Based on the laser parameters, we have also investigated strategies to drill high aspect-ratio holes free of debris. Thermal damage and fractures in the surrounding tissue are two of the main drawbacks of laser ablation, which can be minimized through the appropriate selection of laser parameters.

7883F-128, Session 2

Space simulation of thermal fields generated in bone tissue for application to nanophotothermolysis and nanophotothermalism

R. R. Letfullin, C. Rice, Rose-Hulman Institute of Technology (United States); T. F. George, Univ. of Missouri-St. Louis (United States)

The use of nanoparticles in medical applications has been gaining momentum as antibody-conjugated nanoparticles are becoming more and more feasible as a means of targeted delivery of various therapies. Irradiating nanoparticles with light of strongly-absorbed wavelengths allows them to act as heat generation sites. Two therapies utilize these nanoparticles to heat lesions to kill the disease in the form of nanophotothermolysis, which heats the particles just enough to disrupt cell function and trigger cell death; and nanophotothermalism, which heats the particles to such extremes as to destroy the cell membrane. The use of optical wavelengths in the range of 750-1100 nm has been to capitalize on the “optical transparency window” of biotissues between the absorption peaks of hemoglobin in the visible and water in the near-IR. However, further research has shown that a plasmon resonance can greatly affect the absorption characteristics of nanoparticles at the plasmon resonant frequency, allowing for increased absorption characteristics at desirable wavelengths. Thus, other transparency windows may find use in a similar manner, such as nanoparticle heating by RF waves. This paper presents the modeling of 3D thermal fields around nanoparticle absorbers in bone tissue for various frequencies. A comparison of the heating effectiveness across multiple wavelengths is discussed for application to nanophotothermalism and nanophotothermolysis treatments in or near biological hard tissue.

7883F-129, Session 2

Quantitative evaluation of simulated human enamel caries kinetics using photothermal radiometry and modulated luminescence

A. Mandelis, A. Hellen, Y. Finer, Univ. of Toronto (Canada); B. T. Amaechi, The Univ. of Texas Health Science Ctr. at San Antonio (United States)

Detection modalities that can indirectly evaluate the early stages of dental caries are indispensable, as accurate detection and diagnosis initiate treatment plans to encourage remineralization. The development of photothermal techniques to detect thermal waves in biological tissue has occurred with a concomitant advancement in the extraction of material thermophysical and optical properties and knowledge regarding the internal structure of a medium. Photothermal radiometry and modulated luminescence (PTR-LUM) is an emerging non-destructive methodology applied toward the characterization and quantification of human dental caries. The purpose of this investigation was to evaluate the efficacy of PTR-LUM to detect, monitor and quantify human enamel caries in silico. Artificial caries were simulated in extracted human molars (n = 15) using an acidified gel system (pH 4.5) for 10 or 40-days. PTR-LUM frequency scans (1 Hz - 1 kHz) were performed prior to and during demineralization. Transverse Micro-Radiography (TMR) analysis followed at treatment conclusion. A coupled diffuse-photon-density-wave and thermal-wave theoretical model was applied to PTR experimental data to quantitatively evaluate the changes in opto-thermophysical properties of demineralized enamel as a function of time. Higher optical scattering coefficients and poorer thermophysical properties were characteristic of the growing demineralized lesions, as the generated microporosities of the subsurface lesion confined the thermal-wave centroid. Enhanced optical scattering coefficients of demineralized lesions resulted in poorer luminescence yield due to scattering of both incident and converted luminescent photons. PTR-LUM sensitivity to changes in tooth mineralization coupled with optical and thermophysical property extraction illustrates the technique’s potential for non-destructive quantification of enamel caries.

7883F-130, Session 2

Image-guided photoacoustic spectroscopy in diagnosis of osteoarthritis in hands: an initial study

Z. Yuan, Y. Sun, J. Xiao, E. S. Sobel, H. Jiang, Univ. of Florida (United States)

Multispectral photoacoustic tomography was employed to perform a case study of image-guided spectroscopy on osteoarthritis in the finger joints. In this case study, the distal interphalangeal finger joints from two patients and three healthy subjects were routinely scanned by a multispectral photoacoustic imaging systems. Images of tissue physiological parameters including oxy-hemoglobin, deoxy-hemoglobin, oxygen saturation and water content along with tissue acoustic velocity of all the examined joints were simultaneously recovered using a finite element reconstruction algorithm for multispectral photoacoustic measurements. The recovered photoacoustic images appear to show that the osteoarthritis joints have high water values and decreased oxygen saturation as well as increased acoustic velocity compared to the normal joints. Spectral-resolved photoacoustic tomography shows the potential in diagnosis of osteoarthritis and the capability to differentiate disease joints from the healthy ones.
Chirped laser photothermal radiometric cross-correlation studies of demineralized animal bones
A. Mandelis, Univ. of Toronto (Canada)

No abstract available

Three-dimensional imaging of dental hard tissues with Fourier domain optical coherence tomography
Y. Chen, Agiltron, Inc. (United States); Y. Yang, Univ. of Connecticut (United States); J. J. Ma, J. J. Yan, Y. Shou, Agiltron, Inc. (United States); T. Wang, Univ. of Connecticut (United States); J. Zhao, Agiltron, Inc. (United States); Q. Zhu, Univ. of Connecticut (United States)

Dental radiology (X-ray) is widely used as the gold standard for assessment of mineralization levels of hard tissues of dentin and enamel. The limited resolution and contrast however, prevents effective diagnostics of early tooth decay. The projectile image along buccal-lingual direction makes it difficult to identify caries lesions on occlusal surface, where up to 80% of tooth decay originates nowadays. In this paper, we report three dimensional imaging of tooth hard tissue surface using a high speed Fourier domain optical coherence tomography (OCT) system. A fiber probe is specifically designed for in vivo tooth imaging purpose. The probe has a handheld design with only 0.2dB single-pass insertion loss. To accommodate the topology of tooth surface, the probe has a 2.7 mm working distance with a depth of focus better than 2mm. The system is capable of acquiring image of 8 x 8 mm in X Y (lateral dimensions) and more than 5mm in Z (depth/height dimension) in one second. This imaging volume covers the region that most tooth decay occurs.

Tooth samples with demineralization from different orientations are scanned. The lesion area appears to have stronger back scattering intensity. We demonstrate several advantages in three-dimensional volumetric scans, such as indentifying pit area on occlusal surfaces and mapping topology of dentin-enamel junction. Volumetric OCT scan also enables image registration of OCT to itself measured at different time and to other imaging modalities such as micro CT and photograph. OCT and corresponding X-ray images are compared in this study.

Detection of chemical changes in bone after irradiation with Er,Cr:YSGG laser
C. Benetti, Instituto de Pesquisas Energéticas e Nucleares (Brazil); P. A. Ana, Univ. Federal do ABC (Brazil); M. O. Santos, J. S. Rabelo, D. M. Zezell, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

The use of laser for bone cutting can be more advantageous than the use of drill. However, for a safe clinical application, it is necessary to know the effects of laser irradiation on bone tissues. In this study, the Fourier Transform Infrared spectroscopy (FTIR) was used to verify the molecular and compositional changes promoted by laser irradiation on bone tissue. Bone slabs were obtained from rabbits and were polished down to 0.1 mm thick. After an initial analysis by ATR-FTIR spectroscopy, all samples were irradiated using a pulsed Er,Cr:YSGG laser (2780 nm), at energy density of 6.06 J/cm² and at adjusted speed of 13 mm/s to avoid pulse overlapping. After irradiation, samples were analysed once again using ATR-FTIR. In order to verify changes due to laser irradiation, the area under phosphate (1300-900 cm⁻¹), amides (1680-1200 cm⁻¹), water (3600-2400 cm⁻¹), and carbonate (around 870 cm⁻¹ and between 1600-1300 cm⁻¹) bands were calculated, and normalized by phosphate band area (1300-900 cm⁻¹). The statistic used was the hypothesis testing to paired sample T test at 5% significance level. It was observed that Er,Cr:YSGG irradiation promoted a significant decrease in the content of water and amides I and III at irradiated bone, evidencing that laser procedure caused an evaporation of the organic content and changed the collagen structure, suggesting that these changes may interfere with the healing process. In this way, these changes should be considered in a clinical application of laser irradiation in surgeries.

Diffuse reflectance study of the effects of bleaching agents in damaged dental pieces
J. Bante-Guerra, R. A. Trejo-Tzab, J. D. Macias, P. Quintana-Owen, J. J. Alvarado-Gil, Ctr. de Investigación y de Estudios Avanzados (Mexico)

One of the most important subjects of interest in dentistry and teeth preservation is related to the effects of bleaching agents on the integrity of the dental pieces. This is especially crucial when teeth surface has received some damage, related by chemical, biological and mechanical agents or weathering in the case of dental pieces recovered from burial sites. In this work the time evolution of the effects of bleaching agents on the surface of dental pieces is monitored using diffuse reflectance in the visible spectrum. The effects were monitored in teeth previously subject to chemical and mechanical abrasion. After that, samples were stained with coffee and tea immersing the pieces in high concentration solutions. Bleaching was induced using commercial whitening products. It is shown that the time evolution of the reflectance depends strongly on the condition of the surface as well as the thickness of enamel. Additionally the colorimetric analysis of the samples during the bleaching, useful in for comparing with previous studies, is presented. In order to complement our analysis, the effects of the bleaching were also monitored by scanning electron microscopy.

Photoacoustic Radar: Optimal Bandwidth and Chirp Duration for Frequency-domain Imaging
A. Mandelis, B. Lashkari, Univ. of Toronto (Canada)

In our previous work [1] in developing the photoacoustic (PA) radar, it was demonstrated experimentally that the -6 dB bandwidth of the ultrasonic transducer does not necessarily produce the best PA signal-to-noise ratio (SNR), as the optical and acoustic properties of the medium will modify the optimal bandwidth. The effects of these factors are investigated in frequency-domain (FD) PA imaging by employing a one-dimensional as well as an asymmetrical model of the PA effect, and the KLM [2] model for the employed transducers. Linear frequency modulation (LFM) chirps with different bandwidths were utilized and transducer sensitivities were measured to ensure the accuracy of the models. The theoretical conclusions are compared with experimental results and it is shown that the PA effect can act as a low-pass filter in the signal generation. Furthermore, the effective bandwidth lower limit can be affected by absorber and/or laser beam size: in the PA radar, the effect of two-dimensional wave generation appears as a false peak in the cross-correlation signal trace. These effects should be considered in the manipulation of the controllable features that the FD-PA method provides to improve the image quality.

No abstract available
Increasing the chirp duration and the number of successive A-scans for averaging purposes and SNR increase, however, is limited by safety standards impacting laser power and total exposure time. The impact on the SNR of each factor and thus on the maximum detectivity of the system is investigated using the abovementioned models, and experimental results are demonstrated and discussed.

**Efficient delivery of small interfering RNA into injured spinal cords in rats by photomechanical waves**

T. Ando, Keio Univ. (Japan); S. Sato, T. Toyooka, H. Kobayashi, H. Nawashiro, H. Ashida, National Defense Medical College (Japan); M. Obara, Keio Univ. (Japan)

In spinal cord injury (SCI), over-expression of intermediate filament proteins, such as vimentin and glial fibrillary acidic protein (GFAP), disturbs regeneration of injured nerve fibers, resulting in glial scar formation. Thus, silencing of these proteins would be an attractive strategy to the treatment of SCIs, and this can be achieved by an efficient delivery of the relevant small interfering RNAs (siRNAs) into injured spinal tissue. However, no viral methods for efficient gene delivery into spinal cords have not been established yet. In this study, we examined the delivery of fluorescent probe-labeled siRNAs into injured spinal cords in rats by applying photomechanical waves (PMWs). Rat spinal cord was exposed and injured with the MASCIS impactor device. After intrathecal injection of Alexa-Fluor 488-labeled siRNAs, a laser target, 0.5-mm thick natural rubber disk covered with a 1-mm thick transparent plastic sheet for laser-produced plasma confinement, was placed on the dura of the exposed spinal lesion. The target was irradiated with 532-nm, nanosecond laser pulses from a Q-switched Nd:YAG laser to generate PMWs. Animals were sacrificed 5 days after PMW application, and the spinal cords were removed and sectioned to observe fluorescence originating from siRNAs. We observed intense fluorescence from siRNAs in a much broader region of the spinal cords that had been exposed to 10 pulses of PMW generated at a laser fluence of 0.3 J/cm² when compared to the spinal cords with siRNA injection only. There were no significant differences in the results of functional evaluation between rats with the PMW application and those without PMW application. These results demonstrate the capability of PMWs for noninvasive, efficient delivery of siRNA into injured spinal cords.

**Functional near infrared brain imaging with a brush-fiber optode array to improve study success rates on pediatric subjects with cerebral palsy**

B. Khan, The Univ. of Texas at Arlington (United States); C. Wildey, The Univ. of Texas at Dallas (United States); F. Tian, M. I. Romero, The Univ. of Texas at Arlington (United States); M. R. Delgado, N. J. Clegg, L. Smith, Texas Scottish Rite Hospital for Children (United States); H. Liu, The Univ. of Texas at Arlington (United States); D. L. MacFarlane, The Univ. of Texas at Dallas (United States); G. Alexandrakis, The Univ. of Texas at Arlington (United States)

Neuroimaging techniques are useful to study neuroplastic rearrangements that occur due to dysgenesis, early life injury, or response to treatment in children with cerebral palsy (CP). However, the success rate of such studies is currently low for functional magnetic resonance imaging (<50%) due to motion artifacts caused by the patients’ involuntary movements. In recent studies we have demonstrated that functional near infrared (fNIR) imaging is a viable and sensitive method for mapping motor cortex activities in children with CP. The fNIR imaging signal collection success rate was ~70% as the imaging probes were strapped onto the patient’s heads, making the procedure robust to motion artifacts. Importantly, the main reason for the unsuccessful fNIR studies in the remainder ~30% of cases was because dense hair roots hindered optical contact between the probe and the scalp. To address this issue we are developing a head probe for fNIR imaging consisting of custom brush-fiber bundles that thread through hair and thus attain good optical contact. In preliminary studies we have found that, compared to commercially available fiber bundles, light signal throughput is increased by eight-fold while the head probe setup time is reduced by six-fold and the overall patient comfort is increased. However, the larger source and detector brush-fiber areas also degrade spatial resolution. We will present computational techniques that we use as part of the image reconstruction procedure to recover optimal spatial resolution. Validation results will be presented from sensory-motor spatio-temporal activation patterns obtained from children with CP and age-matched controls.

**Simultaneous imaging of light-evoked activities in retinal photoreceptors and inner neurons**

X. Yao, Y. Li, C. E. Strang, F. Amthor, L. Liu, K. T. Keyser, The Univ. of Alabama at Birmingham (United States)

Stimulus-evoked fast intrinsic optical signals (IOSs) in the retina may provide a new method to evaluate the functional connectivity of photoreceptors and inner neurons. In this experiment, we demonstrate the feasibility of IOS imaging of a frog retina slice preparation that allows simultaneous observation of stimulus-evoked responses from the photoreceptors to inner neurons. Robust IOSs were consistently detected from stimulus activated photoreceptor outer segments, inner plexiform layer (IPL), and ganglion cells; and weak IOSs could be occasionally identified in the outer nuclear layer, outer plexiform layer, and inner nuclear layer between the photoreceptor and IPL. At the photoreceptor layer, high magnitude IOSs were mainly confined to the area covered by the visible light stimulus. In comparison, IOSs of the IPL and ganglion layer could spread beyond the image area. High resolution IOS images showed complex spatial distributions of positive and negative IOSs at different retinal layers. At the photoreceptor and ganglion layers, positive and negative optical responses were mixed at a sub-cellular scale. We speculate that the positive and negative signal complexity might result from different types of localized light scattering and transmission changes associated with single activated photoreceptor or ganglion cells. We consistently observed that the IPL was dominated by positive-going optical responses. We hypothesize that the positive signal that dominated the IOS response in the IPL might be related to light scattering changes due to light-regulated release of synaptic vesicles at nerve terminals.

**In-vivo optical measurement of activity-dependent fluorescence change in striatum using synaptophluorin mice**

S. B. Jun, G. Cui, X. Jin, M. D. Pham, C. Thaler, S. S. Vogel, National Institutes of Health (United States); R. Costa, Instituto Gulbenkian de Ciência (Portugal); D. M. Lovinger, National Institutes of Health (United States)

The use of genetically encoded fluorescence probes is rapidly increasing especially in neuroscience. However, it still remains challenging to optically monitor neural activity from deep brain areas in awake animals. In this study, we propose a novel method to monitor neural activity in striatum of synaptophluorin transgenic mice using fiber optics and time-correlated single photon counting. Synaptophluorin is a pH-sensitive EGFP derivative linked to the vesicle protein, providing a probe that increases fluorescence upon exocytosis. A single-mode fiber for excitation and a multimode fiber for detection were assembled and implanted into the dorsolateral striatum region of synaptophluorin mice. Along with the fibers, a multichannel microelectrode array was used to record from neurons in the same striatum region.
implanted to simultaneously record extracellular neural activity. When the animal was anesthetized with the general anesthetic isoflurane, photon emission/detection was decreased by 50%. Electrophysiological recording also showed a decrease in the frequency of single unit activity and a decrease in the amplitude of local field potentials. To examine the fluorescence response to external stimuli in awake, behaving animals, an auditory stimulus of white noise was presented. Transient fluorescence increases were induced by the sound stimulation. The response was coincident with increased gamma oscillation of local field potential. After intraperitoneal injection of baclofen, a GABA receptor agonist, the fluorescence change to the sound stimulation was significantly decreased, indicating that the fluorescence change was sensitive to changes in presynaptic neurotransmitter release. These findings indicate that we can use in vivo photometry to measure fluorescence changes that report physiological changes in brain in the intact animal.

We present in this work biological responses on cultured mouse vestibular and retinal ganglion cells triggered by near infrared laser stimulation. Two techniques have been used to measure ionic exchanges through the neuron membrane: calcium fluorescence imaging and electrophysiological recordings (by whole cell patch clamp method). The stimulation system is based on a pulsed laser diode beam of a few mW launched into a multimode optical fiber positioned at a few micrometers away from the cells. Effects of three different wavelengths (from 1470 to 1875 nm), position and stimulation duration have been investigated. Values of stimulation energy thresholds show that the phenomenon clearly depends on the water optical absorption coefficient. Measurements of the local temperature have been performed. We show that the temperature rise is of the order of magnitude of the sensitivity of heat-activated membrane channels, supporting the hypothesis of a photothermal stimulation of ion channels.

7883G-146, Session 1
Optical imaging of signals evoked by infrared neural stimulation of the rat brain

J. M. Cayce, Vanderbilt Univ. (United States); M. Bouchard, B. Chen, Columbia Univ. (United States); E. D. Jansen, Vanderbilt Univ. (United States); E. M. C. Hillman, Columbia Univ. (United States); A. Mahadevan-Jansen, Vanderbilt Univ. (United States)

Infrared neural stimulation has been well characterized as a novel method to stimulate peripheral nerves without causing damage or inducing a stimulation artifact. Recently, our group has shown that INS can be used to produce intrinsic optical images of the intact brain in response to INS that follow established time courses for intrinsic responses to tactile stimulation of the forepaw and whisker. Single unit recordings made in regions of activation identified by optical imaging indicated inhibition during INS whereas tactile stimulation induced excitatory responses. To further our understanding of INS induced signals in the brain, multilayer optical imaging was used to assess components of the hemodynamic response and calcium dynamics during INS. INS and electrical stimulation was performed in the somatosensory cortex corresponding to the forepaw and hindpaw. INS was performed at 1.875μm light using repetition rates between 10 - 250 Hz for pulse trains ranging between 500 ms to 1000 ms. Electrical stimulation (3 Hz for 3 sec) was applied to the hindpaw or forepaw of the animal through needle electrodes to identify regions of cortex corresponding to the stimulated limb. Electrical stimulation parameters were adjusted to induce a small twitch in the stimulated paw. Optical images (images were collected in 90 Hz (90 Hz for each wavelength of light) for 30 secs under 470 nm (Calcium dye), 530 nm (total Hb), and 632 nm (deoxy Hb) illumination. The resulting images were compared for signal amplitude, spatial precision, and temporal precision between different laser parameters.

7883G-147, Session 2
Infrared laser stimulation of retinal and vestibular neurons

F. Bardin, Univ des Nîmes (France) and Univ. Montpellier 2 (France); J. Bec, Univ. Montpellier 2 (France); E. S. Albert, C. Chabbert, C. Hamel, Institut des Neurosciences de Montpellier (France); G. Dupeyrion, Ctr. Hospitalier Univ. de Nîmes (France); I. Marc, Ecole des Mines d’Ales (France) and Univ. Montpellier 2 (France); M. Dumas, Univ. Montpellier 2 (France)

The study of laser-neuron interaction has gained interest over the last few years not only for understanding of fundamental mechanisms but also for medical applications such as prosthesis because of the non-invasive characteristic of the laser stimulation. Several authors have shown that near infrared lasers are able to stimulate nerves. It is suggested that a thermal gradient induced by the absorption of the laser radiation on cells is the primary effect but the exact mechanism remains unclear.

7883G-138, Session 2
Pacing the embryonic heart with a pulsed laser

M. W. Jenkins, Case Western Reserve Univ. (United States); A. R. Duke, Vanderbilt Univ. (United States); S. Gu, Y. Doughman, H. J. Chiel, H. Fujioka, M. Watanabe, Case Western Reserve Univ. (United States); E. D. Jansen, Vanderbilt Univ. (United States); A. M. Rollins, Case Western Reserve Univ. (United States)

The exquisite control achievable using electrical pacing has been critical to advancing our understanding of cardiac electrophysiology. Unfortunately, electrical pacing of the embryonic heart is invasive and difficult to achieve consistently and without tissue damage. A simple noninvasive technique to control heart rate would allow one to manipulate forces applied to cells in early developing embryos, enabling a new class of experiments exploring the roles of mechanotransduction and electrical activation. Recently, we have demonstrated the ability of pulsed infrared light to pace embryonic quail hearts. A pulsed infrared laser operating at 1.875 μm noninvasively locked the heart rate of quail embryos to the pulse frequency of the laser. A laser Doppler velocimetry (LDV) signal was used to verify the pacing. At low radiant exposures, embryonic quail hearts were reliably paced in vivo without detectable damage to the tissue. Here, we describe thresholds for stimulation, which regions of the heart can be stimulated and the optical parameters necessary for successful pacing. Optical pacing (OP) has great potential as a tool to study embryonic cardiac dynamics and development. OP does not require contact, has high spatial precision and avoids stimulating artifacts in electrophysiological recordings. OP will not only enable a new class of experiments in developmental cardiology, but also may become a useful tool for investigating cardiac electrophysiology, single-cell (cardiomyocyte) dynamics, and cardiac tissue engineering. Furthermore, OP may potentially be capable of pacing the adult heart, which could lead to clinical applications.

7883G-131, Session 2
Analysis of the Thermal Response to Optical Nerve Stimulation

M. A. Mackanos, J. D. Malphrus, A. Mahadevan-Jansen, E. D. Jansen, Vanderbilt Univ. (United States)

A novel method for damage-free, artifact -free stimulation of neural tissue using pulsed, low-energy infrared laser light has been developed. Optical stimulation elicits compound nerve and muscle potentials similar to those induced by a free electron laser. In addition nerve stimulation has been performed using a Ho:YAG laser (2.12 um) and a solid-state laser nerve stimulator (1.87 um). One of the main goals of nerve stimulation research is to determine the relative contributions of thermal effects of the pulsed laser light at varying wavelengths and varying pulse durations. Previous
work has shown that a 6 Degrees C - 10 Degrees C surface temperature change is required for stimulation of the peripheral nerve; however, the laser pulse duration has an important role in the necessary temperature change for nerve stimulation. The goal of this research is to analyze how direct neural activation with pulsed laser light is induced by a thermal transient with varying pulse durations. We will present data comparing the thermal response on tissue phantoms (Ringer's lactate and gelatin) transient with varying pulse durations. We will present data comparing the change in the plasma membrane permeability and corresponding stimulation thresholds were calculated as the effective dose (ED50) outputted by a probit regression. The average threshold radiant exposure was 6.296 J/cm2 for repetition rates and 6.412 J/cm2 for pulse durations. The results show no statistically significant changes in threshold for the range of parameters tested except for the 20 ms pulse duration (7.59 J/cm2). Because the thermal relaxation time is ~90 ms, some heat dissipates during the 20 ms pulse. This higher threshold is therefore consistent with a thermally mediated mechanism of INS. Having shown feasibility in Aplysia, we believe this is a useful model for further studies to unravel the neurobiological mechanisms of INS.

7883G-143, Session 3

Monitoring millimeter wave-induced changes in neuronal activity using the leech ganglion

V. Pikov, Huntington Medical Research Institutes (United States); P. H. Siegel, California Institute of Technology (United States)

While penetrating electrodes are commonly used for focal electrical stimulation in the brain, their invasiveness can be traumatic to the brain tissue. This study attempts to develop a novel method for stimulation of neuronal activity using non-invasive delivery of millimeter waves. Segmental ganglion of the adult leech (Hirudo sp.) provides a simple and attractive model for evaluation of intracellular neuronal activity due to its easy dissection and maintenance ex vivo. Following the dissection, an individual ganglion or a chain of 2-3 ganglia is placed in a small Petri dish. The dish is filled with a leech saline and the ganglia are immobilized by placing a plastic ring over the outward axonal bundles. The millimeter waves are applied at power densities of 1 to 100 µW/cm² from below, through a quartz window in the dish bottom. The power density is quantified with a custom calorimeter placed immediately above the quartz window. The intracellular neuronal activity is monitored using sharp glass electrodes and an intracellular amplifier. The dose dependent change in the plasma membrane permeability and corresponding alteration in the firing rate are observed. The results provide strong evidence for the feasibility of controlling neuronal activity using non-invasive delivery of millimeter waves, and will be explored further for applications in basic neuroscience and treatment of neurological disorders.

7883G-144, Session 3

Depolarization of neuroblastoma cells using ultrashort electric pulses

B. L. Ibye, Air Force Research Lab. (United States); V. V. Nesin, Old Dominion Univ. (United States); G. J. Wilmink, Air Force Research Lab. (United States); A. G. Pakhomov, Old Dominion Univ. (United States)

Ultrashort or nanosecond electrical pulses (USEP) cause repairable damage to plasma membranes of cells through the creation of nanopores. Such nanopores are able to pass ions such as sodium, calcium, and potassium, but remain impermeable to larger molecules such as Trypan blue and propidium iodide. What remains uncertain is whether electromagnetic pulses can, at low doses, stimulate and, at high doses, inhibit action potentials within excitable cells and tissues. In addition, it also remains unclear how sensitive excitable cells are to such stimulus. In this paper, we aim to explore the sensitivity of excitable cells to USEP using electrophysiological and fluorescent imaging techniques to probe for changes in membrane potential and poration of the plasma membrane. Ultimately, such studies will uncover the threshold for stimulation and inhibition and differentiate it from lasting irreversible damage.
Two-Photon stimulation of excitable cells with and without optogenetic sensitization
S. Shivalingaiah, L. Gu, S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Recent advancement in optical stimulation of excitable cells using optogenetics via the use of channelrhodopsin-2 (ChR2) has enabled high temporal precision with low laser power requirement. However, since ChR2 is activated by blue light, which is highly attenuated in tissue, in-depth stimulation of excitable cells in an organ is limited or requires delivery of the light source near the cells of interest. Since the demonstration of in-depth two-photon stimulation (TPS) of ChR2-sensitized cells by us using Calcium imaging, there has been significant interest in measuring electrophysiological responses subsequent to TPS. Here, we report comparison of TPS threshold for excitable cells with and without optogenetic sensitization by co-registering calcium imaging and patch-clamp measurements. Since the average power threshold required in stimulating the ChR2-sensitized cells was found to be considerably higher than that used for two-photon imaging, we probed the damage threshold of cells as a function of power and stimulation period. To achieve minimal photo damage while stimulating the cells to generate action potential we investigated effect of a defocused TPS laser irradiation on the cell membrane. This also enabled evaluation of the minimum area (or ion channels considering ubiquitous distribution of calcium channels in outer membrane of the model cell) requiring activation for generating an action potential. We will present a comparison between focused and defocused TPS in cells with/without ChR2 using calcium imaging to confirm or rule out possible optoporation by TPS laser beam.

Nerve fiber recruitment in the context of hybrid neural stimulation
A. R. Duke, Vanderbilt Univ. (United States); H. Lu, M. W. Jenkins, Case Western Reserve Univ. (United States); M. A. Gault, Vanderbilt Univ. (United States); J. McManus, H. J. Chiel, Case Western Reserve Univ. (United States); E. D. Jansen, Vanderbilt Univ. (United States)

Recently, hybrid neural stimulation combining electrical and optical techniques was demonstrated. By applying a sub-threshold electrical stimulus with infrared neural stimulation (INS), hybrid stimulation was shown to reduce INS thresholds as much as 3-fold while maintaining spatial selectivity; thus overcoming the risk of thermally-induced tissue damage associated with INS and the fundamental lack of spatial specificity associated with electrical stimulation. While the potential of hybrid stimulation is evident, a better fundamental understanding of the interaction between tissue, light and current is necessary before this new stimulation paradigm can be further refined and optimized towards clinical implementation. A key element of this understanding is the spatial superposition of the electrical and optical stimuli. A successful hybrid stimulation paradigm requires accurate recruitment of the same neuron(s) by each modality. The fiber recruitment order of electrical stimulation is known, but recruitment associated with INS is less understood. We will present our investigation of the recruitment of select neurons using INS and electrical stimulation in the context of developing the hybrid stimulation paradigm. Stimulation thresholds are shown to vary with stimulus position to a greater extent than with electrical stimulation. This demonstrates improved selectivity with INS. Successful hybrid stimulation relies on recruitment of a target axon by both stimulation modalities. Our results indicate that the combined selectivity of optical and electrical stimulation will allow hybrid stimulation to offer a highly selective stimulation modality with less optical energy than INS alone.

Optical path of infrared neural stimulation in the guinea pig cochlea
L. E. Moreno, S. M. Rajaguru, A. I. Matic, N. Yerram, A. M. Robinson, C. Richter, Northwestern Univ. (United States)

It has been demonstrated previously that infrared neural stimulation (INS) can be utilized to stimulate spiral ganglion cells in the cochlea. Although neural stimulation can be achieved without direct contact of the radiation source and the tissue, the presence of fluids or bone between the target structure and the radiation source may lead to absorption or scattering of the radiation. Absorption or scattering of radiation may limit the efficacy of INS. The present study demonstrates that only neural structures in the beam path are stimulated. The histological reconstructions of guinea pig cochleae stimulated with INS suggest that the orientation of radiation from the optical fiber determined the site of stimulation in the cochlea. Best frequencies of the neural responses obtained from the central nucleus of the inferior colliculus matched the histological site in the spiral ganglion. Overall, the results indicated that the stimulated structures in the cochlea are the spiral ganglion cells and not the nerve fibers in the center of the modiolus.

Pulse shape effects on cochlear responses during infrared neural stimulation
R. Banakis, A. I. Matic, S. M. Rajaguru, C. Richter, Northwestern Univ. (United States)

Infrared neural stimulation (INS) has been shown to be effective in several neural systems, including peripheral motor nerves, the cortex, and the cochlea. The leading premise for INS mechanism is that neural depolarization occurs secondary to a spatially and temporally restricted photothermal interaction. In other words, a transient temperature rise needs to occur in the tissue to induce neural stimulation. Recent studies have investigated the variability of evoked responses with different optical parameters. In part, this is motivated by an interest in minimizing the temperature increase that occurs in the tissue, which is directly correlated with the amount of energy that is deposited. In this study, we examined the effect of pulse waveform on the evoked responses of INS in the cochlea. Pulse waveforms, such as square and triangular pulses with different rise times, were tested. Energy, power, and temporal properties of each pulse were verified optically. Cochlear compound action potentials (CAPs) were recorded from acutely deafened gerbils. The data were analyzed for CAP threshold and maximum CAP amplitude.

Continuous-wave optical stimulation of the rat prostate nerves using an all-single-mode 1455 nm diode laser and fiber system
S. Tozburnur, The Univ. of North Carolina at Charlotte (United States); G. A. Lagoda, A. L. Burnett, The Johns Hopkins Univ. (United States); N. M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: The cavernous nerves (CN) course along the prostate surface and are responsible for erectile function. Optical nerve stimulation (ONS) has recently been tested as a potential alternative to electrical stimulation for identification and preservation of these nerves during prostate cancer surgery. Continuous-wave (CW), near-infrared laser radiation delivered through 200-µm-core multimode optical fibers was used during these previous studies. This study describes an all-single-mode (SM) laser/fiber ONS system as a compact, inexpensive alternative to multimode (MM) laser/fiber delivery, with improved spatial...
Methods: CW laser radiation from 1455 (infrared) and 660 nm (visible aiming beam) pigtailed, SM diode lasers was combined into a SM fiber (9/125 μm core/cladding). A 3.4-mm-OD probe consisting of the SM fiber and 2-mm-diameter aspheric lens, provided a 1.0-mm-diameter collimated laser beam at the nerve surface over a 25-mm-working-distance. ONS of the CN in 5 rats was performed with 5 stimulations at each power setting. ONS was measured by an intracavernous pressure response (ICP) in the rat penis. A thermal camera recorded CN temperatures during stimulation.

Results: Successful ONS was observed at minimum temperatures of 42 °C using a laser power of 30 mW. ICP signal-to-noise-ratios of up to 4:1, and minimum ICP response times of 3 s were recorded. CN thermal damage did not occur until temperatures of 48 °C.

Conclusions: Continuous-wave infrared optical stimulation of rat cavernous nerves is feasible using an all-single-mode laser/fiber ONS system. Advantages include reduced cost and size, ease-of-use, and improved spatial beam profile for alignment and stimulation.

7883G-191, Session 3

Multi-physics system performance model for numerical simulations of infrared nerve stimulation

M. Keller, B. Norton, Lockheed Martin Aculight (United States); C. Richter, A. Izzo-Matic, S. M. Rajguru, Northwestern Univ. (United States); J. D. Wells, Lockheed Martin Aculight (United States)

Infrared nerve stimulation (INS) is the result of a laser-induced spatio-temporal temperature gradient. A multi-physics system performance model was recently developed by Lockheed Martin Aculight to simulate all physical processes associated with INS; the objective of this model is to identify optimal laser parameters to create a thermal distribution in tissue reaching stimulation threshold levels, while staying below damage thresholds, in a confined volume of axons needed to drive independent physiological responses (e.g. motor function or sensory percept). The model performs the following steps: light propagation from the light source or delivery system with a ray-tracing program; absorption and scattering of photons by tissue via Monte Carlo simulations; and heat generated from both absorption of photons and accumulation/dissipation of device-generated heat in the tissue with a finite difference model. Simulations can study the optically induced thermal field in complex, multi-layered tissue on the basis of any 3-D waveguide geometry and implant design, and reconstructions from histology and device designs can be directly entered into the model and assigned optical, thermal, and mechanical properties for each tissue or material type. The model has undergone preliminary validation by comparing predicted temperature rises with thermal ink studies in a hemi-sected cochlea. To date, it has been used both to predict the maximum number of allowable channels in an INS-based cochlear implant, and to examine relative strengths and weaknesses of performing high-channel-count stimulation of peripheral nerves via extraneural cuff and intrafascicular penetrating array approaches.
Diode laser for endodontic treatment: investigations of light distribution and disinfection efficiency

K. Stock, R. Graser, M. Udart, A. Kienle, R. Hibst, Univ. Ulm (Germany)

Diode lasers are used in dentistry mainly for oral surgery and disinfection of root canals in endodontic treatment. The purpose of this study was to investigate and to improve the laser induced bacteria inactivation in endodontic treatment.

To find out whether high power NIR laser bacterial killing is caused by a photochemical or a photothermal process we heated bacteria suspensions of E. coli by a water bath and by a diode laser (940 nm) with the same temporal temperature course. Furthermore, bacteria suspensions were irradiated while the temperature was fixed by ice water. Killing of bacteria was measured via fluorescence labelling.

In order to optimize the irradiation of the root canal, we designed special fiber tips with radial light emission characteristic by optical ray tracing simulations. Also we calculated the resulting light distribution in dentin by voxel-based Monte Carlo simulations. Furthermore we irradiated root canals of extracted human teeth using different fiber tip geometries and measured the resulted light and heat distribution by CCD-camera and thermography.

Comparison of killing rates between laser and water based heating shows no significant differences. Most important parameter is the maximum temperature.

Irradiation of root canals using fiber tips with radial light emission characteristic by optical ray tracing simulations. Also we calculated the resulting light distribution in dentin by voxel-based Monte Carlo simulations. Furthermore we irradiated root canals of extracted human teeth using different fiber tip geometries and measured the resulted light and heat distribution by CCD-camera and thermography.

In conclusion our experiments show that at least for E. coli bacteria inactivation by NIR laser irradiation is solely based on a thermal process and the heat distribution in root canal can be significantly improved by special designed fiber tips.

Laser scanning dental probe for endodontic root canal treatment

M. Blank, Univ. of Washington (United States); M. Friedrich, Consultant (United States); P. Lee, J. Berg, E. J. Seibel, Univ. of Washington (United States)

Complications that arise during endodontic procedures pose serious threats to the long-term integrity and health of the tooth. Potential complexities of root canals include residual pulp tissue, cracks, and accessory canals. In the case of a failed root canal, successful apicoectomy can be jeopardized by isthmuses, accessory canals, and root microfracture. Confirming diagnosis using a small scanning probe would allow proper treatment and prevent retreatment of endodontic procedures.

An ultrathin and flexible laser scanning endoscope of 1.2 to 1.6mm outer diameter was used in vitro to image extracted teeth with varied root configurations. Teeth were opened using a conventional bur and high speed drill. Imaging within the opened access cavity clarified the location of the roots where canal filing would initiate. Although radiographs are commonly used to determine the root canal size, position, and shape, the limited 2D image perspective leaves ambiguity that could be clarified if used in conjunction with the probe. Direct visualization may avoid difficulties in locating the root canal and reduce the number of radiographs needed. Probes can also separate the tasks of transmitting scanned laser illumination and receiving light; enhancing the imaging utility. A transillumination imaging technique with the separated functions made cracks apparent in the prepared teeth that were otherwise indiscernible with visible light.

Direct visualization of root canal and root end resection procedures using a high-resolution, small endoscope may significantly increase the efficiency and success of endodontic procedures.

Influence of Tm:YAP laser irradiation on tensile strength for bracket debonding

T. Dostálková, Charles Univ. in Prague (Czech Republic); H. Jelinková, J. Šulc, P. Koranda, M. Fibrich, M. Jelinek, Czech Technical Univ. in Prague (Czech Republic); P. Michalik, Charles Univ. in Prague (Czech Republic); M. Nemec, Czech Technical Univ. in Prague (Czech Republic); M. Miyagi, Sendai National College of Technology (Japan)

Laser bracket debonding is based on the principle of degrading the strength of adhesive resin between the tooth and the bracket after laser irradiation. A diode pumped Tm:YAP microchip laser generating a continuous 2-um radiation with the maximum output power of 4W was used. The tensile strength measured was analyzed. Two sets of 20 teeth with two types of ceramic brackets were investigated. Control group was treated without laser radiation. The bracket debonding instrument handle was pull until the bracket was completely separated from the enamel. Second group was irradiated by 1W laser radiation for 60s before debonding. The tensile strength needed for debonding was determined by force gauge. The temperature rise during the debonding procedure was recorded by the digital thermometer. Tooth surface temperature spatial distribution was monitored by the thermal imager-infrared camera. The stereomicroscope and scanning electron microscope were used for the documentation of the enamel surface. From the experiments performed it follows that the debonding tensile strength without laser irradiation had to be in the range from 23.0N (ceramic bracket with metal slot group) to 45.2N (full ceramic bracket group). When the brackets were irradiated the tensile strength was decreased from 13.1N (ceramic bracket with metal slot group) to 26.6N (full ceramic bracket group). The tensile strength after irradiation was about 50% lower in average. Continuously running, compact, diode-pumped Tm:YAP microchip laser having the output power 1W applied for 60s can remove the ceramic bracket without enamel iatrogenic damage.

Fluorescence-based calculus detection using a 405-nm excitation wavelength

O. Brede, F. Schelle, S. Krueger, B. Oehme, C. Dehn, M. Frenzen, A. Braun, Rheinische Friedrich-Wilhelms-Univ. Bonn (Germany)

The aim of this study was to assess the difference of fluorescence signals of cement and calculus using a 405 nm excitation wavelength. A total number of 20 freshly extracted teeth was used. The light source used for this study was a blue LED with a wavelength of 405nm. For each tooth the spectra of calculus and cementum were measured separately. Fluorescence light was collimated into an optical fibre and spectrally analyzed using an echelle spectrometer (aryelle 200, Lasertechnik Berlin, Germany) with an additionally bandpass (fgb 67, Edmund Industrial Optics, Karlsruhe, Germany). From these 40 measurements the median values were calculated over the whole spectrum, leading to two different median spectra, one for calculus and one for cementum. For further statistical analysis we defined 8 areas of interest (AOI) in wavelength regions, showing remarkable differences in signal strength. In 7 AOIs the intensity of the calculus spectrum differed statistically.
significant from the intensity of the cementum spectrum (p < 0.05). A spectral difference could be shown between calculus and cement between 600nm and 700nm. Thus, we can conclude that fluorescence of calculus shows a significant difference to the fluorescence of cement. A differentiation over the intensity is possible as well as over the spectrum. Using a wavelength of 405nm, it is possible to distinguish between calculus and cement. These results could be used for further devices to develop a method for feedback controlled calculus removal.

7884-05, Session 2
Detection of calculus by laser-induced breakdown spectroscopy (LIBS) using an ultra-short pulse laser system (USPL)
F. Schelle, S. Krueger, B. Oehme, C. Dehn, M. Frentzen, A. Braun, Rheinische Friedrich-Wilhelms-Universität Bonn (Germany)
The aim of this study was to assess the detection of calculus by Laser Induced Breakdown Spectroscopy (LIBS). The study was performed with an Nd:YAG laser, emitting pulses with a duration of 8 ps at a wavelength of 1064 nm. We used a repetition rate of 500 kHz at an average power of 5 W. Employing a focusing lens, intensities of the order of 1011 W/cm2 were reached on the tooth surface. These high intensities led to the generation of a plasma. The light emitted by the plasma was collimated into a fibre and then analyzed by an echelle spectroscope in the wavelength region from 200 nm - 900 nm. A total number of 15 freshly extracted teeth was used for this study. For each tooth the spectra of calculus and cementum were assessed separately. Comprising all single measurements median values were calculated for the whole spectrum, leading to two specific spectra, one for calculus and one for cementum. For further statistical analysis we defined 28 areas of interest as wavelength regions, in which the signal strength differed regarding the material. In 7 areas the intensity of the calculus spectrum differed statistically significant from the intensity of the cementum spectrum (p < 0.05).

Thus we can conclude that Laser Induced Breakdown Spectroscopy is well suited as a new method for a reliable diagnostic of calculus. Further studies are necessary to verify that LIBS is a minimally invasive method allowing a safe application in laser-guided dentistry.

7884-06, Session 2
Subgingival calculus imaging based on swept-source optical coherence Tomography
Y. Hsieh, National Chiao Tung Univ. (Taiwan); Y. Ho, S. Lee, National Yang-Ming Univ. (Taiwan); C. Lu, Industrial Technology Research Institute (Taiwan); C. Chuang, National Taiwan Univ. (Taiwan); C. Wang, National Chiao Tung Univ. (Taiwan); C. Sun, National Yang-Ming Univ. (Taiwan)
We demonstrate a non-invasive, non-destructive and non-radiative subgingival calculus detection method based on swept-source optical coherence tomography (SSOCT) at 1310 nm wavelength with Mach-Zehnder interferometer scheme. The refraction index of enamel, dentin, cementum and calculus is 1.625 0.024, 1.534 , 1.570 0.021 and 1.896 0.085 in our measurements, respectively. For feasibility study of clinical calculus diagnosis, a human tooth with calculus under gingival tissue with 0.3 mm thickness was employed as an in vitro sample. Besides, the calculus region was selected for image calibration with post imaging process. Our preliminary results show a high contrast image for subgingival calculus detection.

7884-07, Session 3
Effects of the new 940 diode laser treatment combined with scaling and root planing in the reduction of periodontal pockets: an in-vivo study
A. Fallah, RWTH Aachen (Iran, Islamic Republic of)
Objective: This study compared the effect of 940 Diode laser + scaling and root planing (SRP) versus SRP alone in the reduction of periodontal pockets.
Method: Twenty-four patients were included in the study with 144 random teeth divided in 2 equal groups (each 72 teeth) - control (SRP) and experimental (SRP with laser treatment). There was 4 dental treatment sessions every 7 days and one last assessment session at the 28th day. In both group and in all 4 treatment sessions, the complete SRP with ultrasound techniques and Gracy curettage was performed. In experimental group, we used 940nm diode laser with 1.5W, continuous mode, and 3 mm/sec sweeping motion from the depth of pocket upward to the margin. The whole laser process was done twice with a 2 minutes gap. The same laser process was performed as sham for the control group during the treatment sessions. PPD and BOP were determined before and 28 days after the treatment.

Results: The PPD values at the end of the treatment were lower than the baseline values. The results also showed significant improvement from laser+ SRP group to SRP alone group.

Conclusion: The present data suggest that periodontal pocket treatment with either 940 Diode laser + SRP or SRP alone results in statistically significant improvements in the clinical parameters. The combination of 940 Diode laser irradiation in the gingival sulcus and SRP, was significantly better as compared to SRP alone.

7884-08, Session 4
Development of an OCT probe for in-vivo early caries assessment
M. Hewko, S. Vergnole, F. D’Amours, M. L. Dufour, G. Lamouche, M. G. Sowa, L. Choo-Smith, National Research Council Canada (Canada)
In recent years, we have been developing optical coherence tomography (OCT) for the detection and monitoring of early dental caries. Our previous studies have shown that OCT provides high resolution depth imaging of early demineralization. In addition, we have demonstrated that a fibre-optic probe coupled to a swept-source OCT (SS-OCT) system allows for the collection of useful data with good signal-to-noise ratios and in real-time suitable for future in vivo preclinical studies. The next challenge is the development of appropriate in vivo probes. A rotating OCT catheter probe mounted within a handpiece similar to one commonly used for intra-oral examination was developed for this purpose. Results will be discussed regarding the technical design requirements of the probe and handpiece as well as specifications regarding spot size, focus depth and resolution. Preliminary in vivo OCT images will be presented in order to highlight the advantages and limitations of the current prototype for clinical intra-oral use.

7884-09, Session 4
Thermophotonic lock-in imaging of early demineralized and carious lesions in human teeth
A. Mandelis, N. Tabatabaei, Univ. of Toronto (Canada); B. T. Amaechi, The Univ. of Texas Health Science Ctr. at San Antonio (United States)
A novel dental imaging modality is proposed as an extension of frequency-domain photothermal radiometry. Thermophotonic lock-in imaging (TPLI) is introduced as a dynamic dental imaging modality. It uses photothermal wave principles and is capable of detecting early carious lesions and cracks on occlusal and proximal surfaces as well as early demineralization induced by artificial caries solutions. The increased light scattering and absorption within early carious lesions increases the thermal–wave amplitude and shifts the thermal–wave centroid, producing contrast between the carious lesion and the intact enamel in both amplitude and phase images. Samples with artificial demineralization and natural occlusal and approximal caries were examined in this study. Thermophotonic effective detection depth is controlled by the modulation frequency according to the well-known concept of thermal diffusion length. TPLI phase images are emissivity normalized and therefore insensitive to the presence of stains. Amplitude images, on the other hand, provide integrated information from deeper enamel regions. It was concluded that the results of our non-invasive, non-contacting imaging methodology exhibit higher sensitivity to very early demineralization than dental radiographs and are in agreement with the destructive transverse microradiography mineral density profiles. Moreover, the proposed modality is found to be able to detect the presence of approximal caries by imaging the occlusal surface to the tooth.

7884-10, Session 4

In-vivo near-IR imaging at 1310 nm
D. Fried, M. Staninec, C. L. Darling, C. S. Lee, H. Kang, K. H. Chan, Univ. of California, San Francisco (United States)

We show the first near infrared images of carious lesions on tooth proximal and occlusal surfaces. Two types of near infrared probes were developed and used for in vivo imaging. In this study 34 lesions on proximal surfaces and 15 lesions were imaged on occlusal surfaces. The carious lesions were clearly visible on both tooth surfaces in the near infrared. Lesions on proximal surfaces were chosen for imaging if they were visible on radiographs but were not visible to the clinician. Occlusal lesions were chosen that were scheduled for restoration based on conventional diagnosis that consists of visual and tactile examination. Out of the 34 proximal lesions examined 33 were visible in near infrared images. This study demonstrates that near infrared imaging at 1310-nm is well-suited for caries detection.

7884-11, Session 5

Swept source optical coherence tomography for quantitative and qualitative assessment of dental composite restorations
A. Sadr, Y. Shimada, P. Makishi, I. Hariri, T. A. Bakhsh, Tokyo Medical and Dental Univ. (Japan); Y. Sumi, National Ctr. for Geriatrics and Gerontology (Japan); J. Tagami, Tokyo Medical and Dental Univ. (Japan)

The aim of this work was to explore the utility of swept-source optical coherence tomography (SS-OCT) for quantitative evaluation of dental composite restorations. The system (Santec, Japan) with a center wavelength of around 1300nm and axial resolution of 12µm was used to record data during and after placement of light-curing composites into adhesively treated standard cavities up to 1.7mm in depth in a series of experiments. The Fresnel phenomenon at the interface resulted in brighter areas for gap as small as a few micrometers. An image analysis software was used to import the OCT B-scan data and quantify the extension of these bright areas. The gap extension at the interface was quantified and compared to the observation by confocal laser scanning microscope after trimming the specimen to the same cross-section. There was a strong correlation in the gap extension between from the two techniques. Video imaging of the composite during polymerization could provide information about real-time kinetics of contraction stress and resulting gaps, distinguishing them from those gaps resulting from poor adaptation of composite to the cavity prior to polymerization. Some samples were also subjected to a high resolution microfocus X-ray computed tomography (microCT) assessment; it was found that differentiation of smaller gaps from the radiolucent bonding layer was difficult with 3D microCT. Finally, some clinical examples using a newly developed dental SS-OCT system with an intra-oral scanning probe (Panasonic Shikoku Electronics, Japan) are presented. SS-OCT is a unique tool for clinical assessment and laboratory research on resin-based dental restorations. Supported by GCOE at TMDU and NCGG.

Development of polarization dental imaging modality and evaluation of its clinical feasibility
E. Kim, T. Son, B. Jung, Yonsei Univ. (Korea, Republic of)

Recently, it has become more important to objectively analyze color information of tooth in dentistry because it has been taken an interest in terms of esthetical point of view. In the evaluation of tooth color, the specula reflection caused by saliva on tooth may cause artifacts in analysis. Such artifact was partially solved in this study by developing a polarization dental imaging modality. The clinical validity was evaluated by performing three studies such as shade-guide selection for implant, plaque distribution detection, and evaluation of tooth whitening. In the selection of shade-guide, real tooth and shade-guide color images were obtained. The minimum color difference between shade-guide and tooth was calculated using Euclidian distance. In the plaque distribution detection, teeth disclosing agent was used to differentiate plaque from teeth and images were taken. In the tooth whitening, whiteness indices were calculated using the polarization and non polarization images. Results of this study presented that the new imaging modality could provide reproducible images by effectively removing the specula reflection on teeth surface and therefore, minimize artifacts in the quantitatively analysis of shade-guide selection, plaque detection, and tooth whitening. In conclusion, the polarization dental imaging modality potentially proved its clinical efficacy as a new diagnosis imaging modality.

7884-13, Session 5

A construction of standardized near-infrared hyperspectral teeth database: a first step in the development of reliable diagnostic tool for quantification and early detection of caries
M. Buermen, A. Fidler, P. Usenik, F. Pernuš, B. Likar, Univ. of Ljubljana (Slovenia)

Dental caries is a disease characterized by demineralization of enamel crystals leading to the penetration of bacteria into the dentine and pulp. If left untreated, the disease can lead to pain, infection and tooth loss. Early detection of enamel demineralization resulting in increased enamel porosity, commonly known as white spots, is a difficult diagnostic task. Several papers reported on near infrared (NIR) spectroscopy to be a potentially useful noninvasive spectroscopic technique for early detection of caries lesions. However, the conducted studies were mostly qualitative and did not include the critical assessment of the spectral variability of the sound and carious dental tissues and influence of the water content. Such assessment is essential for development and validation of reliable qualitative and especially quantitative diagnostic tools based on NIR spectroscopy. In order to characterize the described spectral variability, a standardized diffuse reflection hyper-spectral database for teeth was constructed by imaging 12 extracted human teeth with natural lesions of various degrees in the spectral range from 900 to 1700 nm with spectral resolution of 10 nm. Additionally, all the teeth were imaged by x-ray and digital color camera. The influence of water content on the acquired
spectra was characterized by imaging the teeth during the drying process. The images were assessed by an expert, thereby obtaining the gold standard. By analyzing the acquired spectra we were able to accurately model the spectral variability of the sound and carious dental tissues and identify the advantages and limitations of NIR hyper-spectral imaging over x-ray and visible imaging.

7884-14, Session 5

Cross-sectional imaging of extracted jaw bone of a pig by optical coherence tomography

N. Tachikawa, Tokyo Medical and Dental Univ. (Japan); R. Yoshimura, Kitasato Univ. (Japan); K. Ohbayashi, Kitasato Univ. School of Medicine (Japan)

Dental implantation has become popular in dental treatments. Although careful planning is made to identify vital structures such as the inferior alveolar nerve or the sinus, as well as the shape and dimensions of the bone, prior to commencement of surgery, it is not fully free from risks. One of the risks is nerve damage in the jaw, which causes pain in teeth, lips, gums, or chin. The risk is rare as the results of surgeons’ extreme tense care during surgery not to cut bone too much. If a diagnostic tool to objectively measure bone feature and thickness during surgery is available, considerable fraction of the risk may be avoided. We can stop boring bone before surround thickness of bone becomes too close to the sinus. OCT is a candidate, which enables cross-sectional imaging of bone to avoid the risk. We have been developing a discretely swept SS-OCT using SSG-DBR lasers and demonstrated its usefulness for dental application in previous works. In this work, we performed in-vitro cross-sectional imaging of extracted pig’s jaw bone using the SS-OCT. The relatively long wavelength range of 1600nm of SSG-DBR laser is suitable for deeper bone imaging. We found the image penetration length exceeds 2 mm in physical length (3.2 mm in optical length), which satisfies one of the criterions to apply OCT for in vivo diagnosis of bone surrounding implant hole during surgery. A novel diagnostic instrument during implant surgery to avoid bone over-cutting risk is proposed.

7884-15, Session 5

Comparison of short-pulsed CO2 9.6 µm wavelength laser-treated and untreated occlusal surfaces with OCT and polarized Raman spectroscopy

D. Charland, School of Dentistry, Univ. of California, San Francisco (United States); C. Fulton, National Research Council Canada (Canada); B. Rechmann, School of Dentistry, Univ. of California, San Francisco (United States); M. Hewko, M. G. Sowa, National Research Council Canada (Canada); J. D. Featherstone, School of Dentistry, Univ. of California, San Francisco (United States); L. Choo-Smith, National Research Council Canada (Canada); P. Rechmann, School of Dentistry, Univ. of California, San Francisco (United States)

Treatment of occlusal surfaces with a short-pulsed CO2 9.6 µm wavelength lasers has previously been proposed as a method for caries prevention. In order to characterize the surfaces following laser treatment, a sampling of 20 human molars were selected for investigation with optical coherence tomography (OCT) and polarized Raman spectroscopy (PRS). OCT provides high resolution morphological depth imaging while PRS furnishes molecular biochemical specificity and structural orientation. Indentation markings were placed to highlight the edges of two sets of occlusal fissure parts per tooth. One fissure part was subjected to laser treatment using a short-pulsed CO2 laser at 9,600 nm wavelength with a fluence of 3.5 J/cm2, 20 Hz pulse repetition rate, 20 µs pulse duration, angulated handpiece, focus diameter 600 µm while the other fissure was left untreated as control. Using a rotary catheter probe, OCT measurements were acquired from the various fissures to generate circularly mapped OCT depth images. PRS measurements of parallel- and cross-polarized spectra were acquired with a Raman microscope system. OCT and PRS results will be presented from treated and untreated occlusal fissures. Preliminary OCT images show differences in the initial air-tooth interface with PRS results indicating a change in the surface property along with biochemical alterations. Future studies are aimed at comparing these measurements with those acquired after a pH cycling demineralization regime.

7884-16, Session 6

Spectroscopic analysis of both enamel and dentin surfaces following XeCl excimer laser surface treatment

M. E. Gheith, National Institute of Laser Enhanced Sciences (Egypt)

Back ground and objective: The use of Excimer laser for the treatment of enamel and dentin surfaces has considerable potential because the combined characteristics of low wave length and short pulse result in limited heat diffusion and therefore tissue ablation without collateral damage. The aim of this work was to study the effect of Xe Cl Excimer laser 308 nm wave length on the mineral contents of human enamel and dentin surfaces.

Materials and methods: 12 lower anterior teeth were subjected to irradiation with 308 nm lambda: physicists model optex excimer laser and Xe Cl fill, the effects created by laser application were assessed by X-Ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR).

Results: laser treatment on both human enamel and dentin surfaces had positive effects as the spectrum of the treated surfaces appeared similar to the untreated surfaces.

7884-17, Session 6

High-power, diode-dumped Er:YAG for dentistry

C. Hagen, Pantec Engineering AG (Liechtenstein); A. Heinrich, Pantec Biosolutions AG (Liechtenstein); B. Nussbaumer, Pantec Engineering AG (Liechtenstein)

A diode pumped Er:YAG laser system suitable for dental and hard tissue cutting applications is presented. Unlike lamp pumped Er:YAG laser systems, repetition rates as high as 2 kHz can be achieved with average output powers of up to 15 W making possible new treatments. The laser system flexibility in terms of pulse duration (10 to 200 µs) and energy (1 to 50 mJ) combined with the good beam quality ensures precise and fast cuts. The laser system is sealed and highly shock and vibration resistant plus the lifetime of several thousand hours makes it practically maintenance free. The high efficiency (optical to optical) around 10% allows packaging to a fraction of the size of lamp pumped lasers. Even at the highest power levels, a beam quality factor M2 < 20 is maintained allowing fiber coupling in a 200 µm diameter fiber and therefore increasing mechanical flexibility and opening to new application areas like minimal invasive surgery. To demonstrate the device potential, experiments on enamel ablation with different pulse widths and energies were successfully performed and the effects of the high repetition rate and low pulse energy were investigated. Thin channels of 200 micron width were cut employing pulses with 50 mJ energy and no carbonization effect has been observed.

Finally, we present a route to scale the diode pumped Er:YAG laser with minimal changes to 200 mJ and 30 W average power.
Heat generation caused by ablational dentin removal with an Ultra Short Pulse Laser (USPL) System

A. Braun, R. Wehry, O. Brede, M. Frenz, F. Schelle, Rheinische Friedrich-Wilhelms-Univ. Bonn (Germany)

Heat generation during the removal of dental restorative materials may lead to a temperature increase and cause painful sensations or damage dental tissues. The aim of this study was to assess heat generation in dental restoration materials following laser ablation using an USPL system.

A total of 225 specimens of phosphate cement (PC), ceramic (CE) and composite (C) were used, evaluating a thickness of 1 to 5 mm each. Ablation was performed with an Nd:YVO4 laser at 1064 nm, a pulse length of 8 ps and a repetition rate of 500 kHz with a power of 6 W. Ablation was performed with a 600 µm spot size, recording the temperature during the ablation process. All measurements were made employing a heat-conductive paste without any additional cooling or spray.

Heat generation during laser ablation depended on the thickness of the restoration material (p<0.05) with the highest values in the composite group (p<0.05), showing an increase of up to 17 K. A time delay for temperature increase during the ablation process depending on the material thickness was observed in the PC and C group (p<0.05) with highest values for cement (p<0.05).

Employing the USPL system for removal of restorative materials, heat generation has to be considered. Especially during laser ablation next to pulp tissues, painful sensations might occur.

Etching enamel for direct bonding with a 1940-nm thulium fiber laser

A. S. Kabas Sarp, M. Gülsoy, Bogazici Univ. (Turkey)

Background: Laser etching of enamel for direct bonding can decrease the risk of surface enamel loss and demineralization which are the adverse effects of acid etching technique. However, in excess of +5.5°C can cause irreversible pulpal responses. In this study, a 1940-nm Thulium Fiber Laser in CW mode was used for laser etching.

Aim: Determination of the suitable Laser parameters of enamel surface etching for direct bonding of ceramic brackets and keeping that intrapulpal temperature changes below the threshold value.

Material and Method: Polycrystalline ceramic orthodontic brackets were bonded on bovine teeth by using 2 different kinds of etching techniques: Acid and Laser Etching. In addition to these 3 etched groups, there was also a group which was bonded without etching. Brackets were bonded with a material testing machine. Breaking time and the load at the breaking point were measured. Intrapulpal temperature changes were recorded with a K-type Thermocouple. For all laser groups, intrapulpal temperature rise was below the threshold value of 5.5°C.

Results and Conclusion: Acid-etched group (11.73 MPa) significantly required more debonding force than 3- second- irradiated (5.03 MPa) and non-etched groups (3.4 MPa) but the results of acid etched group and 4- second- irradiated group (7.5 MPa) showed no significant difference. Moreover, 4- second irradiated group was over the minimum acceptable value for clinical use. Also, 4- second second irradiation caused a 50% of reduction in time according to acid-etch group. As a result, 1940-nm laser irradiation is a promising method for laser etching.

Selective treatment of carious dentin using a mid-infrared tunable pulsed laser at 6 µm wavelength range

M. Saiki, K. Ishii, Osaka Univ. (Japan); K. Yoshikawa, K. Yasuo, K. Yamamoto, Osaka Dental Univ. Hospital (Japan); K. Awazu, Osaka Univ. (Japan) and Univ. of Fukui (Japan) and Kyoto Univ. (Japan)

Interaction between a nano second pulsed laser with 6 µm wavelength and dental hard tissue was investigated to develop a new caries excavation technique for minimal intervention (MI) emphasized in modern operative dentistry.

Bovine dentin plates (5 x 5 x 1 mm) perpendicular to the tubule direction were prepared. Dentin plates of carious model group were demineralized with lactic acid solution before irradiation experiments. A mid-infrared pulsed laser was obtained by difference-frequency generation (DFG) technique developed by Kawasaki Heavy Industries Ltd. and RIKEN. The wavelength emitted by DFG laser was tuned to 6.02 and 6.42 µm which correspond to absorption bands called amide I and amide II. The pulse width and repetition rate were 5 ns and 10 Hz, respectively. The morphological changes after irradiation were observed with a scanning electron microscope, and the measurements of ablation crater were performed with a confocal laser microscope.

At λ = 6.02 µm and the average power density of 15 W/cm², demineralized caries-like dentin was removed selectively with less-invasive effect on sound dentin. The wavelength of 6.42 µm also showed the possibility of selective removal. Compared to 6.42 µm, high ablation efficiency and small amount of melting were observed at λ = 6.02 µm.

The wavelength of 6.02 µm induced a promising interaction for the selective laser treatment of carious dentin. Development of a compact prototype system is required to realize MI concept in clinic.
Dentin enamel junction characterization by use of Stokes-Mueller formalism

Y. Hsieh, National Chiao Tung Univ. (Taiwan); Y. Ho, S. Lee, National Yang-Ming Univ. (Taiwan); C. Lu, Industrial Technology Research Institute (Taiwan); C. Chang, National Taiwan Univ. (Taiwan); C. Wang, National Chiao Tung Univ. (Taiwan); C. Sun, National Yang-Ming Univ. (Taiwan)

This research provides a preliminary result of teeth characterization based on Stokes-Mueller measurement. A thin tooth cross-section slide including enamel, dentin and cementum is used for ex-vivo sample. Since the difference of directional structure between each layer can be observed in micrograph, the birefringence property on dentin enamel junction (DEJ) was studied in experiment. The Muller matrix and Poincare sphere are measured for quantitative analysis of DEJ. The polarization information indicates the DEJ characterization quantitatively.

Polarization resolved near-IR reflectance imaging of sound and carious dental enamel

C. L. Darling, K. H. Chan, D. Fried, Univ. of California, San Francisco (United States)

A thorough understanding of how polarized near-IR light is reflected from sound and carious dental hard tissues is important for the development of optical imaging devices. New optical imaging tools employing non-ionizing radiation are needed for the detection and assessment of dental caries. In this investigation, an automated system was developed to collect images for the full 16-element Mueller Matrix. The polarized light was controlled by linear polarizers and liquid crystal retarders and the 36 images were acquired as the polarized near-IR light is reflected from the occlusal surface of extracted human whole teeth. Previous near-IR imaging studies suggest that polarization imaging can be exploited to obtain higher contrast images of early dental caries due to the rapid depolarization of incident polarized light by the highly scattering areas of decay. In this study, major differences in the Mueller matrix elements were observed in both sound and demineralized enamel. Polarization resolved optical imaging could be exploited to obtain higher contrast images of dental decay.

Optical coherence tomography monitoring of glucose diffusion and long-term glucose impact on the water permeability of tooth dentin

N. A. Trunina, V. V. Lychagov, V. V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

Optical Coherence Tomography (OCT) monitoring of agent diffusion within tooth tissues is important in a wide context of tooth therapy (diffusion of medicinal preparations) and cosmetics (chemical whitening agents). The present study is aimed at OCT monitoring of glucose diffusion within a human tooth. First, we demonstrate experimentally that the diffusion of glucose solution in intact tooth dentin samples is slower than that of pure water. Second, we study the effect of long-term glucose action on the tooth tissue properties. For this the sample was kept in 35% aqueous glucose solution for 5 days, then washed by distilled water and dried similarly, as in the measurements with intact samples. Glucose incubation of tooth samples was considered to simulate the in vivo conditions incipient in the case of diabetes mellitus for tooth tissue. We found that it resulted in irreversible changes of the tooth dentin properties. The most prominent manifestation of these changes was considerable increase of pure water diffusion rate. The permeability coefficient values of intact and glycated samples were found to be (2.59±1.63)×10^-4 cm/s and (3.86±0.39)×10^-4 cm/s, respectively.

Repair of artificial lesions using an acidic remineralization model

H. Kang, C. L. Darling, D. Fried, Univ. of California, San Francisco (United States)

It is difficult to completely remineralize carious lesions because diffusion into the interior of the lesion is inhibited as new mineral is deposited in the outermost layers. In previous re-mineralization studies employing polarization sensitive optical coherence tomography (PS-OCT), two models of re-mineralization were employed and in both cases there was preferential deposition of mineral in the outer most layer. In this study we are attempting to remineralize the entire lesion and demonstrate that this re-mineralization can be measured using PS-OCT. Artificial lesions approximately 100 µm in-depth were exposed to an acidic re-mineralization regimen and the integrated reflectivity from the lesions was measured before and after remineralization.

Selective removal of dental composite using a rapidly scanned carbon dioxide laser

K. H. Chan, D. Fried, Univ. of California, San Francisco (United States)

In this study a carbon dioxide laser operating at high laser pulse repetition rates integrated with a galvanometer based scanner was used to selectively remove composite from tooth surfaces. A diode array spectrometer was used to measure the plume emission after each laser pulse and determine if the ablated material was tooth mineral or composite. The composite was placed on bovine enamel surfaces in distinct patterns and the carbon dioxide laser was scanned across the surface to selectively remove the composite without damaging the underlying sound enamel. The residual composite and the damage to the underlying enamel was evaluated after scanning the samples using optical coherence tomography and optical microscopy.
Adjunctive dental therapy with blue light emitting toothbrush

E. A. Genina, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); V. A. Titoorenko, Saratov State Medical Univ. (Russian Federation); E. S. Tuchina, G. V. Simonenko, A. N. Bashkatov, V. V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); I. V. Yaroslavsky, G. B. Altshuler, Palomar Medical Technologies, Inc. (United States)

The goal of the study was estimation of the efficacy of the treatment of inflammatory disease of gum mucosa with blue light irradiation. For microbiological and clinical study, blue light emitting toothbrushes (LETBs) with ten optical bristle bundles and photorecycling mirror were used. To find the appropriate radiation parameters for photodynamic inactivation of pathogenic subgingival microflora using LETB, samples of subgingival plaques were used. For clinical trials, sixty subjects with light to moderate gingivitis were enrolled. I group included the volunteers, who were treated by LETBs, II group was control one and included the volunteers, who treated by standard manual toothbrushes. Clinical evaluation of changes in gingivitis compared with the baseline was visually assessed using five standard indices satisfying to American Dental Association Acceptance Program Guidelines: 1) Turesky modification of the Quigley-Hein plaque index (TI); 2) Approximate Hygiene Index (AHI); 3) Löe-Silness plaque index (LSI); 4) Gingival Bleeding Index (GBI); and 5) Gingivitis Index PMA. After the month, the improvement of the tooth state of patient from the first group in comparison with the control one was 32%, 13% and 18% for LSI, TI and AHI, respectively. The differences in removing of the plaque between two groups were statistically significant (LSI: P<0.002; TI: P>0.04; AHI: P=0.03). The improvement of GBI for patients from the active group in comparison with the control one was 31% on the 30th day (P=0.003). Relative decreasing of PMA index after a month was 12% for the first group and the second groups, respectively (P=0.002).

Dental photo-acoustic microscopy

B. Rao, X. Cai, L. Li, Washington Univ. in St. Louis (United States); S. Duong, L. L. Liaw, P. B. B. Wilder-Smith, Beckman Laser Institute and Medical Clinic (United States); L. V. Wang, Washington Univ. in St. Louis (United States)

Early detection and quantitative monitoring of dental decay provides both health and economic benefits, including the potential for chemical remineralization of early lesions and non-destructive evaluation of tooth mineralization status. Photoacoustic microscopy (PAM) utilizes short laser pulses to deposit energy into tissues and sensitively detects the absorbed laser energy in the form of laser generated ultrasonic waves. The ultrasonic waves are much less attenuated by dental tissue than a light wave would be. A dark-field photoacoustic microscope based on a 570 nm pulsed laser and point-by-point mechanical scanning stages was applied for the ex vivo imaging of both sliced dental samples and whole tooth samples. Lesions as well as structures that cannot be identified with light microscopy were identified on the high contrast, high resolution maximum-amplitude-projection (MAP) PAM images of samples for the very first time. Although single-wavelength dark-field PAM has demonstrated high contrast in dental imaging, its specificity is still under investigation. However, Multilength wavelength dark-field dental PAM has the potential to identify the spectral signatures of dental lesions and provide both high sensitivity and high specificity dental imaging in the near future.

Cross polarization optical coherence tomography for diagnosis of oral soft tissues

N. Gladkova, M. Karabut, E. Kiseleva, Nizhny Novgorod State Medical Academy (Russian Federation); N. Robakidze, St-Petersburg Medical Academy of Postgraduate Studies (Russian Federation); A. Muravev, Y. V. Formina, Nizhny Novgorod State Medical Academy (Russian Federation)

We consider the capabilities of cross-polarization OCT (CP OCT) focused on comparison of images resulting from cross-polarization and co-polarization scattering simultaneously for diagnosis of oral soft tissues. CP OCT was done for 35 patients with dental implants and 30 patients with inflammatory intestine diseases. CP OCT images were compared with histological data. Collagen was picrosirius red stained. Only mature collagen type I gave a strong signal in cross-polarization. CP OCT images of gingiva above implant demonstrate features even of weak gingivitis: in a co-polarized image inflammatory edema is visualized as horizontal stripes of low signal intensity and in a cross-polarized image inflammation weakens the signal from collagen which loses its polarization properties. CP OCT also enables measuring gingival thickness above implant in patients with thin gingival biotype. We analysed CP OCT images of inflammatory intestine diseases and found high intensity signal from fibrous collagen of lamina propria in patients with Crohn’s disease and weak signal intensity in patients with nonspecific ulcerative colitis. The signal level in cross-polarization agrees well with intensity of collagen color revealed with PSR staining in polarized light.

Our study showed good diagnostic capabilities of CP OCT for detecting soft tissue pathology in the oral cavity. The cross-polarized images demonstrate the ability of tissue to depolarize, thus enhancing diagnostic capabilities of CP OCT. CP OCT demonstrates clinical capabilities for early diagnosis of inflammatory intestine diseases by the state of oral cavity mucosa and for early detection of gingivitis in patients with thin biotype of gingiva above implant.

The impact of laser irradiation during antimicrobial photodynamic therapy in an artificial biofilm model

M. Schneider, G. Kirfel, M. Berthold, O. Brede, M. Frenzten, A. Braun, Rheinische Friedrich-Wilhelms-Univ. Bonn (Germany)

The aim of the study was to assess the impact of laser irradiation during antimicrobial photodynamic therapy in an artificial biofilm model. Using sterile chambered coverglasses, a salivary pellicle layer formation was induced in 40 chambers. Streptococcus mutans cells were inoculated in a sterile culture medium. Employing a live/dead bacterial viability kit, bacteria with intact cell membranes stain fluorescent green. Each pellicle coated test chamber was filled with 0.5 ml of the bacterial suspension and analyzed using a confocal laser scan microscope within a layer of 10 µm at intervals of 1 µm from the pellicle layer. Phenothiazine chloride (Helbo) was used as a photosensitizer and added to all 40 test chambers. 20 chambers were irradiated with a diode laser (wavelength: 660 nm, output power: 100 mW, Helbo) for 2 min each. An interval of 5 min was used as residence time for the photosensitizer in the remaining 20 chambers. Comparing baseline fluorescence after adding the photosensitizer (median: 3.6 [U], min: 1.1, max: 9.0) with the values after laser irradiation (median: 2.1, min: 0.4, max: 3.4), a decrease of fluorescence could be observed (p<0.05). The non-irradiated group (baseline median: 1.9, min: 0.7, max: 3.6) showed a slight increase of fluorescence after the residence time of the photosensitizer (median: 1.9, min: 0.8, max: 6.0) (p<0.05).

The present study indicates that laser irradiation is an essential part to...
reduce bacteria by antimicrobial photodynamic therapy. The treatment protocol should be followed carefully to obtain a toxic effect on microorganisms.

7884-33, Poster Session

**Near-infrared imaging of teeth at wavelengths between 1200 and 1600 nm**

S. Chung, D. Fried, C. L. Darling, Univ. of California, San Francisco (United States)

Near-IR (NIR) imaging is a new technology that is currently being investigated for the detection and assessment of dental caries without the use of ionizing radiation. Several papers have been published on the use of transillumination and reflectance NIR imaging to detect early caries in enamel. The purpose of this study was to investigate alternative near infrared wavelengths to determine the illumination conditions that yield the highest contrast in both transmission and reflectance imaging modes. Artificial lesions were created on twenty-five tooth sections of varying thickness for transillumination imaging. NIR images were also acquired for fifty whole teeth with occlusal lesions using a Ge-enhanced CMOS image sensor at wavelengths from the visible to 1600-nm produced using a tungsten halogen lamp with several spectral filters.
There are over 3.2 - 4.7 million people in the US suffering from dry eye syndrome (DES) by middle age. DES is a common multi-factorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. The tear system of the human eye is highly regulated and complex. Failure of any aspect will compromise the integrity of the system, resulting DES, which includes burning, foreign body sensation, reflex tearing, and visual distortion. In its most severe forms, dry eye may lead to painful recurrent corneal erosion or to secondary infection causing severe visual loss. For effective dry eye diagnosis and treatment, it is critically important to quantify the tear film thickness and its time dependent changing process. Currently, there is no suitable measurement technique that can quantify the tear film thickness in a live eye with good resolution. In this paper, we report our exploration of the optical reflectometry technique for live eye tear film evaluation. With the capability of nm high resolution measurement and with thickness measurement range of 0.02 µm to 50 µm, the reflectometry technique will have significant impact to the dry eye diagnosis and treatment.

Dry eye syndrome is a common irritating eye disease. Current clinical diagnostic methods are invasive and uncomfortable to patients. A custom designed noncontact infrared (IR) thermal image system was developed to measure the spatial and temporal variation of the ocular surface temperature over a 6-second eye-opening period. We defined two parameters: the temperature difference value (TDV) and the compactness value to represent the degree of the temperature change and irregularity of the temperature distribution on the tear film, respectively.

The TDV is obtained by taking IR images 30 film/s for 6-second, and calculate the averaged temperature in a region of interest (ROI). The difference of averaged temperature in the first image and the last image is TDV. The TDV is grater in dry eye group than in normal control group. The compactness value is obtained from measuring the area of the temperature distribution on the tear film, respectively.

By using these two parameters, we have achieved a linear discrimination result for the dry eye and the normal eye group; the sensitivity is 0.9, the specificity is 0.86 and the receiver operating characteristic (ROC) area is 0.91. The result suggests that the custom designed IR thermal image system may be used as an effective tool for noncontact detection of dry eye.
The instrument is capable to provide 3D information of the cone photoreceptors with negligible eye motion artifacts due to an implemented 3D motion correction on a cellular level. This allows for the in vivo investigation of temporal changes within human cone photoreceptors. Short term (within 8 hours) as well as long term (within 72 hours) changes are investigated.

7885-06, Session 1

**Imaging of hyaloid vessels in mouse embryonic eye with swept source optical coherence tomography**

K. V. Larin, Univ. of Houston (United States)

Throughout much of embryonic development, the mammalian lens is surrounded by a hyaloid vascular system. This vasculature is considered vital for the maturation and growth of the lens. Presence of these vessels does not support the development of the retina; however regression of these vessels after birth is crucial for normal retinal development. Optical Coherence Tomography (OCT) is a three dimensional (3D) imaging modality, which has the capability of producing high resolution (~8µm) images with an imaging depth of up to 3 mm. We tested the capability of OCT to perform live 3D imaging of the hyaloid vasculature in the embryonic eye in utero. Our results suggest that OCT can be used to understand the development and progressive regression of hyaloid vasculature in the vitreous region of the eye.

7885-07, Session 2

**Imaging microscopic structures in pathological retinas using a flood-illumination adaptive optics retinal camera**

C. Viard, Imagine Eyes (France); K. Nakashima, CHNO des Quinze-Vingts (France); B. Lamory, Imagine Eyes (France); M. Paques, CHNO des Quinze-Vingts (France); X. Levêque, Imagine Eyes (France)

**PURPOSE.** This research aimed at characterizing in vivo differences between healthy and pathological retinal tissues at the microscopic scale using a compact adaptive optics (AO) retinal camera.

**METHOD.** Tests were performed in 120 healthy eyes and 180 eyes suffering from 19 different pathological conditions, including age-related maculopathy (ARM), glaucoma and rare diseases such as inherited retinal dystrophies. Each patient was first examined using SD-OCT and infrared SLO. Retinal areas of 4x4 deg were imaged using an AO flood-illumination retinal camera (rtx1, Imagine Eyes, France), based on a Shack-Hartmann sensor and a large-stroke deformable mirror. While the AO system was compensating for the eye’s optical defects, the camera was focused at the cone photoreceptor layer and series of 40 rough images were obtained by exposing the retina to 9 ms near-infrared flashes. Contrast was finally enhanced by registering and averaging rough images using classical algorithms.

**RESULTS.** Cellular-resolution images could be obtained in most cases. In ARM, AO images revealed granular contents in drusen, which could be invisible in SLO or OCT images, and allowed to observe the cone mosaic between drusen. In glaucoma cases, were correlated to changes in cone visibility. In inherited retinal dystrophies, AO helped to evaluate cone cell losses across the retina. Other microstructures, slightly larger in size than cones, were also visible in several retinas.

**CONCLUSION.** AO provided potentially useful diagnosis and prognosis information in various diseases. In addition to cones, other microscopic structures revealed by AO images may also be of interest in monitoring retinal diseases.

7885-08, Session 2

**Advanced capabilities of the multimodal adaptive optics imager**

D. X. Hammer, M. Mujat, N. V. Ifitina, A. H. Patel, E. P. Plumb, D. P. Biss, R. D. Ferguson, Physical Sciences Inc. (United States); M. C. W. Campbell, Univ. of Waterloo (Canada); T. Chui, J. D. Akula, A. B. Fulton, Children’s Hospital Boston (United States) and Harvard Medical School (United States)

We have recently developed several multimodal adaptive optics (AO) retinal imaging system versions, which includes scanning laser ophthalmoscopy (SLO) and Fourier domain optical coherence tomography (FD-OCT) imaging channels as well as an auxiliary line scanning ophthalmoscope (LSO). Some versions have also been equipped with a fluorescence channel and a retinal tracker. We describe the performance of three key features of the multimodal AO system including: simultaneous SLO/OCT imaging, which enables OCT 3-D raster registration using eye movement offsets extracted from the SLO image; a small animal imaging port, which adjusts the beam diameter at the pupil from 7.5 to 2.5 mm for use with small animals ubiquitous in biological research or for extended depth-of-focus imaging in humans; and slow scan Doppler flowmetry imaging using the wide field auxiliary LSO imaging channel. The systems are currently deployed in several ophthalmology clinics and research laboratories and several investigations have commenced on patients with a variety of retinal diseases.

7885-09, Session 2

**AO-OCT with reference arm phase shifting for complex conjugate artifact free imaging of in-vivo retinal structures**

R. J. Zawadzki, D. Kim, UC Davis Medical Ctr. (United States); S. M. Jones, Lawrence Livermore National Lab. (United States); S. Pilli, UC Davis Medical Ctr. (United States); S. S. Olivier, Lawrence Livermore National Lab. (United States); J. S. Werner, UC Davis Medical Ctr. (United States)

We report results on testing performance of AO-OCT system with reference arm phase shifting for complex conjugate artifact free imaging of in-vivo retinal structures. As a complex conjugate artifact removal method we use previously reported technique that requires constant phase shifts between consecutive A-scans. In our system this shifts were generated by continuous beam path length changes from offsetting the pivot point of the scanning mirror placed in the system reference arm. In order to reconstruct complex spectral fringe pattern we used Fourier transformation along transverse axis and the filtering algorithm. The suppression ratio of mirror complex artifact images was assessed with several other metrics measuring feasibility of this approach. Finally potential problems and limitations connected with this acquisition scheme and data processing algorithms will be discussed.

7885-10, Session 2

**3D imaging of cone photoreceptors over extended time periods using optical coherence tomography with adaptive optics**

O. P. Kocaoglu, S. Lee, Q. Wang, A. E. Herde, J. Besecker, W. Gao, R. S. Jonnal, D. T. Miller, Indiana Univ. (United States)

Optical coherence tomography with adaptive optics (AO-OCT) is a highly sensitive, noninvasive modality for 3D imaging of the microscopic retina. The purpose of this study is to advance AO-OCT technology to enable repeated imaging of the same cone photoreceptors over extended
7885-14, Session 3

Velocity ranging in joint spectral and time domain OCT imaging with resonant scanner


We will demonstrate a method of enlarging the flow imaging range in a Doppler OCT technique with utilization of segmented scanning. Introduction of data acquisition segments with small number of A-scans acquired over a very short time periods allows for imaging of both fast and slow flows. We have implemented this idea by utilization of a resonant scanner and a galvanometric XY scanner. The resonant scanner produces acquisition segments while the galvanometer scanner performs the object scanning. Depending of the implemented scanning trajectory different flow ranges can be imaged. In particular slow flow detection is improved in comparison to methods utilizing the galvanometric scanners only. Measurements can be performed to minimize the examination time and maximize the area where flow can be imaged. In addition averaging techniques can be used to improve flow and structural images.

Imaging was performed in a flow model and in the human retina using ultrahigh speed (212 000 axial scans/s) spectral/Fourier domain OCT instrument with a CMOS camera. Light source with 820nm center wavelength and FWHM=70nm provided the axial imaging resolution was 4.3µm. Data analysis was performed using the method of Joint Spectral and Time domain OCT.

7885-15, Session 3

Stable absolute flow estimation with Doppler OCT based on virtual circumpapillary scans


We propose an algorithm to extract the angles of retinal vessels for the correction of flow measurements in circumpapillary Doppler OCT scans. Firstly, we register a volume scan to two reference scans in order to determine its anatomically correct structure. The fast scanning axis of the reference scans is orthogonal to the one of the volume acquisition. Then, vessels within the volume are manually segmented and their angles are calculated and stored in a look-up table. For flow assessment circumpapillary scans are recorded and located within the reference volume by using the vessel shadows from projections along the fast axis between the retinal pigment epithelium and the outer receptor layer. The associated angles can then be found immediately from the look-up table. In addition typical D-OCT artifacts as fringe washout and phase-wrapping are corrected under the assumption of a parabolic flow profile. Mean velocity and pulsatility (PI) and resistance indices (RI) are calculated. Repeatability measurements of flow parameters on different vessels including arteries of a healthy subject show a low coefficient of variation of the flow velocity of 3 to 8.5%.

7885-16, Session 3

Retinal blood flow measurement with ultrahigh-speed swept-source/Fourier domain optical coherence tomography

B. Baumann, B. M. Potsaid, J. J. Liu, M. F. Kraus, Massachusetts Institute of Technology (United States); D. M. Huang, Doheny Eye Institute (United States); J. Hornegger, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); J. S. Duker, Tufts Medical Ctr. (United States); J. G. Fujimoto, Massachusetts Institute of Technology (United States)
We present a robust method for measuring total retinal blood flow using ultrahigh-speed swept source / Fourier domain optical coherence tomography (OCT). Detecting abnormal retinal blood flow is an important parameter in severe ocular diseases such as glaucoma or diabetic retinopathy, two leading causes of blindness. The prototype swept source OCT system operates at an axial scan rate of 200 kHz, which makes it possible to measure axial flow velocities up to ~50 mm/s. This ability to measure this high velocity range enables the assessment of blood flow in the central retinal vessels. The high axial scan rate also facilitates fast acquisition of densely sampled data sets with reduced motion artifacts. The detection of high flow velocities is also enabled by the fact that swept source OCT is less sensitive to signal loss from phase washout as compared to spectral / Fourier domain OCT. Imaging at 1050 nm allows enhanced penetration for imaging retinal vessels deep in the optic nerve.

Three-dimensional (3D) OCT data sets of the papilla were acquired and en-face Doppler images were extracted and analyzed using a novel algorithm. From the flow velocity information in a single cross-sectional en-face Doppler image, absolute blood flow was computed for single vessels as well as for the total retina. The results indicate that ultrahigh speed Doppler OCT can be a promising tool for clinical assessment of absolute retinal blood flow.

7885-17, Session 3

**Extended volume retinal vascular imaging with phase contrast optical coherence tomography**

J. P. Fingler, California Institute of Technology (United States); D. M. Schwartz, Univ. of California, San Francisco (United States); S. E. Fraser, California Institute of Technology (United States)

We present recent developments from a phase variance based motion contrast method of retinal vascular OCT imaging, called phase contrast optical coherence tomography (PC-OCT). Using a 25 kHz spectral domain optical coherence tomography (SDOCT) system, the vascular visualization capabilities of this contrast method are demonstrated with composite images created from multiple data sets. Wide field vascular images extending over the fovea and optic nerve head are presented as well as microvascular retinal images over the fovea to demonstrate the trade-offs between imaging speed and vascular visualization.

7885-18, Session 3

**Comprehensive OCT imaging of retinal microvasculature without adaptive optics**


Novel CMOS detector based spectral OCT has recently demonstrated its ability to image at acquisition speeds of 200kHz comprehensive details due to the virtual lack of motion artifacts. We applied this system to the retina and achieved high resolution imaging with 5um x 5um transverse and axial resolution. Such resolution allows observing microscopic details such as photoreceptor cone mosaic, nerve fiber bundles, and the capillary bed without applying adaptive optics instrumentation. Doppler methods have been successfully applied to extract the capillary network. We demonstrate how already on the intensity level para-foveal capillary structures can be segmented using learning post-processing algorithms.

7885-19, Session 3

**Microvasculature imaging by using double-beam Doppler optical coherence angiography**

S. Makita, M. Yamanari, Univ. of Tsukuba (Japan); B. Cense, Utsunomiya Univ. (Japan) and Univ. of Tsukuba (Japan); M. Miura, Tokyo Medical Univ. Kasumigaura Hospital (Japan) and Univ. of Tsukuba (Japan); Y. Yasuno, Univ. of Tsukuba (Japan)

Micro-vasculature changes occur in several pathologies of the eye such as choroidal neovascularization (CNV). Almost all of vascular diseases cause poor vision. In addition, some capillary abnormalities indicate early symptoms of pathologies.

In this study, we demonstrate micro-vasculature imaging of the posterior human eye with high-sensitive Doppler optical coherence angiography (OCA). Polarization-multiplexed dual-beam spectral-domain optical coherence tomography was used. High-contrast and high-sensitive phase-resolved blood flow images were obtained without birefringence artifacts, which were present in our previous dual-beam Doppler OCA system. Light from a source which has 840 nm central wavelength and 50 nm bandwidth (FWHM) were devided into two polarization states to obtain two OCT signals.

The scanning rate of two line scan cameras were 27 kHz. Although the observation time for Doppler frequency measurement was more than 20 times larger comparing to that of conventional phase-resolved Doppler OCT, one volume consisting 512 x 256 axial scans was acquired within 5 s.

Two eyes of two pathological cases and 5 eyes of 4 normal subjects were examined. Choroidal blood flow signals were detected at choroidal lesions, also visible as hyper-fluorescent areas in indocyanine green angiography (ICGA). Ultrahigh-sensitive blood flow imaging revealed the micro-vasculature of capillary-level vessels at the posterior eye and the optic nerve head. At the macular region, foveal avascular zones were observed. Choroidal blood flow in high-sensitive Doppler OCA corresponding to ICGA at CNV lesion might indicate possibility of the detection of CNV with Doppler OCA. Its superior flow sensitivity enables visualization of capillary-level fine vasculature.
An improved method of laser Thermokeraotoplasty to correct presbyopia
E. I. Maguen, J. J. Salz, Cedars Sinai Medical Ctr. (United States); M. Berry, NTK Enterprises, Inc. (United States); K. J. Rodgers, Vision Rejuvenation Ctr. (Bahamas); H. T. Glenn, Advanced Eye Centers, Inc. (United States)

Purpose: To evaluate the safety and effectiveness of the NTK Optimal Keratoplasty (Opti-K™) device for treating eyes with plano presbyopia to achieve uncorrected near visual acuity (UNVA) improvement. Methods: 68 eyes with plano presbyopia (MRSE = -0.25 to +0.88 D; mean add: 1.96 ± 0.37 D) of 37 patients (30 female, 7 male; mean age: 50.2 ± 5.4 y) were treated by the NTK Opti-K™ device using a cw Thulium fiber LASER, 193 µm with a sapphire applanation window for cornea epithelium protection. in order to achieve UNVA improvement. Follow-up (f/u) extends to 24m post-Op.

Results: Safety - no adverse events, loss of lines of corrected visual acuity (CDVA, CNVA) or significant induced astigmatism have been observed. Epithelial protection and comfort have been excellent. Effectiveness - geometric mean (gm) UNVA improvement was 4 lines at 1day post-Op. Gm UNVA improvement diminished over time, regressing to 2 lines by 12m post-Op. Gm distance visual acuity (UDVA) improved at most f/u times. “Optimal keratoplasty” has been achieved - corneas have been reshaped to improve UNVA while preserving (and, in most cases, improving) UDVA. Successful patient neuroadaptation to “multifocality” or “blended vision” produced by optimal keratoplasty occurred immediately post-Op.

Conclusions: In eyes with plano presbyopia, optimal keratoplasty (Opti-K™) appeared to be safe and effective for improving UNVA while retaining UDVA. Although UNVA improvement regresses over time, patients have elected to have additional Opti-K™ treatments when needed.

OCT-guided femtosecond laser system for cataract surgery
D. V. Palanker, Stanford Univ. School of Medicine (United States); G. Schuele, OptiMedica Corp. (United States); N. Friedman, Stanford Univ. School of Medicine (United States); D. E. Andersen, OptiMedica Corp. (United States); M. S. Blumenkranz, Stanford Univ. School of Medicine (United States); J. Batlle, R. Feliz, Centro Láser (Dominican Republic); J. H. Talamo, Harvard Medical School (United States); G. R. Marcellino, OptiMedica Corp. (United States); B. Seibel, Seibel Vision Surgery (United States); W. Cubertson, Bascom Palmer Eye Institute (United States)

Cataract surgery is a manual procedure highly dependent on the surgical skills and complicating factors. We developed and tested a system including OCT and fs laser to improve the precision and reproducibility of cataract surgery. A long-range OCT automatically discerns the anterior and posterior surfaces of the lens and cornea for planning of capsulotomy; lens segmentation and corneal incisions are then performed using a co-registered fs laser. Capsular strength following laser capsulotomy and mechanical capsulorhexis were compared on cadaveric eyes, and retinal safety was verified on rabbits. 50 patients have undergone cataract surgery using the fs laser system. Eyes were examined ophthalmoscopically, and extracted capsules were analyzed using histology and SEM. Capsular strength after laser capsulotomy was nearly twice stronger than after manual capsulorhexis: 124 mN vs. 66 mN. Average deviation from intended size in laser capsulotomy was eight times better than with manual procedure: 32 µm vs. 240 µm. Roundness of laser capsulotomy improved by a factor of 6 compared to capsulorhexis: deviation of 1.5% vs. 10%. Histology and SEM of incised capsules showed smooth edges. Lens segmentation facilitates its disassembly into easily separable quadrants and nucleus fragmentation reduces the perceived hardness of the nuclear sclerotic cataract by two grades, making its emulsification much easier and faster. Multi-planar corneal incisions provide for unique self-sealing wound constructions. No retinal damage or other laser-related adverse events have been observed. This integrated system offers a previously unattainable exactitude that promises improved centration of IOLs and correction of residual corneal astigmatism.

Sutureless closure of scleral wounds in animal models by the use of laser-welded biocompatible patches
F. Rossi, P. Matteini, Istituto di Fisica Applicata Nello Carrara (Italy); L. Menabuoni, I. Lenzetti, Azienda USL 4 (Italy); R. Pini, Istituto di Fisica Applicata Nello Carrara (Italy)

The common procedures used to seal the scleral or conjunctival injuries are based on the traditional suturing techniques, that may induce foreign body reaction during the follow up, with subsequent inflammation and distress for the patient. In this work we present an experimental study on the laser welding of biocompatible patches onto ocular tissues, for the closure of surgical or trauma wounds. The study was performed ex vivo in animal models. A penetrating perforation of the ocular tissue was performed with a surgical knife. The wound walls were approximated, and a biocompatible patch was put onto the outer surface of the tissue, in order to completely cover the wound as a plaster. The patches were prepared with a biocompatible and biodegradable polymer, showing high mechanical strength, high elasticity, high permeability for vapour and gases and rather low biodegradation. During preparation Indocyanine Green (ICG) was included in the biopolymeric matrix, so that the films presented high absorption at 810 nm. Effective adhesion of the membranes to the ocular tissues was obtained by using diode laser light emitted from an 810 nm diode laser and delivered by means of an optical 200 micrometer core diameter optical fiber, to produce spots of focal film/tissue adhesion, due to the photothermal effect at the interface. The result is an immediate closure of the wound, thus reducing post-operative complications due to inflammation. The same procedure was performed by the use of a patch of a human amniotic membrane (HAM), previously stained with a water solution of ICG.

Lowering threshold energy for femtosecond laser pulse photodisruption through turbid media using adaptive optics
A. Hansen, T. Ripken, Laser Zentrum Hannover e.V. (Germany); R. R. Krueger, The Cleveland Clinic (United States); H. Lubatschowski, Laser Zentrum Hannover e.V. (Germany)

Focussed femtosecond laser pulses are applied in ophthalmic tissues to create an optical breakdown and therefore a tissue dissection through photodisruption. The threshold energy for the optical breakdown depends on the photon density in the focal volume which can be influenced amongst others by the size of the focal volume. For an application in the posterior eye segment, the aberrations of the anterior eye elements cause a distortion of the wavefront and therefore an increased focal volume which reduces the photon density and thus raises the threshold.
energy. This leads to an increased peripheral damage which is especially critical for applications in the vicinity of the retina. The influence of adaptive optics on lowering the threshold energy by refining a distorted focus was investigated. The adaptive optics system designed for correcting eye aberrations with the use of a Hartmann-Shack-sensor and a deformable mirror can measure and correct for aberrations introduced by a turbid object with low optical quality. The effect of the laser pulses on a retina model were examined microscopically and compared for the aberration corrected and uncorrected case. A reduction of the threshold energy was shown when using adaptive optics. The lowered threshold energy allows for tissue dissection with reduced peripheral damage. This offers the possibility for moving femtosecond laser surgery from corneal or lental applications in the anterior eye to vitreal or retinal applications in the posterior eye.

7885-26, Session 5
Realtime temperature determination during retinal photocoagulation on patients
R. Brinkmann, Univ. zu Lübeck (Germany); S. Koinzer, Univ. Schleswig-Holstein (Germany); K. Schrott, L. Ptaszynski, M. Bever, A. Baade, Medizinisches Laserzentrum Lübeck GmbH (Germany); J. Roider, Univ. Eye Hospital (Germany); R. Birngruber, Medizinisches Laserzentrum Lübeck GmbH (Germany)

Retinal photocoagulation is an established treatment for a variety of retinal diseases, most commonly applied for diabetic retinopathy. The damage extent of the retinal photocoagulations depends on the temperature increase and the time of irradiation. So far, the temperatures are unknown due to intraocular variations in light transmission and RPE/choroidal pigmentation. Thus in practice, often too large burns are produced, which can lead to extended scotoma and bleeding in the worst case. In order to control the coagulation, we measure the temperature increase during the photocoagulation process by optoacoustics. Additionally to the cw treatment laser beam, short laser pulses are applied to excite thermoelastic transients. These pressure waves can be measured with a modified contact lens at the cornea. The change in the acoustic transients can be used to calculate the present temperature at the retinas. In this talk we present the very first data determined on patients, which are achieved with a modified photocoagulation laser (Carl Zeiss Meditec, Visulas, 532 nm). First patients measured showed typical coagulation temperatures between 65 and 75 °C within the macular and the periphery. We will show the temperature - spot size correlations in clinical practice and will further discuss the accuracy of the technique and automatic online dosimetry control.

7885-27, Session 5
Dynamics of micro bubble clusters in retina phantoms
A. Fritz, Medizinisches Laserzentrum Lübeck GmbH (Germany); A. Zegelin, Univ. zu Lübeck (Germany); L. Ptaszynski, Medizinisches Laserzentrum Lübeck GmbH (Germany); H. Stoehr, Univ. zu Lübeck (Germany); R. Birngruber, Medizinisches Laserzentrum Lübeck GmbH (Germany); R. Brinkmann, Univ. zu Lübeck (Germany)

Selective retina treatment (SRT) is a laser based method to treat retinal diseases associated with disorders of the retinal pigment epithelium (RPE) while preserving photoreceptors and choroid. Applying microsecond laser pulses to the 100-200 strongly absorbing melanin granules inside the RPE cells induces transient micro bubbles which disrupt the cells. Aim of this work is to understand bubble dynamics in clusters with respect to the influence of the adjacent retina. Bubble dynamics were investigated in vitro on porcine RPE explants and on a floppy disc based RPE model. To both models a 200 µm thick layer of agarose gel was applied in order to simulate the mechanical properties of retina. Different laser pulse durations from 3 ns (532 nm, Nd:YAG) to 1.7 µs (527 nm, Nd:YLF) were used. The bubbles were investigated interferometrically (fiber interferometer @ 830 nm) and with high speed photography (25 ns flash duration). Bubble sizes, velocities and lifetimes were measured. The results show that with retina phantoms the bubble formation threshold was reached at 2.5 times higher irradiation than without retina phantom for 1.7 µs laser pulses. The 3 ns laser pulses where almost not influenced by the agarose layer. Irradiation twofold over bubble formation threshold resulted in 3.5 times longer bubble lifetimes for µs laser pulses but only two times longer lifetimes for ns pulses. The results are in good agreement with investigations of different pulse durations in-vivo in rabbit eyes. It has to be investigated if the results can be transferred to human eyes.

7885-28, Session 5
Optoacoustic temperature determination and automatic coagulation control in rabbits
K. Schrott, Medizinisches Laserzentrum Lübeck GmbH (Germany); S. Koinzer, Univ. Schleswig-Holstein (Germany); L. Ptaszynski, S. Luft, A. Baade, M. Bever, Medizinisches Laserzentrum Lübeck GmbH (Germany); J. Roider, Univ. Eye Hospital (Germany); R. Birngruber, R. Brinkmann, Medizinisches Laserzentrum Lübeck GmbH (Germany) and Univ. zu Lübeck (Germany)

Retinal laser coagulation is a reliable treatment for many retinal diseases like macula edema and diabetic retinopathy. The selection of the laser parameters is so far based upon post treatment evaluation of the lesion size and strength. Due to local pigment variations the same laser parameters often lead to overtreatment. Optoacoustics allow a non invasive monitoring of the retinal temperature increase during retinal laser irradiation by analyzing the temperature dependent pressure amplitude, which is induced by probe laser pulses. A 523 nm / 75 ns Nd:YLF was used as a probe laser at a repetition rate of 1 kHz, and a 532 nm cw treatment laser for coagulation (Zeiss Visulas). A contact lens (Mainster Focal Grid) was modified with an ultrasonic transducer to detect the pressure waves at the cornea. Temperatures were determined for irradiations leading to soft lesions and to no visible lesion. Based on these data the threshold for denaturation was evaluated. An algorithm was found to calculate the irradiation time, which is needed for a soft lesion formation. By this it was possible to provide a real-time dosimetry by automatically switching of the treatment laser after the calculated irradiation time. Automatic controlled coagulations appear softer and more uniform. Arrhenius parameters were evaluated from the experimental threshold data as a frequency factor of 1 E44 / s and activation energy of 273 kJ / mol.

7885-29, Session 6
Extended-depth optical coherence tomography for anterior segment imaging
M. Ruggeri, S. Ulthorn, F. Manns, J. A. Parel, Bascom Palmer Eye Institute (United States)

Non-invasive imaging of ocular structures and quantitative assessment of biometric parameters is essential for diagnosis and treatment of disorders that affect the human eye. There are several non invasive imaging modalities that allow cross-sectional imaging and ocular biometry as Scheimpflug photography, slit scanning topography, magnetic resonance imaging (MRI), ultrasound biomicroscopy (UBM) and optical coherence tomography (OCT). As a non-invasive imaging technique Fourier Domain OCT (FD-OCT) offers high axial resolution, high sensitivity, fast imaging speed and 3D imaging of ocular structures. Although OCT shows
many advantages compared to other techniques, the axial range of the current FD-OCT implementations must be extended in order to image the entire anterior segment of human eye. Long range depth scanning requirements can be achieved by designing a spectrometer for FD-OCT systems offering very high spectral resolution. We describe our work on the extended depth OCT imaging of ocular structures of the human anterior segment and we demonstrate that FD-OCT provides images of the whole anterior segment of the human eye. The OCT system capability together with the algorithm for correcting image distortions is significant for human ocular biometry. Although images of the whole human anterior segment were presented, the axial range is still limited for imaging the entire anterior segment of some subjects. In order to address this issue we are currently working on further increasing the imaging depth, which would also facilitate the alignment procedure during imaging.

7885-30, Session 6

Full-range imaging of the whole anterior segment of eye by high-speed optical frequency domain imaging using a reflective Fabry-Perot tunable laser

H. Furukawa, H. Hiro-Oka, R. Yoshimura, D. Choi, M. Nakanishi, A. Igarashi, Kitasato Univ. (Japan); K. Ohbayashi, Kitasato Univ. School of Medicine (Japan); K. Shimizu, Kitasato Univ. (Japan)

One of the interesting capabilities of anterior segment OCT is non-invasive non-contact observation of accommodation. Accommodation occurs through the combined change in the crystalline lens shape and thickness, and in distances between major refractive surfaces. Therefore dynamic simultaneous imaging of the whole anterior segment (cornea, iris, anterior chamber and crystalline lens), while eye accommodates, is required for clinical investigation of accommodation. Recently successful such sort of imaging was reported in two works with SD-OCT methods. However, to overcome the limited imaging depth in SD-OCT, a method to eliminate complex ambiguity was used in one work combining two images and a dual channel dual focus SD-OCT was used in the other work losing the image of central crystalline lens region. We developed a high-speed long-depth range optical frequency domain imaging (OFDI) system employing a commercially available high-speed reflective Fabry-Perot tunable laser. The source spans from 1250 to 1360 nm with an average output power of 16 mW. The source is shipped with a k-sampling clock output for 5mm imaging depth. To take advantage of the long coherence length (13 mm) of the laser and to realize longer imaging depths, we introduced an external Mach-Zehnder interferometer to generate k-clock for 6 or 12 mm depth ranges. The fast A-scan rate of 20,000 per second with the depth range of 12 mm enabled dynamic OFDI, and the combined dynamic change of the crystalline lens thickness and distances between major refractive surfaces following accommodation was demonstrated for a healthy eye and an eye with IOL without removing complex ambiguity using single-beam single-focus optical system.

7885-31, Session 6

Ultra-high speed 1050-nm swept source ophthalmic OCT imaging at 100,000-200,000 axial scans per second

B. M. Potsaid, B. Baumann, J. J. Liu, M. F. Kraus, Massachusetts Institute of Technology (United States); S. Barry, A. E. Cable, Thorlabs Inc. (United States); D. M. Huang, Doheny Eye Institute (United States); J. S. Duker, New England Eye Ctr. (United States); J. Hornegger, Friedrich-Alexander-Univ. Erlangen-Nürnberg (United States); J. G. Fujimoto, Massachusetts Institute of Technology (United States)

We demonstrate 1050nm ultrahigh speed swept source/Fourier domain OCT for ophthalmic imaging using a short cavity swept laser at 100kHz to 200kHz axial scan rates. Densely sampled 500x500 axial scan data sets of the optic nerve head imaged at 100kHz show deep penetration into the choroid and optic nerve head. The 3.5um axial resolution enables delineation of fine retinal layers. At 200kHz, large 12x12mm2 3D data sets can be acquired that include both the macula and disc. Small area imaging at 200kHz shows individual cone photoreceptors in certain regions of the retina. Imaging of the anterior eye can also be performed. In a 500x500 axial scan volume of the anterior angle, outflow features, such as the trabecular meshwork and Schlemm's canal can be visualized. An extended imaging range configuration of the instrument achieves 7.5mm depth range in tissue by using a 1 MSPS sampling rate. The cornea, iris, and front of the lens can be visualized in a single image. The improvements in imaging speed and depth range provide important advantages for ophthalmic imaging. The ability to rapidly acquire 3D-OCT data over a wide field of view promises to simplify examination protocols. The ability to image fine structures can provide detailed information on focal pathologies. The large imaging range and improved image penetration at 1050nm wavelengths promises to improve performance for instrumentation which images both the retina and anterior eye. These advantages suggest that swept source OCT at 1050nm wavelengths will play an important role in future ophthalmic instrumentation.

7885-32, Session 6

The effect of collimator lenses on the performance of an optical coherence tomography system

P. Fält, Univ. of Eastern Finland (Finland) and Utsunomiya Univ. (Japan); R. J. Zawadzki, UC Davis Medical Ctr. (United States); B. Cense, Utsunomiya Univ. (Japan)

In order to balance an adaptive optics Fourier-domain optical coherence tomography (AO-Fd-OCT) performance in patients with low quality ocular media, the effect of using collimator lenses with different focal lengths on the performance of an AO-Fd-OCT system is studied. A near-infrared broadband superluminescent diode (836 nm; bandwidth: 112 nm) is used as a light source. Light is guided into the sample, reference and detection arms via single mode fibers. In vivo AO-Fd-OCT scans of a healthy human retina are taken separately for 50, 25, 18.2 and 9 mm focal length collimator lenses (in the sample arm). A long focal length collimator lens ultimately provides a relatively wide collimated beam width, and thus, a smaller spot size on the retina. On the downside, the spot size on the fiber-tip for the returning light is relatively large and the fiber coupling efficiency is poor. With a short focal length collimator lens, the collimated beam entering the eye is relatively narrow, resulting in a large spot on the retina and poor resolution. But, a short focal length lens produces a small spot size on the fiber-tip, improving the collection of reflected light into the fiber. Results indicate that a stronger signal can be obtained from the retina with relatively short focal length collimators, albeit at a lower resolution. This is due to a large spot size on the retina and improved light collection on the fiber-tip. The results might have applications in the OCT-imaging of challenging cases.

7885-33, Session 6

Comparison of in-vitro retinal full-field swept source and Fourier domain OCT

J. R. Fergusson, Cardiff Univ. (United Kingdom); B. Považay, B. Hofer, W. Drexler, Medizinische Univ. Wien (Austria)

Weakly scattering tree shrew retina has been imaged in vitro with full field swept source optical coherence tomography and compared with images of the same tissue sample imaged with a Fourier domain OCT system. The full field swept source system utilizes a 50.8nm bandwidth light source (BS840, Superlum) centred at 850nm with Labview written software and crosstalk suppression to achieve ~8µm of axial resolution and 8µm of transversal resolution. Volumetric images of retinal tissue...
with dimensions of 1mm\(^3\) (504x500x512 voxels (horizontal by vertical by axial)) were recorded in 1.7 seconds (equivalent of ~150,000 A-scans per second) with a measured signal to noise ratio of 83dB. From the 20mW of SLD optical power available, 5.5mW illuminates the sample, giving an energy density of 0.935J/cm\(^2\), approximately half the energy density of the DFOCT system. The dynamic range of the recorded images was 23dB.

The same retina was also imaged with an 800nm 20 kHz Fourier domain OCT system (1.3mW, giving an energy density of 1.625J/cm\(^2\)) for comparison, acquiring a volume (500x500x2048 voxels) in 12.5 seconds with a signal to noise ratio of 93dB. The image dynamic range was 26dB with an axial resolution of 4μm.

It has been shown that full field swept source OCT can generate 3D images with comparable SNR and acquisition speeds to traditional Fourier domain OCT system. Furthermore diffuse illumination allows higher optical intensity to safely be applied to in vivo retinal tissue than scanning Fourier domain OCT.

7885-34, Session 7

Effect of accommodation on peripheral refraction when modified by a novel contact lens design to manipulate peripheral defocus

A. Ho, P. Lazon de la Jara, Brien Holden Vision Institute (Australia) and The Vision Cooperative Research Ctr. (Australia) and The Univ. of New South Wales (Australia); A. Martinez, The Univ. of New South Wales (Australia) and CIBA Vision, Asia Ctr. of Excellence (Singapore); J. Kwan, Brien Holden Vision Institute (Australia); C. Fedtke, Brien Holden Vision Institute (Australia) and The Vision Cooperative Research Ctr. (Australia) and The Univ. of New South Wales (Australia); S. Delgado, CIBA Vision, Asia Ctr. of Excellence (Singapore); B. Holden, The Brien Holden Vision Institute (Australia) and The Vision Cooperative Research Ctr. (Australia) and The Univ. of New South Wales (Australia); P. Sankaridurg, Brien Holden Vision Institute (Australia) and The Vision Cooperative Research Ctr. (Australia) and The Univ. of New South Wales (Australia)

No abstract available

7885-35, Session 7

Telescopic vision contact lens

E. J. Tremblay, Univ. of California, San Diego (United States); R. D. Beer, Pacific Science & Engineering Group, Inc. (United States); A. Arianpour, J. E. Ford, Univ. of California, San Diego (United States)

Previous visual aids for age-related macular degeneration (AMD) are either optics worn on glasses or head-mounted optics, requiring motion of the head to orient the lenses towards an object of interest, or optics surgically inserted into the eye. In this talk, we present design and proof of principle experimental results for a contact lens visual aid for AMD that provides magnification to the user without surgery or external head-mounted optics.

Our contact lens optical system is designed to provide a combination of telescopic and unmagnified vision using two independent optical paths. The magnified outer optical path incorporates a telescopic arrangement to achieve 2.8x magnification on the eye, while a central clear aperture functions as a conventional unmagnified contact lens.

We will present the experimental outcome of two preliminary designs: (1) A simplified afocal noncontact design intended to be tested in front of a conventional camera, and (2) A contact lens fit to a scale laboratory model of the human eye. The noncontact optic is 13 mm in diameter, less than 1.5 mm thick and is made from rigid Calcium Fluoride, for low risk fabrication. The contact optic is 8 mm in diameter, less than 1.2 mm thick conformal to the cornea and is made from PMMA. Future prototypes will move to rigid gas permeable polymers better suited for wear on the eye. In addition to the optical design and experimental results, we will discuss the psychophysiological aspects of such a contact lens and the associated possibilities of multiplexed vision and image switching.

7885-36, Session 7

A prosthetic eye which reacts to light

J. Lapointe, Ecole Polytechnique de Montréal (Canada) and Advanced Photonics Concepts Lab. (Canada); A. Harhiria, Ecole Polytechnique de Montréal (Canada); J. Durette, Oculo-Plastik, Inc. (Canada); S. Beaulieu, Univ. de Sherbrooke (Canada); A. Shaat, Ain Shams Univ. (Egypt); P. R. Boulous, Hôpital Maisonneuve-Rosemont (Canada) and Univ. de Montréal (Canada); R. Kashyap, Ecole Polytechnique de Montréal (Canada) and Advanced Photonics Concepts Lab. (Canada)

The eye is a vital organ not only in terms of vision but also as an important component of facial expression. Loss of this organ is unaesthetic and has a great psychological impact on the patient. Around 100k-200k people/year lose an eye. A realistic prosthetic eye, with hand painted iris and sewn-in implant, which is capable of following the real eye movements, is provided for most of anophthalmic patients in order to preserve their psychological well being. However, the reaction of an ocular prosthesis is limited by the immobility of the pupil. Our solution is to use a liquid crystal display (LCD) to vary the pupil diameter as a function of the ambient light. The dynamic pupil is controlled by a novel, entirely autonomous and self-powered passive electronic circuit using a specific photodiode. The size of the photodiode in the high voltage configuration matches the minimum opening of the pupil. The first LCD surviving the rugged conditions of the ocular prosthesis manufacturing process has been demonstrated for the first time to our knowledge. A design for a complete prosthesis with a dynamic pupil has been proposed and a standard device for the mass production of ocular prostheses is presented. Finally, we have shown that a practical solution for an autonomous self-powered prosthetic dynamic pupil is possible, given the constraints of size, fabrication process, weight, cost and manufacturability on a mass scale. We will discuss the operation of this device for a fully working prototype of the dynamic ocular prosthesis.

7885-37, Session 7

Photovoltaic retinal prosthesis

J. Loudin, Stanford Univ. (United States); K. Mathieson, Univ. of Glasgow (United States); T. I. Kamins, L. Wang, L. Galambos, Stanford Univ. (United States); A. Sher, Univ. of California, Santa Cruz (United States); D. V. Palanker, Stanford Univ. School of Medicine (United States)

Electronic retinal prostheses seek to restore sight to patients suffering from retinal degenerative disorders. Implantable electrode arrays apply patterned electrical stimulation to surviving retinal neurons, producing visual sensations. All current designs employ inductively coupled coils to transmit power and/or data to the implant. We present here the design and initial testing of a photovoltaic retinal prosthesis fabricated with a resolution as high as 256 pixels/mm\(^2\). Photodiodes within each pixel of the subretinal array directly convert light to stimulation current, avoiding the use of bulky coil implants, decoding electronics, and wiring, and thereby reducing surgical complexity. A goggles-mounted camera captures the visual scene and transmits the data stream to a pocket processor. The resulting images are projected into the eyes by video goggles using pulsed, near infrared (~900 nm) light. Prostheses with three pixel densities (16, 64, and 256 pix/mm\(^2\)) are being fabricated, and tests indicate a charge injection limit of 1.62 mC/cm\(^2\) at 25Hz. In
vitro tests of the photovoltaic retinal stimulation using a 512-element microelectrode array have recorded stimulated spikes from the ganglion cells, with latencies in the 1-100ms range, and peak irradiance that is more than 100 times below the IR retinal safety limit. Elicited retinal response disappeared upon the addition of synaptic blockers, indicating that the inner retina is stimulated, rather than the ganglion cells directly and raising hopes that the prosthesis will preserve some of the retina’s natural signal processing.

7885-74, Session 7

**Pupillometer-based objective chromatic primery**

M. Belkin, Y. Rotenstreich, Tel Aviv Univ. (Israel); A. Skaat, A. Kolker, S. Melamed, Tel Aviv Univ. (USA); G. Atar-Ferman, Tel Aviv Univ. (Israel)

**Purpose:** Using the pupillary Light Reflex (PLR) for objective perimetry

**Methods:** The PLR of 25 normal individuals and 16 retinitis pigmentosa (RP) patients eyes were measured in each of 13 different visual field points for short and long wavelength stimuli at stimulus size V3c, in light intensity of 39.8 cd/m² and duration of 1000 ms. Ratios of the PLR diameter for the short and long wavelengths were calculated for each spot. Results: The average ratio were in the normal subjects 0.41 in RP patients of seeing area of the visual fields 0.62 and in the non-seeing area 0.97 which was significantly different (p<0.001). Conclusions: The PLR ratios of the short and long wavelength stimuli were significantly higher in areas of visual field defects in RP patients.

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7885-38, Session 8

**Understanding the impact of aberrations in three-dimensional vision with the binocular adaptive optics visual simulator**

E. J. Fernández, P. M. Prieto, P. Artal, Univ. de Murcia (Spain)

Displays capable for three-dimensional (3-D) vision are currently experiencing a renewed commercial interest. In this context many aspects remain poorly understood, especially those pertaining the impact of aberrations on 3-D vision. In particular, the possible degradation in presence of aberrations distinct of defocus of some visual functions associated to binocular vision, such as stereoaucity, has never been explored so far. We have devised a binocular adaptive optics visual simulator which permits the study of stereoaucity with simultaneous and independent full control of the aberrations of both eyes. The corrector is a single liquid crystal on silicon (LCOS) spatial light modulator, simultaneously operating on the aberrations from the two eyes of the observer in open loop. Two classic 3-D stereocuity tests, the three needle test and random-dot stereogram, have been programmed for exploring the performance of the instrument. The set-up has been operated for measuring stereoaucity in two subjects with varying amounts of defocus and trefoil aberration. First experimental results show a complex relationship between the eye’s aberrations and stereopsis. The latter seems to exhibit a larger tolerance to aberrations than monocular acuity. This instrument may provide some light on the actual importance of aberrations on 3-D vision, which might have an impact in the design of systems and displays dedicated for stereo-perception of images. In addition, the apparatus can be operated as an adaptive optics binocular phoropter, producing 3-D stimuli.

7885-39, Session 8

**Compact adaptive optics scanning laser ophthalmoscope with high efficiency wavefront correction method using dual LCOS-SLM**

F. Hirose, K. Nozato, K. Saito, Y. Numajiri, Canon Inc. (Japan)

A novel and compact adaptive optics scanning laser ophthalmoscope (AO-SLO) for high resolution retinal imaging using two liquid crystal phase modulators (LCOS-SLMs) is described. LCOS-SLM is able to compensate large aberration with phase wrapping method. However, it basically compensates only one polarization component. As a result, the light efficiency of AO-SLO with LCOS-SLM was comparably low. In order to overcome the problem, we have adopted two LCOS-SLMs to compensate two polarization components in the AO-SLO. We designed a compact optical system occupying 500mm × 370mm with aspherical mirrors and used a wavefront sensor and the two LCOS-SLMs for measurement and correction of aberration, respectively. The LCOS-SLMs are aligned as their directions of the polarization components to be modulated are orthogonal each other. As a result, the aberration which is composed of both polarization components is compensated. In AO control we used custom software made with C++, which calculates the 2D data to control the LCOS-SLMs on the basis of the aberration of the eye sensed by the wavefront sensor. The software controls AO closed loop between the LCOS-SLMs and the wavefront sensor, and the same 2D data for each LCOS-SLM are basically output. The AO closed loop update frequency is about 10Hz. With the AO-SLO we observed photoreceptors and blood flow in capillaries at the parafoveal region of human healthy subjects. These results were in good agreement with histology in detail, and the signal was much stronger than that with single LCOS-SLM.

7885-40, Session 8

**Toward real-time wavefront sensor-less adaptive optics using a graphical processing unit (GPU) in a line scanning system**

D. P. Biss, A. H. Patel, R. D. Ferguson, M. Mujat, N. V. Iftimia, D. X. Hammer, Physical Sciences Inc. (United States)

Adaptive optics scanning laser ophthalmoscopes (AOSLO) generally use some type of wavefront sensor to detect wavefront aberrations and determine the appropriate phase profile to apply to an adaptive element, such as deformable mirror. These instruments have been highly successful in reducing aberrations in retinal imaging systems and improving image contrast and resolution, but they have not seen widespread adoption in a clinical setting. In general AOSLO systems tend to be expensive and complicated instruments when compared to other non-AO based fundus imaging systems.

We are adapting a wavefront sensor-less AO algorithm to our line-scanning laser ophthalmoscope (LSO). This algorithm utilizes the image’s spatial frequency content to optimize a metric based upon the system optical transfer function. This method can be computationally intensive requiring multiple Fast-Fourier Transforms to be performed on the image data. The computation time is an issue in retinal imaging, as saccades and micro-tremors in the eye can alter the field of view and cause vignetting.

To overcome these problems we have implemented this algorithm on a NVIDIA GPU to decrease the computation time. We have seen a three-fold decrease in computation time by moving the algorithm from a MATLAB environment to the GPU. While the current computation rate is 0.17 Hz we have begun to investigate the effect on spectral density and algorithm execution time of using smaller strips of the line-scan image. Acceptable condition for real-time operation of the algorithm is correction on the scale of the frame rate of the camera (tens of Hz).
7885-41, Session 8

Retinal imaging system with adaptive optics enhanced with pupil tracking

B. Sahin, Imagine Eyes (France) and National Univ. of Ireland, Galway (Ireland); B. Lamory, X. Leveccq, L. Vabre, Imagine Eyes (France); C. Dainty, National Univ. of Ireland, Galway (Ireland)

Adaptive optics enables the acquisition of high-resolution images of the human retina, but eye and head movements decrease the efficiency of aberration correction. Using an AO system with pupil tracking, we aim to demonstrate the effect of motion on AO correction and also the effectiveness of correction using pupil tracking.

A new control algorithm was integrated into an Adaptive Optics (AO) retinal camera to measure and correct for the eye’s aberrations in vivo using pupil tracking. Using both pupil tracking and the eye’s aberration data, changes of the eye’s aberration during the original measurement are simulated. Correlation analysis of the simulations and the original measurements shows that the fluctuations in pupil movements are correlated with the changes in pupil movements and other factors such as tear film or internal refractive variations. The average RMS of an healthy subjects eye’s aberrations before correction was 1.8±0.5 μm. The average residual RMS under static correction was 0.4±0.1 μm. The average residual RMS with adaptive closed loop corrections based on WFS only and based on pupil tracking only were 0.12±0.07 and 0.2±0.1 μm respectively. The average RMS of the simulated wavefront and the difference between simulated and the original wavefront were 0.3±0.1 and 0.2±0.1 μm. The pupil moved 60±70 μm in average. AO correction based on an initial wavefront measurement and pupil tracking alone resulted in a lower residual RMS than a static correction. A better correction is hoped to be achieved by using both wavefront sensing and pupil tracking data in closed loop.

7885-42, Session 8

Low-coherence wavefront sensing for AO imaging in rodent eyes

R. D. Ferguson, D. X. Hammer, M. Mujat, N. V. Iftimia, N. Lue, D. P. Biss, A. H. Patel, Physical Sciences Inc. (United States); J. D. Akula, Children’s Hospital Boston (United States)

Rodent models of human eye disease have significantly increased the demand for, and the value of, new in vivo animal fundus imaging modalities with cellular resolution. The small eye of the mouse with its relatively large pupil provides among the largest numerical apertures of any mammal eye, and consequently has the potential to provide superior retinal image resolution. However, due to the relatively poor optical quality of mouse eyes in particular, even high resolution imaging can’t fully exploit the large N.A. and select layers for precision adaptive optics (AO) correction. It is not deformable mirror technology that currently falls short, but conventional wavefront sensing technology (i.e. Shack-Hartman or SHWS). We describe recent progress toward the development and demonstration of a new, compact multimodal AO imaging platform optimized for rodent imaging, and novel alternatives to SHWS including a low-coherence wavefront sensing (LCWS) techniques. Recently, coherence-gated wavefront sensing using a virtual Shack-Hartmann Sensor was described by Rueckel and Denk [2006] for microscopy. Our low-coherence wavefront sensor (LCWS) operates similarly with a dedicated SLD source (~14nm FWHM) and a reference arm mirror driven axially with a piezo-electric PZT stage. The LCWS recovers the phase map representing the wavefront by phase unwrapping, resulting directly in a deformable mirror surface correction applied directly to the mirror. Initial work has shown that this depends critically on wavefront sensor camera speed to capture stable fringes, speckle smoothing with scanning, and proximity to adequate initial AO correction. An alternative coherence-gated strategy for wavefront sensor-less AO depth and focus control based on simultaneous AOSLO and AO-OCT images in a graphical processing unit (GPU) implementation is also described.

7885-43, Session 9

Motion correction of optical coherence tomography volumes in three dimensions on a per A-Scan basis using orthogonal scan patterns

M. F. Kraus, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany) and Massachusetts Institute of Technology (United States); B. M. Potsaid, Baumann, Massachusetts Institute of Technology (United States); V. Manjunath, New England Eye Ctr. (United States); M. A. Mayer, R. Bock, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); J. S. Schuman, Univ. of Pittsburgh Medical Ctr. (United States); J. S. Duker, New England Eye Ctr. (United States); J. G. Fujimoto, Massachusetts Institute of Technology (United States); J. Hornegger, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany)

The development of high speed optical coherence tomography (OCT) has made it possible to capture densely sampled 3D volume data in a matter of seconds. One key application is the generation of high quality in vivo imaging data of the human retina. Since the whole volume is not captured at once, eye movement during the scan process leads to distortion. We use two or more volumetric scans with orthogonal fast scan directions that are registered in order to find and correct the unknown eye motion. Registration is performed by optimizing a global objective function using one dense displacement field for each input volume and special regularization based on the time structure of the acquisition process. After optimization each volume is undistorted and a single merged volume is constructed that possesses superior integrity and signal quality compared to the input volumes. Experiments on multiple OCT systems, including ultrahigh resolution and ultrahigh speed spectral and swept source instruments at 800 nm and 1050 nm, imaging both healthy and diseased eyes, show that the algorithm is able to correct motion in all three dimensions. Both slow drift, as well as saccadic movement is corrected. The resulting volumes do not show any visible motion artifacts. Using a graphics card for acceleration, the process takes as little as 25s for two 300 by 200 axial scan volumes. These methods promise to facilitate the acquisition of large 3D-OCT data sets and improve measurement reproducibility without the need for active eye tracking or motion free reference images.

7885-44, Session 9

Stabilized simultaneous retinal optical coherence tomography at 800 and 1060 nm

B. Považay, Medizinische Univ. Wien (Austria); I. Böttcher, Heidelberg Engineering GmbH (Germany); B. Hofer, W. Drexler, Medizinische Univ. Wien (Austria)

Retinal imaging is demonstrated with a combined 800/1060 nm eye-tracking optical coherence tomography system. While current commercial optical coherence tomography devices for retinal imaging are operated at the 800 nm wavelength region, research has shown that the influence of scattering at longer wavelengths is reduced and penetration through cataract as well as into the subretinal tissue of the choroid and sclera is improved at the 1060 nm water absorption window. Tracking and stabilization of eye movement together with averaging further removes speckle, suppresses noise and increases the signal to achieve unprecedented image quality. By acquiring at the two wavelength ranges at exactly equal imaging conditions the spectral, penetration and contrast differences are visualized and discussed.
Segmentation of retinal layers in volumetric OCT scans of normal and glaucomatous subjects

K. A. Vermeer, The Rotterdam Eyehospital (Netherlands) and i-Optics BV (Netherlands); J. van der Schoot, H. G. Lemij, The Rotterdam Eyehospital (Netherlands); J. F. de Boer, The Rotterdam Eyehospital (Netherlands) and Vrije Univ. Amsterdam (Netherlands)

Volumetric scans of current OCT devices can contain on the order of 50 million pixels. Manual assessment of these volumetric scans in the clinic is therefore not feasible. In addition, quantitative measurements in these volumetric scans are often needed. Automatic segmentation of the scans is therefore required.

In this paper, a fully automatic retinal layer segmentation algorithm is presented, based on voxel-classification. First, each voxel is segmented by data from a local neighborhood, producing a feature vector. These feature vectors are used as inputs for a support vector machine (SVM), which classifies each voxel as above or below each interface. Finally, a level set method regularizes the result, producing a smooth surface within the 3-dimensional space.

Volumetric scans of 10 healthy and 8 glaucomatous subjects were acquired with a Spectralis OCT. Each scan consisted of 193 B-scans, 512 A-lines per B-scan (5 times averaging) and 496 pixels per A-line. Two B-scans of each healthy subject were manually segmented and used to train the SVM. One B-scan of each glaucomatous subject was manually segmented and used only for error estimation.

The root-mean-square errors for the healthy eyes were 3.4, 13.2, 8.5 and 6.0 µm for the vitreous/NFL, NFL/GCL, IPL/INL and RPE/choroid interfaces, respectively, and 3.9, 14.9, 11.9 and 5.6 µm for the glaucomatous eyes. Based on the segmentation, retinal and NFL thickness maps and blood vessel masks were produced.

Performance of automated versus manual segmentation of retinal lesions by polarization sensitive OCT


Segmentation of retinal lesions has become an important research field for quantitative applications of optical coherence tomography (OCT). It is required both for diagnostic lesion grading and for follow up studies of various kinds. Several algorithms have been developed for this purpose. They are essentially based on variations of backscattered intensity between layers or at the boundaries of layers. Intensity-based algorithms are, however, sensitive to various factors like illumination conditions, presence of vessels, or phase washout caused by ocular motions.

Polarization sensitive (PS) OCT provides additional information on tissue, allowing direct tissue identification by intrinsic contrast mechanisms. E.g., the retinal nerve fiber layer is birefringent while the retinal pigment epithelium (RPE) acts as depolarizer, i.e. scrambles the polarization state. We recently used the latter effect to develop improved algorithms for segmenting the RPE and adjacent lesions. We now report on further refinements of this technique and on an evaluation of algorithm performance by comparison to segmentation by expert readers. We used a spectral domain PS-OCT/SLO system for the measurements. The instrument operates at a wavelength of 840 nm and records 20000 A-scans/s. Three parameters can be measured simultaneously: reflectivity, retardation, and optic axis orientation. In addition, spatially resolved Stokes vectors can be measured from which the degree of polarization uniformity (DOPU) can be derived.

Automatic segmentation of SDOCT images from multiple ophthalmic applications congruent with expert manual segmentation

S. J. Chiu, J. Y. Choi, F. LaRocca, A. N. Kuo, C. A. Toth, J. A. Izatt, S. Farsiu, Duke Univ. (United States)

Accurate detection of anatomical and pathological structures in Spectral Domain Optical Coherence Tomography (SDOCT) images is critical for the study and diagnosis of ocular diseases. Only recently have algorithms been developed to automate the segmentation process. These works, however, focus mainly on the segmentation of a particular anatomical or pathological feature. We extended a general segmentation framework based on graph theory and dynamic programming, which we introduced previously for segmenting retinal layers in normal eyes. We broadened the application of our framework by incorporating prior information about the morphology of the ocular structures. We applied this technique to segment images from several different ophthalmic SDOCT applications, including normal retina, Level 3 aged-macular degeneration (AMD) retina with drusen, advanced AMD retina, pediatric retina (with and without edema), and cornea. The underlying algorithm for the segmentation of these various images was identical, with modifications made to the graph weights, search space, and segmentation order for each image category. Results show that our algorithm accurately segmented layers in retinal and corneal SDOCT images with varying degrees and types of pathology. Furthermore, our automatic segmentation matched an expert grader more closely than a second grader for all image targets. This is highly encouraging for not only reducing the time and manpower required to segment images in ophthalmic studies, but also for offering an extensible yet integrated algorithm for the segmentation of different ocular diseases.

In-vivo quantitative assessment of outer retinal degeneration in a rat retinal model with UHROCT and a novel semi-automated segmentation algorithm

S. Hariri, A. Akhlagh Moayed, D. Lee, S. Shakeel, Univ. of Waterloo (Canada); S. Boyd, St. Michael's Hospital (Canada); K. K. Bizheva, Univ. of Waterloo (Canada)

A high speed (47,000 A-scan/second), high resolution FD-OCT system, operating in the 1060nm wavelength range was used to acquire in vivo 3D images of normal and damaged rat retinas and to monitor and quantify non-invasively outer retinal degeneration over time. The OCT system provided 3µm axial resolution in the rat eye and ~100dB sensitivity at 1.3 mW power of the imaging beam. Images of the healthy normal rat retinas show clear visualization of all retinal layers, as well as the capillary network of the inner and mid-retina. Images acquired from the degenerated retinas show partial or full disintegration of the external limiting membrane (ELM), inner and outer segments (IS/OS) of the photoreceptor layer and damage to the retinal pigment epithelial (RPE) layer. A novel semi-automated segmentation algorithm was used for layer segmentation and thickness measurement. Statistical analysis of the individual layer and total retina thickness was obtained over 4 eyes. Surface thickness maps were generated to show the spatial distribution of the retinal degeneration over time.
In vivo investigation of cornea and anterior segment using office-based polarization sensitive swept-source optical coherence tomography

Y. Lim, M. Yamanari, S. Fukuda, Y. Kaji, T. Kiuchi, Univ. of Tsukuba (Japan); M. Miura, Tokyo Medical Univ. Kasumigaura Hospital (Japan); T. Oshika, Y. Yasuno, Univ. of Tsukuba (Japan)

Thirteen eyes of 7 cases of keratoconus, 3 eyes of 3 subjects who were treated by trabeculectomy, and 4 eyes of 2 rabbits on which trabeculectomy was performed, were investigated by an office-based polarization sensitive swept-source optical coherence tomography (PS-SSOCT). The office based PS-OCT which simultaneously measures intensity and phase retardation images was developed based on our PS-OCT using a 1.3 um high speed wavelength-swept laser and a customized scanning head.

En face phase retardation maps of the corneal back surfaces of the keratoconus patients were extracted from volumetric OCT data. In the phase retardation maps, inhomogeneous changes of the phase retardation were observed. These changes might be caused by corneal lamellar structural changes. The positions of the changes of phase retardation were different depending on individual patients, and did not have clear relationship to corneal topometry. In the 3 human trabeculectomy measurements, the regions of the fibrosis around the blebs were visualized with PS-OCT. Patients with high intraocular pressure had larger area of fibrosis. Animal models of rabbits were measured in 4 cases before the surgery and 0, 8, and 14 days after the surgery of trabeculectomy. Phase retardation images showed fibrosis in postoperative 8-, and 14-day eyes.

Using PS-SSOCT, the structural and birefringent changes of lamellar in the cornea and the formation of fibrosis around the blebs were visualized. PS-SSOCT would potentially be useful to study and diagnose the corneal diseases, and the functioning of blebs.

Polarization sensitive optical coherence tomography at 840 nm and 1030 nm in ophthalmology

T. Torzicky, E. Götzinger, M. Pircher, S. Zotter, M. Bonesi, C. K. Hitzenberger, Medizinische Univ. Wien (Austria)

In our current work we are investigating polarization sensitive optical coherence tomography (PS-OCT) systems in the 840 nm and the 1 µm wavelength region and compare their performance especially for ophthalmologic applications. It has been previously shown that PS-OCT in the 840 nm wavelength region can be used in ophthalmology for detection of pathologic changes as in glaucoma or age-related macular degeneration.

The wavelength region of 840 nm allows for high resolution imaging, but is limited for imaging retinal layers beyond the retinal pigment epithelium (RPE) like choroid and sclera. Therefore OCT imaging in the 1 µm wavelength region has recently gained popularity for ophthalmic applications due to the fact that it allows enhanced visualization of choroid and sclera.

For our comparison we use a spectrometer based PS-OCT system in the 840 nm region which has been presented previously and has been used for imaging of a variety of retinal diseases. Furthermore we developed a novel PS-OCT system working in the 1 µm region. With this 1 µm PS-OCT system polarization characteristics of retinal layers beyond the retinal pigment epithelium are investigated.

Inner-layer-based birefringence measurement of RNFL using PS-OCT

Q. Wang, Indiana Univ. (United States); B. Cense, Utsunomiya Univ. (Japan); O. P. Kocaoglu, W. Gao, R. S. Jonnal, S. Lee, D. T. Miller, Indiana Univ. (United States)

Birefringence of the retinal nerve fiber layer (RNFL) has been suggested a sensitive indicator of tissue health, with changes reported to precede clinically detectable vision loss. A leading method to measure birefringence in the eye is polarization-sensitive optical coherence tomography (PS-OCT), which measures simultaneously the double pass phase retardation (DPPR) and thickness of the RNFL. PS-OCT measurement of birefringence is based on a least squares fit of the DPPR across the full thickness of the RNFL. While this slope method has been rigorously validated, it requires averaging of A-lines (which reduces lateral resolution of the instrument) and a thick RNFL for accurate slope fits.

We propose a new method for post-processing of PS-OCT images that does not share these limitations. Instead of fitting to the DPPR slope, a comparison is made between Stokes vectors immediately anterior and posterior of the RNFL. This comparison leaves only the birefringence contribution of the RNFL. In this study we validate the new method and confirm its advantages by comparing it to the traditional slope method. Comparison is realized using subject data acquired with PS-OCT.

Dependence of the retinal reflectance on illumination angle and retina location

W. Gao, B. Cense, O. Wang, O. P. Kocaoglu, R. S. Jonnal, S. Lee, D. T. Miller, Indiana Univ. (United States)

A research-grade spectral-domain optical coherence tomography system was developed to measure the directional properties of the retina as a function of retinal eccentricity. Particular attention was given to the photoreceptor layer (PL), Henle’s fiber layer (HFL), and retinal nerve fiber layer (RNFL). A horizontal stationary scan intersected the fovea with a...
scan width of 8 degree over the experiment. B-scans were acquired at each pupilary position along the horizontal and vertical meridians. The PL reflection was found sensitive to entry beam positions on the pupil, and showed considerable variability of directionality over the retina, which is consistent with the optical Stiles-Crawford effect. Reflections from HFL and RNFL were found not only sensitive to the illumination angle, but also the meridian of beam entry in the pupil. A significant variation in apparent thickness of HFL is observed, whereas that of the RNFL shows little.

7885-54, Session 10
Retardation of Henle’s fiber layer measured with polarization-sensitive optical coherence tomography
D. T. Miller, Q. Wang, J. Besecker, W. Gao, S. Lee, O. P. Kocaoglu, R. S. Jonnal, Indiana Univ. (United States); B. Cense, Utsunomiya Univ. (Japan)

The structured organization of Henle’s fiber layer (HFL) is well known to exhibit birefringence, which has been suggested as a sensitive indicator of retinal disease. To investigate this possibility, we have developed a method based on polarization-sensitive optical coherence tomography (PS-OCT) to measure the double pass phase retardation (DPPR) of Henle’s fiber layer. Stationary horizontal and vertical B-scans (15°; 1000 A-scans) and volumes (15° x 15°; 100 x 1000 A-scans) were recorded at an A-scan rate of 25 kHz. Both imaging scan patterns were centered on the fovea. We developed a two step process that consisted of determining Stokes vectors on surfaces that straddled HFL and comparing the vector sets to determine the DPPR contribution of HFL. In this process, birefringence contributions from all other layers are removed. PS-OCT measurements were performed on ten healthy young subjects (20 to 35 years of age), ten healthy old subjects (50 to 65 years of age) and five glaucoma patients. In the healthy subjects, B-scans through the fovea center generated characteristic M-shaped curves of DPPR: a minimum near the fovea center; maxima (DPPR = 15°-25°) proximal to the rim of the fovea pit; and decreased values extending out from the rim. In the glaucoma patients, DPPR was found reduced. These are the first measurements to our knowledge in which the retardation of Henle’s fiber layer was axially resolved and quantified in the living retina.

7885-55, Poster Session
Critical evaluation of the ultrasonic pachymetry for “in vitro”corneas
V. A. Cacciacarro Lincoln, L. Ventura Schiabel, S. J. Faria e Sousa, Univ. de São Paulo (Brazil)

The measurement of central corneal thickness (CCT) is vastly useful for diagnostic and therapeutical evaluation. There are various techniques to measure CCT and the ultrasound pachymetry is currently the most common. This study was undertaken to determine the precision and correlation of measurements obtained by mechanical and ultrasound pachymetry.

The ultrasound pachymetry was determined using an A-scan pachymeter. The probe tip was held perpendicular on the central cornea by a device that descends smoothly to avoid excessive pressure and instability.

The mechanical pachymetry was determined using a micrometer with a tip of 2mm diameter, 1 micron precision and digital display. The tip was held perpendicular on the central cornea by an apparatus that retains the cornea stabilized. A 10x optics magnification and a digital video camera displayed the image of the tip touching the cornea on the laptop screen to assist the manual control of approach and full contact.

Eight human corneas were obtained from cadaveric eyes. Measurements using both systems were undertaken by three different users. Each user performed five blinded measurements, which was taken note by a different user. The results were averaged.

The results for the measurements in our mechanical set up, as well as in the ultrasound system, presented an average standard deviation of 33 microns, which was referred to be as systematic error among users (due to positioning; centering; pinching). However the difference in measurements between both systems was 100 microns, which possibly refers to the imprecision of ultrasonic pachymetry for measuring in vitro corneas.

7885-56, Poster Session
Portable prototype for ultraviolet analysis of donated corneas
L. Ventura Schiabel, V. A. Cacciacarro Lincoln, H. Schiabel, S. J. Faria e Sousa, Univ. de São Paulo (Brazil)

The cornea has a natural UVA and UVB protection, so as to avoid further exposure of the inner optical components of the eye. As technology improves human vision, some procedures currently performed might be causing the decrease of the natural UV protection.

In order to provide means for clinical studies of these effects on the corneas, a portable system has been developed to be manipulated by the physicians and to endow with two types of UVA/UVB protection evaluation for: 1. Regularly donated corneas; 2. Corneas submitted to removed corneal lamellae, simulating refractive keratotomy.

The prototype consists of an optical dual beam system, composed by an UV lamp UV sensors for measuring the transmittance of the cornea at the 300nm - 400nm range.

The system was built so as the reference sensor and cornea measuring sensor are delivered by the same amount of radiation, simultaneously, in order to avoid fluctuations of the signal. As the lamp emits the UV radiation the voltage delivered on both sensors are collected providing the amount of transmittance of UVA/UVB as percentage. The system performs 500 measurements/s and provides ±0.25% precision for the transmittance.

The system has been correlated to a CARY17 spectrophotometer for acrylic samples and to a HR4000CG-UV-NIR Ocean Optics spectrophotometer for donated human corneas. The correlation factor corneas the is 0.98981 and 0.985, respectively.

7885-57, Poster Session
Optical clearing of rabbit bulbar conjunctiva by 40%-glucose solution
A. N. Bashkatov, E. A. Genina, E. A. Zubkina, A. Parkheychuk, V. V. Tuchin, N.G. Chernyshevskiy Saratov State Univ. (Russian Federation)

We present experimental results on the optical properties of the rabbit bulbar conjunctiva controlled by administration of a hyper-osmotic optical clearing agent, such as 40%-glucose solution. Administration of the chemical agent induces diffusion of matter and as a result equalization of the refractive indices of collagen and ground material. Results of experimental study of influence of the glucose solution on transmittance spectra of rabbit bulbar conjunctiva are presented. The increase of transmittance of the rabbit bulbar conjunctiva samples under action of the glucose solution was demonstrated. Glucose diffusion coefficient in rabbit conjunctiva was estimated.

7885-58, Poster Session
Laser welding of chitosan-GNRs films for the closure of a capsulorhexis
F. Rossi, P. Matteini, F. Ratto, Istituto di Fisica Applicata Nello
We found that the aspect ratio obtained from the 2D FFT analysis can be transformed (2D FFT) to obtain the distribution of collagen fiber orientations. The SHG images are later analyzed with 2D fast Fourier transform (WFS) to obtain the distribution of collagen fiber orientation in freshly enucleated porcine eyes. The lens was aspirated, then the capsule bag was used to deliver single spots (200 micrometer core diameter optical fiber) of local capsule/patch adhesion. Then the bag was refilled with silicon oil. The result is an immediate closure of the capsular tissue, with high transparency and flexibility. The laser welded chitosan-GNR films are an innovative and highly stable solution to be exploited for the treatment of capsular breaks and for the implementation of a lens refilling procedure.

7885-62, Poster Session
Quantitative analysis of collagen fiber orientation in with two-dimensional fast Fourier transform
W. Lo, National Taiwan Univ. (Taiwan) and National Cheng Kung Univ. (Taiwan); C. Hsueh, W. Chen, National Taiwan Univ. (Taiwan); S. Chen, National Cheng Kung Univ. (Taiwan); H. Tan, Chang Gung Memorial Hospital (Taiwan) and Chang Gung Univ. (Taiwan); C. Dong, National Taiwan Univ. (Taiwan)

The purpose of this study is to investigate the structural features of the corneal stroma by second harmonic generation (SHG) microscopy. Since collagen can be induced to generate strong second harmonic generation (SHG) signals, multiphoton excitation provides direct visualization of collagen orientation within corneal stroma. In this work, we collected both forward and backward SHG signals at different depths across the cornea specimens. The SHG images are later analyzed with 2D fast Fourier transform (2D FFT) to obtain the distribution of collagen fiber orientations. We found that the aspect ratio obtained from the 2D FFT analysis can be used for the quantitative determination of fiber orientation and that this approach may be used for the diagnosis of pathological corneas.

7885-63, Poster Session
Quantitative surface curvature measurement by a traditional camera and a wavefront image sensor (WIS)
J. Ren, X. Cui, C. Yang, California Institute of Technology (United States)

A method that allows users to quantitatively measure the surface curvature of a remote object based on our newly developed wavefront image sensor (WIS) and a traditional photographic camera is reported. In traditional photography, only the intensity of light field at the image plane is recorded. An inherent capability of our WIS is the separation of intensity and phase information of a light field. By placing the sensor on the image plane, we can gain the extra wavefront information, the normalized phase gradient, of the image. We notice that this wavefront at the image plane is actually related to the wavefront at the object plane. The relationship between them is determined by the optical configuration of the imaging system. We used a calibration procedure to acquire this relationship, where a collimated beam as a reference is continuously tilted and scanned over the scope of the camera. By measuring the wavefront of the image and applying the wavefront relationship between image and object planes, we can compute the wavefront of the object plane and further deduce the surface normal of the object with the properties of the surface reflection. In a proof-of-concept demonstration, a set of concave mirrors (smooth surfaces) with different focal lengths (50-200 mm), are imaged. The results agree well with their real values.

7885-64, Poster Session
Endoscopic device for functional imaging of the retina
D. T'so, S. Lohani, B. Martell, SUNY Upstate Medical Univ. (United States); E. S. Barriga, P. Soliz, VisionQuest Biomedical LLC (United States)

Non-invasive imaging of retinal function based on the recording of spatially distributed reflectance changes evoked by visual stimuli has to-date been performed primarily using modified commercial fundus cameras. We have constructed a prototype retinal functional imager, using a commercial endoscope (Storz) for the frontend optics, and a low-cost back-end that includes the needed dichroic beamsplitter to separate the stimulus path from the imaging path. This device has been tested to demonstrate its performance for the delivery of adequate NIR illumination, intensity of the visual stimulus and reflectance return in the imaging path. The current device was found to be capable of imaging reflectance changes of 0.1%, similar to that observable using the modified commercial fundus camera approach. The visual stimulus (a 505 nm spot of 0.5 s) was used with an interrogation illumination of 780 nm, and a sequence of images captured. At each pixel, the image signal was subtracted and normalized by the baseline reflectance, so that the measurement was ∆R/R. The typical retinal activity signal observed had a ∆R/R of 0.3-1.0%. The noise levels were measured when no stimulus was applied and found to vary between ± 0.05%.

Functional imaging has been suggested as a means to provide objective information on retina function that may be a preclinical indicator of ocular diseases, such as age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy. The endoscopic approach promises to yield a significantly more economical retinal functional imaging device (Endo-f-imager) that would be clinically important.
3D assessment of mechanical wave propagation in the crystalline eye lens using PhS-SSOCT
K. V. Larin, Univ. of Houston (United States)

According to the most widely accepted theory of presbyopia, the age-related loss of accommodation is attributed to gradual loss of lens elasticity. The stiffness of the lens could be assessed by measuring the propagation of mechanically induced waves on its surfaces. Here we present results for the volumetric assessment of mechanical waves propagating on both surfaces of the crystalline lens measured with the Phase-Sensitive Swept Source Optical Coherence Tomography (PhS-SSOCT) technique. The results indicate that the system could detect vibrations of as small as 0.3 µm induced on the crystalline lens, and hence, PhS-SSOCT could be potentially used to assess stiffness of a crystalline lens.

Adaptive prediction of human eye pupil position and effects on wavefront errors
A. Garcia Rissmann, C. Kulcsar, Y. El Mrabet, H. G. Raynaud, Univ. Paris 13 (France); B. Sahin, B. Lamory, Imagine Eyes (France)

The effects of human eye movement on image quality in a retinal imaging instrument are studied in this paper. Indeed, even when a patient is fixing a target, residual movements are still present, due to eye movements themselves (drifts, tremor, saccades) or to head movements. When retinal imaging is performed using an adaptive optics (AO) system as in INOVEO project or in [1], substantial gain is foreseen when accounting for eye pupil motion.

A pupil tracker, developed by Imagine Eyes®, provides pupil position measurements at 80Hz sampling rate. Experimental data have been collected on 25 patients, showing high variability in patterns of pupil displacements. We evaluate wave-front errors obtained by computing the root mean square of the difference between a wave-front and a displaced wave-front. The results confirm that pupil movements have to be compensated. In an AO loop, there is inevitably a delay between wave-front measurement and correction, so that compensation means prediction.

We investigate several ways of predicting pupil movement, either by retaining the last value given by the pupil tracker, or by performing prediction position thanks to auto-regressive (AR) models with parameters updated on-line (adaptive recursive least-squares). We show that significant improvement is obtained with adaptive AR modeling, and explain why low order AR models have to be favored.

[1] Control design and performance assessment for iPhot AO retinal imaging system
C. Kulcsár, S. Meimon, H.-F. Raynaud, J.-M. Conan, A. Garcia, X. Lévecq, B. Lamory

Submitted to this session

Measuring the retina optical properties using a structured illumination imaging system
A. Basiri, The Catholic Univ. of America (United States); Q. D. Nguyen, M. Ibrahim, The Johns Hopkins Univ. (United States); J. C. Ramella-Roman, The Catholic Univ. of America (United States)

Patients with diabetic retinopathy (DR) may experience a reduction in retinal oxygen saturation (SO2). Close monitoring with a fundus ophthalmoscope can help in the prediction of the progression of disease. In this paper we present a noninvasive instrument based on structured illumination aimed at measuring the retina optical properties including oxygen saturation.

The instrument uses NIR illumination, a fast acquisition camera, and a splitter system that allows for contemporaneous collection of images at two different wavelengths. This scheme greatly reduces eye movement artifacts.

Structured illumination is achieved with several binary illumination masks fabricated using laser micro-machining. A near-sinusoidal projection pattern is ultimately achieved at the image plane by appropriate positioning of the binary masks. The Carre’ algorithm is utilized for the reconstruction of the tissue µa and µs'. The system was calibrated using optical phantoms of known optical properties.

Correlation of spatial intensity distribution of light reaching the retina and restoration of vision by optogenetic stimulation
S. Shivalingaiah, M. Bhalerao, L. Gu, S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Stimulation of retinal neuronal cells using optogenetics via use of chanelrhodopsin-2 (ChR2) and blue light has opened up a new direction for the restoration of vision with respect to treating Retinitis pigmentosa. In addition to targeting a specific retinal layer using genetic engineering, delivery of threshold level of blue light onto the retina is required to generate action potential, since blue light is significantly attenuated in tissue. To predict the excitation of ChR2 in the retina, requires an accurate representation of stimulating-light transport within the eye. This is due to the different absorption and scattering as well as histological properties of various layers. We report on the measurement of intensity and distribution of light reaching the retina of mouse models for Retinitis pigmentosa and compared those results with Monte Carlo simulation. The behavioral patterns of the mice with their retina expressing Thy1-ChR2-YFP specifically in retinal ganglion cells were found to correlate with stimulation intensity. Further, in order to restore vision, spatially distributed intensity patterns needed to be projected and matched with the targeted retinal neurons. To achieve this task, a blue LED array was developed to be mounted in front of the mouse eye having ChR2 expression. We present recent advances and challenges in the optogenetic treatment and image formation capability of the optogenetically treated eye from the point of view of spatial resolution.

Smart polymers containing substituted coumarin side groups enable photo-induced tuning of focal length of intraocular lenses
M. Schraub, N. A. Hampp, Philipps-Univ. Marburg (Germany)

A general problem of cataract surgery is that after IOL implantation in more than 80% of all cases vision is not perfectly restored and the prescription of viewing aids is required. Reasons for this suboptimal result are that biometric data, e.g. the curvature radii of the cornea,
cannot be determined with the desired precision and further wound healing and post-operative migration of the IOL cannot be fully predicted. In this study we present next generation polymers comprising substituted coumarins in the side chains. Their refractive indices can be changed non-invasively by a photo-induced intermolecular $[2\pi + 2\pi]$ cycloaddition reaction between coumarin moieties. Derivatisation of the coumarins with halogens increased the dimerization rates while decreasing the energy dose needed for photochemical dimerization of the coumarin side groups. These polymeric materials show photoinduced changes in the refractive indices of up to $\Delta n > 0.02$, which in turn enables one to tune the focal length of a standard IOL by more than two dioptres. IOLs made of these novel polymers allow 96% of all cataract patients to gain optimal vision.

7885-72, Poster Session

**Wavefront conjugated ray tracing aberrometry**

V. V. Molebny, National Taras Shevchenko Univ. of Kyiv (Ukraine)

The optical system of the eye, due to its dual role as an object of measurement and as a part of the aberrometer, creates uncertainty of the results for both ingoing and outgoing techniques. Local time-separated wave front conjugation is proposed for ray tracing aberrometry based on the acousto-optic control of the tilt of the beam at the entrance into the eye. Modeling was made with ZEMAX for rotation non-symmetric modes of Zernike decomposition. The eye is interpreted in paraxial approximation, where aberrations are added as Zernike Standard Sag surfaces. Ray tracing technique is more susceptible for errors than Hartmann-Shack aberrometer. In the proposed solution for ray tracing aberrometer, two sets of 2D acousto-optic deflectors are installed in series, the first one being used for changing the height of the beam, the second one - for tilting the beam and directing it in the same point of the pupil, in which it entered at the primary probing. The proposed principle of local wave front conjugation allows to decrease the measurement error by an order per iteration. Depending on the type of aberration, it typically means that the error as high as about 20% of the actual value will be reduced to 2% after the first iteration, and to 0.2% after the second iteration. In most cases, a single iteration can be enough.

7885-73, Poster Session

**In-vivo 3D imaging of the human upper eyelid with ultrahigh resolution optical coherence tomography**

K. K. Bizheva, P. Lee, D. Lee, S. Shakeel, L. Sorbara, N. Hutchings, T. L. Simpson, Univ. of Waterloo (Canada)

In-vivo 3D images of Meibomian glands (MG) in the human upper eyelid are presented for the first time. Volumetric tomograms of the human tarsal plate were acquired with a 1060nm UHROCT system with ~3µm x 10µm (axial x lateral) resolution at 47,000 A-scans/s. The OCT tomograms reveal the spatial distribution of the MGs’ acini and ducts (in healthy subjects), and accumulation of heterogeneous, highly scattering biological material and clear fluids in inflamed glands. Non-invasive, volumetric, high resolution morphological imaging of the human tarsal area could have a significant impact in the clinical diagnosis of inflammatory and non-inflammatory lid pathologies.
Assessing the biological consequences of PDT: not as easy as it looks

D. H. Kessel, M. Price, Wayne State Univ. (United States)

Studies carried out in the context of photodynamic therapy (PDT) often rely on supposedly specific fluorescence probes for reporting on levels of different reactive oxygen species (ROS) as well as on the specificity of agents reported to quench or amplify pathways involved in ROS interactions. Typical experiments carried out in vitro generally involve incubations carried out in an atmosphere of 20% oxygen that is seldom encountered in vitro. Studies designed to assess the relevance of ROS probes have revealed that these are seldom as specific as is advertised, that altering ROS pathways is not unambiguous and that use of lower levels of oxygen may provide much more information on phototoxicity. No fluorescent probe was found to be specific for any ROS, including agents said to be relatively specific for hydrogen peroxide, superoxide anion radical, hydroxyl radical or singlet oxygen. Manganese porphyrins considered to be superoxide dismutase (SOD) mimics also show catalase activity and can quench singlet oxygen. Incubation of murine cell lines in 2-5% oxygen alters cellular levels of both SOD and catalase. These results illustrate the need for evaluating any analytical method and in vitro protocol before concluding that it is pertinent to the goals of a PDT project.

PDT simultaneously with inhibition of EGFR and c-Met pathways enhances treatment outcomes in experimental pancreatic cancer

L. Z. Zheng, B. Q. Spring, P. R. Rai, Z. Mai, Massachusetts General Hospital (United States); S. P. Pereira, Univ. College London (United Kingdom); B. W. Pogue, Dartmouth College (United States); T. Hasan, Massachusetts General Hospital (United States)

Studies by a number of groups have helped unravel molecular mechanisms involved in cancer growth and metastasis and during photodynamic therapy (PDT). Our strategy has been to exploit these mechanistic data to develop targeted PDT. Of these, the concept of developing treatments that involve a cytotoxic therapy combined with targeted interventions of two or more cross-talking pathways responsible for cancer development and metastasis, has been attractive. Results and implications from testing this approach combining PDT with simultaneous inhibition of the epidermal growth factor receptor (EGFR) and the cellular mesenchymal epithelial transition factor (c-Met) pathways in an orthotopic model of pancreatic cancer will be discussed.

Synthesis, photophysical, tumor imaging, and PDT efficacy of long-wavelength photosensitizers derived from bacteriochlorophyll-a

R. K. Pandey, Roswell Park Cancer Institute (United States)

While PDT has been around for more than 20 years, its potential as a novel diagnostic and therapeutic approach is just beginning to be explored. Although the challenges to discovering the full potential of PDT are complex, the benefits to current and future patients can be significant. The information provided by tumor targeted multifunctional photosensitizers when combined with diagnostic imaging allows for additional localization of tumors and assessment of the effectiveness of the therapy, thus assisting the surgeon in performing tumor resection more completely via the “see and treat” approach.

Fluorescence tomography and PDT dosimetry

S. Davis, Dartmouth College (United States)

No abstract available

Combined modality approaches: dimethyl celecoxib, a non-cyclooxygenase-2 inhibitor, blocks surviving expression and enhances photodynamic therapy responsiveness

C. J. Gomer, A. Ferrario, S. Lim, F. Xu, M. Luna, Childrens Hospital Los Angeles (United States)

An increasing number of laboratories have shown that Photodynamic Therapy (PDT) effectiveness can be improved by employing a variety of combined modality approaches. Specifically, molecular and biological therapies targeting the tumor vasculature and/or tumor cell death pathways enhance PDT responsiveness. We and others have demonstrated that the combination of PDT with celecoxib improves long-term tumoricidal activity without increasing normal tissue phototoxicity. However, side effects arising from the use of coxib based cyclooxygenase-2 (COX-2) inhibitors, including cardiovascular injury induced by reduced prostaglandin levels, have decreased the potential clinical applications of this class of compounds. Recent studies have demonstrated that the tumoricidal action of coxibs such as celecoxib can involve non-COX-2 mediated mechanisms. The coxib derivative, dimethyl celecoxib (DMC), does not inhibition COX-2 but does exhibit cytotoxic properties comparable to the COX-2 inhibitor, celecoxib. We examined the effectiveness of DMC in modulating PDT response in a murine mammary carcinoma model in both cell culture assays and when growing as solid tumors in syngeneic mice. We observed that DMC enhanced the in vitro ER stress response of PDT, blocked the expression of survivin, and increased both apoptosis and cytotoxicity of cells exposed to combination protocols. DMC also enhanced the in vivo tumoricidal effectiveness of PDT without altering PGE2 levels. Our data demonstrates that DMC improved PDT by increasing growth inhibition and apoptosis without modulating COX-2 catalytic activity and suggests that targets such as survivin may be involved in coxib-mediated tumoral enhancement of PDT.
An outstanding problem in cancer therapy is the battle against treatment resistant metastatic disease. The vast majority of cancer-related deaths are not associated with primary tumors, but with the multitude of metastatic lesions that eventually become treatment resistant. Numerous physiological influences contribute to the rise of therapy-resistant cells, with perfusion, pH and hypoxia playing major roles in stymieing the delivery and efficacy of therapeutics. The lack of oxygen known as hypoxia, in particular, renders most chemotherapeutic agents ineffective, and substantially reduces the therapeutic index of the majority of PDT regimens. In our studies using an in vitro model of metastatic ovarian cancer, we have observed both limited uptake and significantly reduced therapeutic efficacy of carboplatin and BPD-PDT in the hypoxic and acidic cores of large (>250 micrometer) nodules. To overcome the problem of limited delivery, and impart cytotoxicity even in completely anoxic environments, we chose the cationic methylene blue derivative ETNBS to treat model ovarian cancer lesions. A primarily type-I photosensitizer, we found that ETNBS is rapidly and selectively taken up into the cores of the ovarian nodules, and is able to kill otherwise treatment-resistant cell populations. Impressively, ETNBS was able to retain its cytotoxic potential even under completely anoxic conditions. Imaging studies with time-lapse OCT (TL-OCT) have revealed an inside-out pattern of cell death, with apoptosis being the primary mechanism of cell death. ETNBS derivatives and their liposomal constructs were also administered, with the goal of understanding the effects of charge and delivery vehicle on uptake and cytotoxicity.

**7886-07, Session 2**

**Imaging growth and photodynamic therapy response in a 3D pancreatic co-culture model**

J. P. Celli, I. Rizvi, L. Z. Zheng, B. Q. Spring, A. O. Abu-Yousif, S. A. Elrington, Massachusetts General Hospital (United States); A. Blanden, Massachusetts General Hospital (United States) and Binghamton Univ. (United States); T. Hasan, Massachusetts General Hospital (United States)

Pancreatic ductal adenocarcinoma is a lethal disease that is often unresectable by the time of diagnosis and is typically non-responsive to chemo- and radiotherapy, resulting in a five year survival of only 3%. Tumors of the pancreas are characterized by a rigid fibrous stroma rich in ECM proteins, which, aside from posing a physical barrier to drug penetration, seems to also play an important yet poorly understood role in tumor growth and therapeutic response. We introduce a three-dimensional in vitro co-culture of pancreatic cancer cells and normal human fibroblasts in which the interaction of these two cell types leads to formation of millimeter sized multicellular tumor nodules with dense fibrotic stroma resembling the ECM-rich hypovascular nodules in human patients. This platform recapitulates physiological tumor-stroma interactions absent in traditional cell culture, and is yet conducive to longitudinal imaging and high-content quantitative interrogation of growth and therapeutic endpoints that would not be feasible in animal models. Inspired by promising clinical and preclinical results of photodynamic therapy (PDT) for pancreatic cancer we leverage this model system to examine cytotoxic and molecular response to PDT. We specifically evaluate the potential for PDT-based combination therapies targeting the secreted enzyme lysyl oxidase, which is involved in matrix remodeling and is associated with metastasis. This combination of biomedical optics for imaging and therapeutics with biologically-rich model systems creates a new approach to design and evaluate treatment strategies for this otherwise deadly disease.

**7886-08, Session 2**

**Photodynamic therapy mediated synergistic enhancement of chemo and biological therapies in a 3D model for micrometastatic ovarian cancer**

I. Rizvi, J. P. Celli, C. L. Evans, A. O. Abu-Yousif, A. Muzikansky, S. A. Elrington, Massachusetts General Hospital (United States); B. W. Pogue, Dartmouth College (United States); D. M. Finkelstein, T. Hasan, Massachusetts General Hospital (United States)

The development and translational potential of therapeutic strategies for cancer is limited, in part, by a lack of biological models that capture critical determinants of tumor growth and treatment response. It is also becoming increasingly evident that no single treatment will be curative for this complex disease. Rationally-designed combination regimens that impact multiple targets provide the best hope of significantly improving clinical outcomes for cancer patients. Rapidly identifying treatments that cooperatively enhance treatment efficacy from the vast library of candidate interventions is not feasible, however, with current systems. There is a vital, unmet need to create cell-based research platforms that more accurately mimic the complex biology of human tumors than monolayer cultures, while providing the ability to screen therapeutic combinations more rapidly than animal models. We have developed an in vitro 3D tumor model for micrometastatic ovarian cancer, which in conjunction with quantitative image analysis routines to batch-process large datasets, serves as a high throughput reporter to screen rationally-designed combination regimens. We use this system to assess mechanism-based combination regimens with photodynamic therapy (PDT), which sensitizes ovarian cancer cells to chemo and biological agents, and has shown promise in clinic trials for the treatment of ovarian carcinomatosis. We show for the first time that PDT-synergistically enhances the efficacy of low-dose carboplatin in a sequence-dependent manner and demonstrate a passage-dependent synergistic response with PDT and Erbitux®. The principles described here could inform the design and evaluation of mechanism-based therapeutic options for a broad spectrum of metastatic solid tumors.

**7886-09, Session 2**

**Quantitatively determining binding of targeted agents in vivo by imaging dual-probe (targeted and nontargeted) injection can improve efficacy of therapeutic agent delivery**

K. S. Samkoe, S. K. Hextrum, H. H. Yang, K. J. Sexton, S. Srinivasan, J. A. O’Hara, Dartmouth College (United States); T. Hasan, Wellman Ctr. for Photomedicine (United States); B. W. Pogue, Dartmouth College (United States)

Molecular expression imaging was analyzed with a dual-probe injection using a non-targeted probe, and one targeted to epidermal growth factor receptor (EGFR), combined with pharmacokinetic analysis of the imaging data. Targeted agents to probe tumor expression often lead to higher uptake as compared to normal tissues; however, inherent transport properties such as enhanced vascular permeability and increased retention from non-functional lymphatics also contribute. Quantification of targeted binding could assess true image contrast as well as efficacy in therapeutic agent delivery. A pharmacokinetic analysis for the dual-probe injection was developed and tested using two tumor lines that overexpress EGFR: an AsPC-1 orthotopic pancreatic cancer model that has limited drug uptake due to high stromal content, and a subcutaneous U251 glioma model that has a much lower stromal content. IRDye800CW (LI-COR Biosciences) conjugated to EGF and the IRDye700DX-carboxylate were used as the targeted and non-targeted probes, respectively. The probes were injected simultaneously and their distribution monitored on a fluorescent flatbed scanner. It was found...
that the U251 tumor showed higher binding rates (5.0x10^4 s^-1) than the AsPC-1 tumor (4.2x10^4 s^-1) as expected; however, the normal pancreas demonstrated much higher binding rates of 8.1x10^4 s^-1 than both tumor types, even though the tumors are thought to be increased in expression. These binding rates have been confirmed ex vivo and this demonstrates the ability to measure and monitor therapeutic uptake within a tumor. This technique has important indications for molecular targeting including combined-PDT treatment, where understanding molecular response may be critical to applying suitable adjuvant molecular therapies.

7886-10, Session 2

Combination of PI3K inhibitors with photodynamic therapy in endothelial and prostate cancer cell lines

B. A. Fateye, B. Chen, Univ. of the Sciences in Philadelphia (United States)

Photodynamic therapy (PDT) is often used in combination with other cancer treatment modalities such as surgery and chemotherapy for better treatment outcome. Molecule-targeted agents are an emerging class of anticancer agent that is showing great promise in cancer treatment. We report here the interaction between PDT and small molecule kinase inhibitors targeting the PI3K/Akt/mTOR pathway that is dysregulated in a majority of human cancers. We found that PDT was more effective in inducing cell death in the SVEC endothelial cells as compared to both PC-3 and DU145 prostate tumor cells. SVEC cells also had a better response to two pan-PI3K/mTOR inhibitors (LY294002 and BEZ235) than two tumor cell lines. Interestingly, the combination of pan-PI3K/ mTOR kinase inhibitors with PDT treatment induced a more than additive cell death in the SVEC endothelial cells, but not in either PC-3 or DU145 prostate cancer cell lines. Our results demonstrate that SVEC endothelial cells are more responsive to both PDT and PI3K/mTOR inhibitor treatments than PC-3 and DU145 cells and the combination of PDT and PI3K/mTOR inhibitors induces more endothelial cell death. The greater enhancement of efficacy of PDT in endothelial cells compared with tumor cells by combination with PI3K inhibition further indicates the advantage of targeting tumor vasculature.

7886-11, Session 2

Signaling from lysosomes to mitochondria sensitizes head and neck cancer cells to photodynamic treatment: role of Mitoferrin 2

H. Hung, G. Quilogue, J. Lemasters, A. Nieminen, Medical Univ. of South Carolina (United States)

Previously, we showed that photosensitizers that localize to lysosomes are more effective in killing cancer cells than ones directed to mitochondria after photodynamic treatment (PDT). Here, we investigated the interactions between lysosomes and mitochondria in promoting PDT cell killing efficiency. Three head and neck cancer cell lines (UMSCC1, UMSCC14 and UMSCC22) and the epidermoid carcinoma A431 cell line were exposed to Pc 4 (mitochondria-targeted)-PDT. The 4 cell lines responded differently: UMSCC1 and UMSCC14 head and neck cancer cells were more resistant, whereas UMSCC22 and A431 cells were more sensitive to Pc 4-PDT. In non-erythroid cells, mitoferrin 2 is an iron transporter in the mitochondrial inner membrane. PDT-sensitive cells expressed higher mitoferrin 2 protein levels compared to the PDT-resistant cell lines. These results suggest that increased mitochondrial iron due to increased mitoferrin 2 may explain greater sensitivity of UMSCC22 and A431 cells to PDT. A major source of chelatable iron that can be mobilized to translocate into mitochondria is the lysosomal/ endosomal compartment, which receives iron by receptor-mediated endocytosis of transferrin, and increased iron in the culture medium increased PDT cell killing. Bafilomycin inhibits the acidic vacuolar proton pump to collapse the lysosomal/endosomal pH gradient compartments, thereby inducing lysosomal iron release into the cytosol. Bafilomycin enhanced cell killing of both resistant and sensitive cells. Taken together, the data suggest that mitoferrin 2, lysosomal iron release and mitochondrial iron uptake act synergistically to induce PDT-induced cell killing. Furthermore, mitoferrin 2 represents a possible biomarker, and cancers expressing higher mitoferrin 2 protein levels may benefit more from PDT.

7886-12, Session 3

Folate receptor targeted Type-1 photosensitizer bioconjugates for tumor visualization and phototherapy


In our continuing efforts toward the development of targeted Type 1 phototherapeutic agents, an azide-based Type 1 photosensitizer (with peak absorption at 425 nm), a fluorophore (with peak absorption and emission between 500 and 620 nm respectively), and a dual diagnostic-therapeutic probe consisting of the fluorophore and the photosensitizer units were prepared and independently conjugated to two vectors that target folate receptors. The vectors targeting folate receptors are folic acid and FOLR1 affinity purified polyclonal antibody. Folate receptors are over expressed in many types of cancers, including ovarian, breast, and cervical. In vivo binding and blood flow studies with both ovarian cancer cells were conducted to determine selective uptake of these conjugates. In vivo studies using the nude mouse xenograft model were conducted to determine selective uptake and tumor destruction, and to evaluate the differences between the bioconjugates containing a single hapten (targeted therapy only) and the one containing a dual probe (targeted diagnostic-therapeutic pair).

7886-13, Session 3

Non-contact monitoring of blood flow dynamics associated with BPD photodynamic therapy in vivo using diffuse correlation spectroscopy

Y. Ti, Univ. of Pennsylvania (United States); A. L. Maas, E. Glatstein, The Univ. of Pennsylvania Health System (United States); A. G. Yodh, Univ. of Pennsylvania (United States); T. M. Busch, The Univ. of Pennsylvania Health System (United States)

Within seconds of beginning illumination photodynamic therapy (PDT) can increase or decrease tumor blood flow in a protocol-dependent manner that is predictive of treatment efficacy. We are examining how characteristic patterns in vascular dynamics during PDT are related to other pathophysiological changes through comparative studies in tumors treated with two different benzoporphyrin derivative (BPD)-PDT protocols. BPD-PDT is generally applied with a short interval between photosensitizer administration and light delivery to create a vascular response or with a longer interval to lead to more direct tumor cytotoxicity. In this study two groups of nude mice bearing H460 human tumor xenografts received intravenous BPD doses of 0.25 mg/kg and 1 mg/kg with drug-light intervals of 15 minutes and 3 hours, respectively. Tumor was exposed to 135 J/cm2 at 890nm. Diffuse correlation spectroscopy (DCS) was used to continuously monitor tumor blood flow dynamics during PDT. Relative blood flow (rBF) was calculated by normalization to pre-PDT flow values and reported over the 30 minutes of PDT, as well as for 10 minutes at 3 hours after PDT. Significant blood flow decrease was observed after PDT in mice treated with both protocols. However, short and long drug-light intervals led to unique patterns of blood flow change during PDT. In tumors treated with the long drug-light interval PDT created an initial, partially reversible decrease in rBF, while, surprisingly, no acute rBF reduction was apparent in tumors treated with a short drug-light interval. Studies comparing the consequences of
blood flow dynamics on tumor oxygenation and outcome measures are in progress.

7886-14, Session 3
A dynamic model for ALA-PDT of skin: analysis of the correlation of fluorescence and singlet oxygen luminescence to spatial distribution of singlet oxygen

B. Liu, McMaster Univ. (Canada); M. S. Patterson, T. J. Farrell, Juravinski Cancer Ctr. (Canada)

Both Singlet Oxygen (1O2) Luminescence Dosimetry (SOLD) and photosensitizer fluorescence photobleaching are being investigated and applied as dosimetric tools during ALA induced PpIX PDT of skin diseases. However, the correlation of both SOLD data and PpIX fluorescence to 1O2 distribution are difficult to interpret because of the temporal and spatial variations of light fluence rate, photosensitizer concentration and oxygen concentration. A model for ALA-PDT of normal human skin was developed to investigate the dynamic behavior of the essential parameters of PDT and the interpretation of detected signals. The model incorporates Monte Carlo simulations of excitation light fluence and both SOLD and PpIX fluorescence signals. 1O2-mediated photobleaching mechanism, ground-state oxygen (O2) diffusion and perfusion, a cumulative 1O2-dependent threshold vascular response and any initial distribution of PpIX. The simulated time-resolved evolution of the instantaneous PpIX fluorescence and cumulative SOLD signals are examined as functions of irradiance and related to the time-resolved distribution of cumulative 1O2 production at various depths. These correlations provide insight into the PDT ‘dose’ distribution and the potential of SOLD and photobleaching as dosimetric tools.

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7886-15, Session 3
Photosensitizer nanocarriers modeling for photodynamic therapy applied to dermatological diseases

I. Salas-García, F. Fanjul-Vélez, N. Ortega-Quijano, Univ. de Cantabria (Spain); M. López-Escobar, Univ. Hospital Marques de Valdecilla (Spain); J. L. Arce-Diego, Univ. de Cantabria (Spain)

Photodynamic Therapy (PDT) involves the therapeutic use of photosensitizers in combination with visible light. The subsequent photochemical reactions generate reactive oxygen species which are considered the principal cytotoxic agents to induce cell death. This technique has become widely used in medicine to treat tumors and other nonmalignant diseases. However, there are several factors related to illumination or the photosensitizer that limit an optimal treatment outcome. The use of nanoparticles (NPs) for PDT has been proposed as a solution to current shortcomings. In this way, there are NPs that act as carriers for photosensitizers, NPs that absorb the light and transfer the energy to the photosensitizer and NPs that are themselves photodynamically active.

In dermatology, the use of topical photosensitizers produces a time dependent inhomogeneous distribution within the tumor, where the stratum corneum is the main barrier to the diffusion of the photosensitizer to the deeper layers of skin. This produces an insufficient photosensitizer accumulation in tumor tissues and therefore, a low therapeutic efficiency in the cases of deep lesions.

This work focuses in the use of NPs as photosensitizer carriers to improve the actual topical drug distribution in malignant skin tissues. We present a mathematical model of PS distribution in tumor tissue using NPs that takes into account parameters related to nanoparticles binding. Once the concentration profile of NPs into tissue is obtained, we use a photochemical model which allows us to calculate the temporal evolution of reactive oxygen species according to PS distribution calculated previously from NPs profile.

7886-16, Session 4
Photodynamic therapy and the treatment of head and neck malignancies

M. A. Biel, Univ. of Minnesota, Twin Cities (United States)

Photodynamic therapy has been successfully used to treat various cancers of the head and neck. Four hundred sixty nine patients with neoplastic diseases of the larynx, oral cavity and pharynx have been treated with PDT with follow-up to 250 months. Those patients with primary or recurrent carcinoma in situ and T1 carcinomas obtained a complete response after one PDT treatment and 88% remain free of disease. Patients with T2 and T3 carcinomas treated with PDT obtained a complete response but in most cases they recurred locally, many with normal overlying mucosa. This is due to the inability to adequately deliver laser light to the depths of the tumor despite aggressive use of interstitial implantation. Intraoperative adjuvant PDT was used in 19 patients with recurrent head and neck cancers and only two developed local recurrences.

PDT is effective for the curative treatment of early carcinomas of the head and neck. It may also be of benefit as an adjuvant intraoperative treatment of large recurrent tumors.

7886-17, Session 4
Biomarkers in phototherapy of esophageal neoplasia

K. K. Wang, Mayo Clinic (United States)

Endoscopic ablation therapy for Barrett’s esophagus and early cancer has become accepted as a clinical alternative to esophagectomy. Nonetheless, there is an approximately 10 percent failure rate of endoscopic ablative therapies such as photodynamic therapy. The failure of these treatments results in unnecessary morbidity from therapy, risk of complications, as well as potential progression of disease. We initially investigated these failures retrospectively in 126 patients and found that of the biomarkers predicting treatment failure, p16 inactivation was significantly associated with non-response. This was with treatment with photodynamic therapy with 200 joules of energy at a wavelength of 630 nanometers. The odds ratio was 0.32 with a confidence interval of 0.1-0.96. Sodium porpimer was administered at a dose at 2 mg per kg intravenously 48 hours prior to photoradiation. This was found to occur regardless of the mechanism of inactivation, either through promoter methylation or loss of heterozygosity. We further established this prospectively in a randomized trial of 90 patients. We have now extended these observations to those patients with early invasive carcinoma in which p16 copy abnormalities were found in 45 percent of early cancers. This was associated with lack of response to PDT while overall evidence of genomic instability was not associated with failure of therapy.

7886-18, Session 4
Photodynamic therapy of pancreatic cancer and elastic scattering spectroscopy of the duodenal mucosa for the detection of pancreaticobiliary malignancy

The diagnosis and treatment of pancreaticobiliary malignancy is of major interest to our group. Building on prior work (Bown et al, Gut 2002; 50: 549-57), we undertook a phase I study of verteporfin photodynamic therapy in patients with locally advanced, unresectable, pancreatic cancer. We also initiated an optical diagnostic study using elastic scattering spectroscopy (ESS) of the normal-appearing peripancreatic duodenal mucosa in vivo to investigate the hypothesis of a field effect in pancreaticobiliary malignancy. In a phase I dose escalation study, patients were treated with intraluminal verteporfin PDT via a single fibre, to determine its general safety profile and the optimum treatment parameters needed to achieve effective and safe necrosis of tumor. With increasing light doses, there was a linear increase in the extent of tumour necrosis around the fibre, without serious adverse events. Follow-up studies using multiple fibres are planned. In 30 patients with benign or malignant pancreaticobiliary disease undergoing clinically-indicated endoscopy, ESS spectra were collected from the normal-appearing duodenal antrum and a diagnostic algorithm generated by principle component and linear discriminant analysis. Pooled data from duodenal sites distal to the ampulla gave a sensitivity of 86% and a specificity of 72% (82% AUC) for the detection of malignancy, whereas those from the peripancreatic region had a sensitivity of 77% and a specificity of 61% (72% AUC); antral measurements were not able to discriminate with such accuracy. These early results suggest that ESS of the duodenal mucosa could represent a novel minimally invasive diagnostic test for pancreaticobiliary malignancy.

5-Fluorouracil as an enhancer of aminolevulinate-based photodynamic therapy in skin cancer models in vivo: new use for a venerable agent

E. V. Maytin, S. Anand, C. Wilson, The Cleveland Clinic (United States)

5-Fluorouracil (5-FU) was developed in the 1950s as an anticancer drug and is now widely used to treat many cancers, including colon and breast carcinoma. 5-FU causes fluoronucleotide misincorporation into RNA and DNA, inhibits thymidylate synthase, and leads to growth arrest and apoptosis. For skin precancers (actinic keratoses; AK), 5-FU is prescribed as a topical agent and was essentially the only option for treating widespread AK of the skin prior to FDA approval of photodynamic therapy (PDT) in 1999. PDT is now gradually replacing 5-FU as a preferred treatment for AK, but neither PDT nor 5-FU are effective for true skin cancers (basal or squamous cell), particularly for tumors >1 mm in depth. In our ongoing work to improve the efficacy of PDT for skin cancer, we previously showed that PDT efficacy can be significantly enhanced by preconditioning tumors with methotrexate (MTX), which leads to increased production of protoporphyrin IX (PpIX) in target cells. However, because MTX must be given orally or intravenously, it is considered unacceptable for widespread human use due to potential toxicity. MTX and 5-FU exert similar effects on the thymidylate synthesis pathway, so we reasoned that topical 5-FU could be a potential alternative to MTX. In this paper, exploratory studies that test 5-FU as a preconditioning agent for PDT are presented. In two different models of cutaneous squamous cell carcinoma (one superficial and one deep), 5-FU significantly enhances PpIX accumulation, and therefore emerges as a new candidate agent for combination therapy with PDT.

Investigating the mechanism of action of targeted gallium corrole for breast cancer photodynamic therapy using multimode optical imaging

J. Hwang, J. Lubow, D. Chu, Cedars-Sinai Medical Ctr. (United States); Z. Gross, Technion-Israel Institute of Technology (Israel); H. B. Gray, California Institute of Technology (United States); D. L. Farkas, Spectral Molecular Imaging, Inc. (United States); L. K. Medina-Kauwe, Cedars-Sinai Medical Ctr. (United States)

We recently developed a novel therapeutic particle, HerGa, for breast cancer treatment and detection. HerGa consists of a tumor-targeted cell penetration protein noncovalently assembled with a gallium-metalated corrole. The corrole is structurally similar to porphyrin, emits intense fluorescence, and has been proven highly effective for breast tumor treatment preclinically without light exposure. Here, we have explored the possibility of testing HerGa as a photosensitizer for photodynamic therapy and have investigated its mechanism of action using multimode optical imaging. Using two-photon excited confocal fluorescence imaging, we have observed that HerGa disrupts the mitochondrial membrane potential in situ, which is substantially augmented by light exposure. In addition, spectral and fluorescence lifetime imaging were utilized to both validate the mitochondrial membrane potential disruption and investigate HerGa internalization, allowing us to optimize the timing for light dosimetry. Finally, we examined whether photoexcitation of HerGa can produce singlet oxygen, which in turn can cause oxidative cell damage. We observe here using advanced multimode optical imaging that light at a specific wavelength promotes HerGa cytotoxicity through singlet oxygen production, which is likely to cause disruption of mitochondrial function. Thus, we can identify for the first time the capacity of HerGa as a photosensitizer for photodynamic therapy and reveal its mechanism of action in the therapeutic intervention of human breast cancer.
Determining how uncertainties of optical properties affect light dose calculations for PDT

J. Sandell, J. C. Finlay, T. C. Zhu, The Univ. of Pennsylvania Health System (United States)

The effectiveness of treatment depends on several factors including an accurate knowledge of optical properties of the tissue to be treated. In order to correctly determine the needed light dose, the values of tissue optical properties must be well known and understood. In this study we consider how the uncertainties in the measured values of optical properties affect the uncertainty in light dosimetry. By using phantoms of known optical properties we determine the uncertainties for several algorithms currently in the literature. We calculate the light fluence uncertainties using a finite element model solution to the diffusion equation in a cavity geometry. Using these uncertainties we conduct a statistical analysis, building a three dimensional space of uncertainties in $\mu_a$, $\mu_s'$, and light dosage. By being able to characterize this space we gain an understanding of the trend in uncertainties and determine which algorithms provide the most accurate light dose.

Modeling of PDT kinetics in cell killing

I. Gkigkitzis, East Carolina Univ. (United States); C. Yang, Y. Feng, Tianjin Univ. (China); J. Q. Lu, X. Hu, East Carolina Univ. (United States)

Photodynamic therapy (PDT) provides an effective option for treatment of tumors and other diseases. A type II PDT involves multiple molecular species in its cell killing process and oxygen plays a critical role. Clear understanding of molecular pathways and particularly the kinetics of photosensitizers, oxygen and receptors in cells is fundamentally important to interpret experimental results of PDT. Previously we have established a numerical model with a group of six rate equations to numerically investigate the molecular kinetics involved in PDT by obtaining the concentration evolution of molecules in both ground and excited states in time domain. The existing model, however, does not account for the possible diffusion of oxygen. In this work, we extended the model with oxygen diffusion in a spherical cell. Different oxygen diffusion mechanisms through cell membrane and inside the cell have been investigated within the context of PDT. We found that the widely accepted cell model of oxygen diffusion using the Michaelis-Menten term can be significantly improved by the extended model. Furthermore, this model allows quantitative study of PDT on various rate and diffusion parameters in details and thus furnishes a powerful platform for modeling cell killing through PDT. We have investigated the self-consistency and the range of parameters of the rate equations and the dependence of photobleaching and triggering of the cell killing on initial photosensitizer concentration and incident light fluence. These results will be presented and their implication on PDT study in cultured cells will be discussed.

A method for fluorescence-guided surgery providing an estimate of depth in multispectral near-infrared subsurface imaging

F. Leblond, P. A. Valdes, Dartmouth College (United States); A. Kim, Ontario Cancer Institute (Canada); S. C. Davis, Z. Ovanesyan, V. Krishnaswamy, Dartmouth College (United States); B. C. Wilson, Ontario Cancer Institute (Canada); A. Hartov, B. W. Pogue, K. D. Paulsen, Dartmouth College (United States); D. W. Roberts, Dartmouth Hitchcock Medical Ctr. (United States)

Fluorescence-guided surgery for intracranial tumor resection relies on optical contrast from the endogenous molecule, protoporphyrin IX (PpIX). Exogenous administration of the precursor delta-aminolevulinic acid (ALA) overloads the heme cellular pathway and leads to selective accumulation of PpIX in neoplastic tissues. The work that is presented consists in the development of a fluorescence-ratio detection method that can be readily applied to localize sub-surface pathologies during wide-field intra-surgical fluorescence imaging, facilitating more complete tumor resections guided by ALA-induced PpIX fluorescence. An analytical derivation is presented providing a closed form mathematical expression that can be used to estimate the depth of fluorescent molecules based on the spectral deformation of diffused near-infrared measurements and prior tissue optical properties characterization. Experimental data acquired for tissue-simulating phantoms with a broad-beam non-contact multi-spectral imaging system are presented. The results provide a feasibility analysis showing that depth correlates with the ratio of fluorescence at two different wavelengths in accordance with the analytic expression derived using diffusion theory. The method has been used intra-operatively for in vivo data acquisition during brain tumor resection procedures. Results are presented along with an assessment of the method in terms of its potential to improve the extent of malignant tissue that is removed during surgery. Although the application described is for surgery, the proposed approach can also be used in other pre-clinical and clinical applications where luminescence contrast depth assessment is required in a multi-pixel sub-surface imaging geometry.
The effects of photodynamic therapy on lung cancer were monitored continuously during HPPH mediated pleural PDT following lung cancer removal. Blood flow and oxy-, deoxy-hemoglobin concentrations in lung cavity tissues were measured using Diffuse Correlation Spectroscopy (DCS) and Diffuse Optical Spectroscopy (DOS), respectively. A probe that combines DCS and DOS was sewn onto the diaphragm muscles in 5 patients. The chest cavity was filled with Intralipid during the PDT, and this Intralipid was replaced occasionally if the signal absorption increased as blood leaked into the Intralipid. A surgeon delivered light to the cavity using a light wand at each corner of chest cavity and near the organs exposed in the cavity. Substantial inter-patient variations were found in blood flow and hemoglobin changes. However, an overall decrease in oxy-hemoglobin and increase in deoxy-hemoglobin was observed in 4 of the patients. Furthermore, significant differences in signal fluctuations were observed in a patient who experienced serious illness after the PDT. Longitudinal changes in the optically measured physiological parameters (blood flow, oxy-, deoxy-, total-hemoglobin concentrations) of each subject will be presented during the session.

Assessment of biophysical tumor response to PDT in pancreatic cancer using localized reflectance spectroscopy

M. E. Isabelle, W. S. Klubben, V. Krishnaswamy, Dartmouth College (United States); J. A. O’Hara, P. J. Hoopes, Dartmouth Hitchcock Medical Ctr. (United States); S. P. Pereira, Univ. College London (United Kingdom); T. Hasan, Massachusetts General Hospital (United States); B. W. Pogue, Dartmouth College (United States)

Biophysical changes such as inflammation and necrosis occur immediately following PDT and may be used to assess the treatment response to PDT treatment in-vivo. This study uses localized reflectance measurements to quantify the scatter changes in tumor tissue occurring in response to verteporfin-based PDT treatment in xenograft pancreas tumors. Nude mice were implanted with subcutaneous AsPC-1 pancreatic tumors in matrigel, and allowed to establish solid tumors near 100 mm3 volume. The mice were sensitized with 1mg/kg of the active component of verteporfin (benzoporphyrin derivative, BPD), one hour before light delivery. The optical irradiation was performed using a 1 cm cylindrical interstitial diffusing tip fiber with 20J of red light (690nm). Tumor tissue was excised and imaged, from two days to seven weeks, after PDT treatment. The tissue sections were stained and analyzed by a veterinary pathologist, who provided information on tissue regions of interest. This information was correlated with scattering differences in the images and the degree of necrosis and inflammation involvement were identified in the scatter images. The results from these studies help demonstrate the effectiveness of Reflectance Spectroscopy to evaluate PDT treatment response and to further understand changes that are occurring in the tissue ultra-structure, and enable more effective treatment planning. These results are relevant to the verteporfin-based PDT trial for treatment pancreatic cancer in non-surgical candidate cases (VERTPAC-1 University College London, PI Pereira), where individualized assessment of damage and response could be beneficial, if this study is proven to be a well-controlled imaging tool.

In-vitro photodynamic therapy of MG-63 osteosarcoma cells mediated by aminolevulinic acid

V. M. Rossi, Pacific Univ. (United States) and Oregon State Univ. (United States); B. M. White, M. J. Newton, Pacific Univ. (United States); S. L. Jacques, Oregon Health & Science Univ. (United States); P. J. Baugher, Pacific Univ. (United States)

This is an in vitro study of photodynamic therapy (PDT) in the MG-63 line of human osteosarcoma cells, as mediated by aminolevulinic acid (ALA). The primary goal of this work is to determine the feasibility and effectiveness of treating osteosarcoma through PDT. In addition, this work is aimed at determining whether the resuming cell death occurs through apoptosis or cellular necrosis. The MG-63 cells are treated with increasing concentrations of ALA from 0.1-10 mM ALA, leading to the accumulation of the photosensitizer protoporphyrin IX (PpIX) within the cells. After incubation periods of 4 and 24 hours in ALA, the cells are illuminated by 0-10 J/cm2 of 636 nm light in order to activate the PpIX and induce oxidative damage to the cells. Light is administered by an 8x12 array of LED’s, which are controlled by an Arduino Duemilanove microcontroller board in order to assure ease of use along with accurate levels of exposure. Controls for this experiment include 0 J/cm2 of light exposure for all experimental concentrations of ALA, as well as illuminating cells that have not been incubated in ALA at all experimental levels of illumination. MG-63 cells are analyzed through fluorimetry, MTT and crystal violet assays, and optical scatter imaging in order to determine the means and effectiveness of ALA mediated PDT of osteosarcoma.

Study of photosensitizers pharmacokinetics in mouse tumor model by transillumination fluorescence imaging in vivo

M. V. Shirmanova, I. V. Balalaeva, M. A. Sirotkina, N.I. Lobachevsky State Univ. of Nizhni Novgorod (Russian Federation); A. G. Orlova, I. V. Turchin, Institute of Applied Physics (Russian Federation); E. V. Zagaynova, Nizhny Novgorod State Medical Academy (Russian Federation)

Fluorescence diagnostics and photodynamic therapy with photosensitizers have shown the effectiveness against superficial tumors of different localization. Currently, in preclinical studies of new photosensitizers ex vivo methods are commonly used which are labor and time consuming and require a lot of animals. This work demonstrates the possibility of in vivo investigation of pharmacokinetics of photosensitizers in tumor by means of fluorescence transillumination imaging. The experiments were performed on transplantable mouse cervical carcinoma using three drugs: photosens, alasens and fotoditazin. Fluorescence images of animals were received in vivo by means of the setup developed at the Institute of Applied Physics (Russia). Animals were scanned in transillumination configuration by a single source (semiconductor laser) and detector (FMP) pair. For quantitative assessment of the photosensitizer concentration in tumor tissue the fluorescence signal was calibrated using tissue phantoms. Based on fluorescence imaging, we evaluated in vivo the main pharmacokinetics parameters: maximum tumor-uptake, half-life in tumor, clearance. The results on kinetics of photosensitizers in tumor obtained by transillumination imaging in vivo agreed with data of standard methods ex vivo - fluorescence spectroscopy and confocal microscopy. The described approach may become indispensable for rapid and cost-effective study of new photosensitizers in small animals.
Conference 7886: Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XX

7886-31, Poster Session

**NADH fluorescence lifetime increase corresponded to photodynamic therapy induced cell death**

G. Su, Y. Wei, H. Wang, National Yang-Ming Univ. (Taiwan)

Photodynamic therapy (PDT) has been applied on cancer therapy. The combination of photosensitizers with their activating light causes cells into the pathway of the apoptotic and/or necrotic cell death. Noninvasive detection of cell death and/or differentiation of cell death pathways is important for the optimization of a range of cancer therapies including PDT. In our recent research, we used two-photon fluorescent lifetime imaging microscopy (FLIM) to detect the change of the cellular NADH fluorescence lifetime in the staurousporine-induced apoptosis but not in the high dose H2O2-induced necrosis. In this study, we investigated the NADH fluorescence lifetime change due to ALA-PDT induced cell death in the H1299 cell. A fixed drug dose (1 mM) and incubation time (4 hrs) were used. Light dose was varied at 1, 2, and 6 J/cm2 with a fixed fluence rate of 10 mW/cm2. Cell viability showed the dependency on the light fluence and post treatment time. Under these treatment doses, we observed the DNA fragmentation with respect to the induction of apoptosis, as revealed by flow cytometric analysis with less than the G1 content of DNA. The light dose 2 J/cm2 showed increased sub-G1 contents. We then employed FLIM to measure the NADH lifetime and observed the cellular morphology after the PDT treatment. The NADH lifetime of cell treated with the light dose 2 J/cm2 increased within 2 hours, but the dose 1 J and 6 J/cm2 showed no change. The cell morphology of cell treated with light dose 1 J and 2 J/cm2 became more round than controls and the dose 6 J/cm2. These findings suggest that the change of the average NADH lifetime and morphology may be a valuable indicator to detect PDT-induced cell death.

7886-34, Poster Session

**Effect of fatty acids on the complexion of proteins with porphyrins**

G. V. Gyulkhandanyan, Institute of Biochemistry (Armenia)

Porphyrins binding and transport to tumor is the one of the central tasks of photodynamic therapy of tumor (PDT). The main carriers of porphyrins (photosensitizers) in the blood are lipoproteins, serum albumin and hemoglobin. In studying the phenomenon of complexion of proteins with ligands must take into considering the real conditions that exist in the organism and, in particular, take into considering the presence of fatty acids in blood. Up to date the role of fatty acids (palmitic and stearic) in the binding of porphyrins with proteins not been determined. A key step in solving of these problems is to determine the binding constants of porphyrin-protein pairs and effect of fatty acids on this process. The most direct and sufficiently accurate methods of solving such problems are complementary methods of absorption and fluorescence spectroscopy. The results of spectral studies on the binding of porphyrins to serum albumin and hemoglobin in the presence of fatty acids demonstrated a significant decrease in the degree of binding pair porphyrin-albumin and porphyrin-hemoglobin with increasing concentrations of fatty acids in solution. The results lead to the conclusion that for hemoglobin the presence in a solution of fatty acids on binding to the porphyrins affected more significantly than for serum albumin. Thus, in natural conditions, when in the blood presented fatty acids the preference between hemoglobin and serum albumin in the binding and in the transport of porphyrins should be given to serum albumin.

7886-35, Poster Session

**Preparation, characterization, and cellular studies of photosensitizer-loaded lipid nanoparticles for photodynamic therapy**

F. P. Navarro, Commissariat à l’Énergie Atomique (France); D. Bechet, Univ. Henri Poincaré Nancy (France); T. Delmas, Commissariat à l’Énergie Atomique (France); C. Frochot, P. Couleaud, R. Vanderesse, Univ. Henri Poincaré Nancy (France); I. F. Texier-Nogues, A. C. Couffin, F. Vinet, Commissariat à l’Énergie Atomique (France); M. Barberi-Heyob, Univ. Henri Poincaré Nancy (France)

PhotoDynamic Therapy (PDT) has been established as a potent and less invasive treatment for different kinds of cancer. Among various attempts to enhance the therapeutics efficacy of PDT, the specific delivery of the PhotoSensitizer (PS) in the tumor is expected to increase its clinical applications, since unwanted accumulation, especially in the skin, impairs the patients’ quality of life (prolonged cutaneous photosensitivity). The aim of this study was to engineer Lipid Nanoparticles (LNP) with different sizes and various PS contents, using simple, solvent-free and easily scale up manufacturing processes. Meso-tetra (hydroxyphenyl) chlorin (mTHPC) is one of the most potent photodynamically active substances in clinical use and it has been successfully applied in the treatment of various indications, such as the head and neck, prostate, pancreatic cancers. Here, a derivative of mTHPC was efficiently incorporated into the lipid core of LNP leading to a large range of stable and reproducible mTHPC-loaded LNP with narrow size distribution. The photophysical and photochemical properties of mTHPC-loaded LNP were studied by measuring absorbance and fluorescence spectra, colloidal stability, particle size and zeta potential, as well as singlet oxygen luminescence. The photocytotoxicity of three selected mTHPC-loaded LNP (25 nm, 45 nm and 95 nm of diameter respectively) was evaluated on MCF-7 cells in comparison to free mTHPC under irradiation at 652 nm with adjusted times to reach a range of light fluence (from 1 to 5 J/cm2). All the physical-chemical, photophysical and biological measurements performed allow us to conclude that LNP is a promising nano-drug delivery system for PDT.

7886-32, Poster Session

**Differences in uptake pattern and PDT efficacy of EtNBS derivatives and liposomal delivery vehicles in an in-vitro 3D ovarian cancer model**

Y. J. Park, Korea Advanced Institute of Science and Technology (Korea, Republic of); C. L. Evans, Wellman Ctr. for Photomedicine (United States)

The effective treatment of metastatic cancer continues to be a challenge due the highly invasive nature of metastatic lesions and their propensity to develop therapeutic resistance. Disseminated peritoneal carcinomas are particularly difficult to treat due to their widespread nature, various implantations sites, and their range of size distributions from micronodules to large scale occult lesions. Optimal therapeutic strategies for peritoneal metastases should have both rapid uptake and penetrate throughout cancerous lesions. Using in vitro models of ovarian cancer, we have found that the cores of tumor nodules are both hypoxic and acidic, rendering most chemotherapeutic agents ineffective, and considerably reducing the therapeutic efficacy of the majority of photodynamic therapy (PDT) regimens. PDT using EtNBS, a cationic photosensitizer, is a promising approach to treating these nodules as it rapidly accumulates into the typically resistant nodule cores, and is effective even in completely anoxic environments. The purpose of this study was to investigate the effects of structural alterations of EtNBS as well as different delivery modalities on the uptake and treatment response of model in vitro ovarian cancer nodules. We used two derivatives, EtNBS-OH and EtNBS-COOH, and found that they have different uptake and spatial localization pattern than EtNBS. In addition, liposomal constructs encapsulating EtNBS and its derivatives were constructed. These liposomal regimens exhibited evenly distributed spatial patterns which were different from the localization patterns of EtNBS and its derivatives. In the future, we will expand our work to in vivo models to ultimately gain further insight for application to the clinic.
Development of an optical fluorescence imaging system for photodynamic therapy

M. M. Costa, C. Kurachi, V. S. Bagnato, L. Ventura, Univ. de São Paulo (Brazil)

Optical fluorescence has been applied in several medical areas, such as oncological diagnosis and detection of dental caries, due to its high sensitivity, simplicity and fast response. Non-invasive and non-destructive assessment of tissues using this technique is very attractive for clinical diagnosis. However, a simple and compact fluorescence image system is needed for clinical application that can be applied in several medical areas. Thus, this study describes the development of a wide-field fluorescence imaging device for visualization of photosensitizer in Photodynamic Therapy. This device consists of a power supply control unit for individual control of ultraviolet-blue light sources and a handheld unit that uses a compact light source, composed of two high-intensity light-emitting diodes (LEDs) and a LED Concentrator Lens. The handheld unit has five optical filters for fluorescence images excitation and emission. Images were acquired using a digital camera equipped with a 74-mm macro lens coupled to the device. For satisfactory fluorescence signal a reasonable optical power is necessary - in this case, 60 mW/cm². Another factor is the excitation range; for this system, a very efficient illumination band was achieved between 390-460 nm. The optical filters allowed a high fluorescence signal with satisfactory contrast for fluorescence images visualization. The detection system for fluorescence imaging was effective, allowing one to obtain efficient fluorescence images with excellent quality and good contrast. The developed handheld system showed a good clinical performance.

In-vivo validation of high-frequency ultrasound-guided fluorescence tomography system to improve delivery of photodynamic therapy

A. Palival, The Cleveland Clinic (United States); S. Torosean, J. D. Gruber, J. A. O’Hara, B. W. Pogue, Dartmouth College (United States); T. Hasan, Massachusetts General Hospital (United States); E. V. Maytin, The Cleveland Clinic (United States)

Photodynamic therapy (PDT) for skin cancer is sometimes only partially effective, due to inadequate levels of the fluorescent drug (photosensitizer, PS) and to heterogeneous distribution of PS within the tissue. To image the PS distribution within skin tumors, we have developed a fluorescence tomography system (FTS) that combines a fluorescence detection array with a high frequency ultrasound (HFUS) transducer. In this paper we describe in vitro and in vivo validation of this system. The target fluorophore for detection was Protoporphyrin IX (PPIX). Validation experiments were performed in vivo using a subcutaneous tumor model in which A431 tumor-bearing mice were treated with 5-aminolevulinic acid to induce production of PPIX. FTS reconstructions were compared with standard histology and with data from bulk tumor slices imaged ex vivo on a fluorescence scanner. Reconstructed images obtained from the FTS were correlated with the histology and the ex vivo scans, confirming several-fold increases in PPIX fluorescence in the skin and in the tumor relative to the surrounding tissues, could be detected. Our data demonstrate the feasibility of using the FTS for subsurface imaging of PPIX in skin carcinoma in vivo. Future aims are to use this device for individualized treatment planning, to improve overall patient responses to PDT.

Quantitative time domain fluorescence tomography for monitoring cancer therapy in vivo

W. Mo, D. J. Rohrbach, U. Sunar, Roswell Park Cancer Institute (United States)

It is important to verify the efficacy of photodynamic therapy (PDT) using imaging techniques. Fluorescence optical tomography (FOT), which emerged recently as a novel imaging modality, provides a feasible visualization approach for PDT evaluation. However, acquiring in situ fluorescence mapping is challenging, due to the short optical propagation distances, requirements of dense spatial, temporal and spectral sampling, and high dynamic range. We present in this article a quantitative FOT approach which offers system improvements on these aspects. Our prototype FOT system uses a pulsed laser at 660 nm as the excitation source, and a ultra-fast gated image intensifier as the detector. The acquired time-resolved signals of emission and excitation were converted to the frequency domain, and amplitude and phase information was extracted. 3D tomographic mappings of fluorescence yield and lifetime were reconstructed simultaneously. Tissue-like phantom experiments were introduced for system calibration and characterization. The results show the quantitative accuracy of yields and the lifetime of the phantoms has been realized in the 3D image reconstruction. As an in vivo verification, we utilized 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-a (HPPH) as a PDT drug. Nude mice with subcutaneous A549 human non-small cell lung carcinoma (NSCLC) tumors were injected with HPPH 24 hours before treatment. Images were acquired before and after PDT treatments, respectively. The changes of HPPH concentration and lifetime within the tumor region were quantified from the reconstructed images. Our results clearly show that HPPH selectively accumulated in the tumors 24-hours post HPPH injection compared to surrounding normal tissue. Meanwhile, the drop of HPPH concentration after PDT clearly indicated significant PDT-induced photobleaching in the tumor region.

Photodynamic therapy outcome model using fluorescence spectroscopy

J. D. Vollet Filho, L. T. Moriyama, C. Grecco, Univ. de São Paulo (Brazil); J. Ferreira, Univ. do Vale do Paraíba (Brazil); C. Kurachi, V. S. Bagnato, Univ. de São Paulo (Brazil)

Photodynamic Therapy (PDT) is a treatment modality for treatment of malignant and pre-malignant lesions. One of the difficulties related to treatment is the incomplete elimination of a lesion. Since there is a relation between the bleaching of the high-fluorescent PS molecules and the extent of damage, this study aims to develop a model that predicts depth of necrosis obtained by PDT application using tissue optical characteristics, light fluence, fluence rate, and fluorescence collected during treatment. Wistar rats underwent PDT application in healthy livers using the PS Photogem® (Photogem®, Moscow, Russia), a hematoporphyrin derivative, with drug-light interval of 30 minutes. Fluence rates used were 150 mW/cm² and 350 mW/cm², for different fluences (50-250 J/cm²). A diode laser (630 nm) was used as a light source for PDT, and fluorescence was induced using a 532 nm excitation laser, and collected during treatment irradiation. Thirty hours after treatment, animals were killed and liver samples were removed to evaluation of depth of necrosis. A model that predicts, under pre-established conditions, PS bleaching and depth of necrosis was obtained. A parameter - obtained by multiplying the normalized fluorescence by depth of necrosis values was also determined. This parameter shall allow one to predict depth of necrosis by knowing - and collecting fluorescence. Moreover, the developed model can be easily usable in clinical practice. Therefore, the obtained model is a simple approach to evaluate damage extent in tissues, with reasonable success. Acknowledgements: CAPES (Ph.D. scholarship), FAPESP (CePOF - CePID program), CNPq (INOF - INCT program).
Binding of cationic porphyrins to serum proteins

G. V. Gyulkhandanyan, A. G. Gyulkhandanyan, L. Z. Gyulkhandanyan, Institute of Biochemistry (Armenia); A. G. Tovmasyan, R. K. Ghazaryan, Yerevan State Medical Univ. (Armenia)

The affinity of serum albumin, lipoproteins and hemoglobin to porphyrins indicates their big role for these proteins as endogenous carriers of photosensitizers used in PDT. Cationic porphyrins are an important form of compounds widely used in photodynamic therapy of tumors (PDT). The new water-soluble meso-substituted cationic 4-pyridylporphyrins and metalloporphyrins (Zn, Ag, Co, et al.) with different peripheral functional groups (oxyethyl, butyl, allyl, and methallyl) were synthesized previously. Via two independent methods of optical spectroscopy (fluorescence and absorption spectroscopy) we investigated the interaction of 8 cationic porphyrins with carrier proteins of blood (serum albumin/SA and hemoglobin), as well as with mitochondrial protein - cytochrome c. It was shown that serum albumin and hemoglobin are equally well bind the cationic porphyrins. Depending on the structure of lateral functional groups different porphyrins variously are associated with SA. Thus, porphyrins with oxyethyl- and butyl-functional groups and their Zn-derivatives “strongly” associated with serum albumin (K = 1.3 - 4.1.105 M-1), whereas porphyrins with allyl- and methally-functional groups and their Zn-derivatives have a relatively “weak” affinity to SA. Binding constants of cationic porphyrin to SA (0.95 - 3.5.105 M-1), obtained by fluorescence spectroscopy, indicate good coincidence between the two spectral methods. Obtained for hemoglobin binding constants of porphyrins (1.7 - 5.3.105 M-1) comparable with the results of other authors. The highest affinity for the cationic porphyrin showed cytochrome c (2 times higher than SA). We assume that the high affinity of cationic porphyrins to cytochrome c is important for start of apoptosis in PDT.

MS2 bacteriophage as a delivery vessel of porphyrins for photodynamic therapy

B. A. Cohen, A. E. Kaloyeros, M. Bergkvist, Univ. at Albany (United States)

The challenges associated with Photodynamic Therapy (PDT) include the packaging and site-specific delivery of therapeutic agents to the tissue of interest. Encapsulation of PDT agents inside targeted virus capsids is a novel concept for nanoscale packaging and site-specific targeting. The icosahedral MS2 bacteriophage is one potential candidate for a packaging-system having an exterior diameter of ~28 nm with large pores, allowing the introduction of small molecules into the capsid. MS2 also has many lysines available on the exterior capsid for conjugation of targeting ligands. Previous work by the present investigators has successfully demonstrated RNA-based self-packaging of a heterocyclic PDT agent (meso-tetrakis(para-N-trimethylanilinium)porphine, TMAP) into the MS2 capsid. Packaging photoactive compounds in confined spaces could result in energy transfer between the molecules upon photoactivation. Such energy transfer can reduce the production of radical oxygen species (ROS), a key component in photodynamic therapy, and as a consequence impact the efficacy of PDT treatment. Findings are presented herein from an investigation of ROS generation of TMAP encapsulated within the MS2 capsid compared to free TMAP in solution. Monitoring of ROS production of encapsulated and free TMAP upon photoactivation via a specific singlet oxygen assay revealed the impact on ROS generation between packaged porphyrins in comparison to free porphyrin in an aqueous solution. The energy transfer of MS2-packaged TMAP is evaluated with fluorescent quenching and fluorescent life-time studies. Future work will study the ability of MS2-packaged porphyrins to generate ROS in vitro and subsequent cytotoxic effects on cells in culture.

Pheophorbide mediated photodynamic therapy against human epidermoid carcinoma cells (A431)

W. Li, Chung Yuan Christian Univ. (Taiwan)

The objective of this study was to characterize the death mechanism of human epidermoid carcinoma cells (A431) triggered by photodynamic therapy (PDT) with pheophorbide. First of all, significant inhibition on the survival of A431 cells (~20%) was observed when an irradiation dose of 5.1 J/cm2 combined with 125 ng/ml of pheophorbide was applied. Survival rate of human keratinocyte cells was over 70% under the same PDT parameters, suggesting that pheophorbide killed cancer cells selectively. Mitochondria were the main target sites where pheophorbide accumulated. Formation of reactive oxygen species (ROS) was detected after PDT. Addition of antioxidant N-Acetyl cysteine prevented ROS production and increased cell survival thereafter. The decrease in cellular ATP level was also observed at 6 hrs after PDT. Typical apoptotic cellular morphology and a collapse of mitochondrial membrane potential occurred after PDT. The loss of mitochondrial membrane potential led to the release of cytochrome c from the mitochondria to the cytosol, followed by activation of caspase-9 and caspase-3. The activation of caspase-3 resulted in poly(ADP-ribose) polymerase (PARP) cleavage in A431 cells, followed by DNA fragmentation. The expression of p21WAF1/CIP1 and the population of sub-G1 cells also increased after PDT. In conclusion, the results demonstrated that pheophorbide possessed photodynamic action against A431 cells, mainly through apoptosis mediated by mitochondrial intrinsic pathway triggered by ROS.
Mechanisms of tumor necrosis in photodynamic therapy with a chlorine photosensitizer: experimental studies

V. A. Privalov, A. V. Lappa, E. N. Bigbov, Chelyabinsk State Univ. (Russian Federation)

A PDT (photodynamic therapy) experiment on 118 inbred white mice with transplanted tumors is performed to reveal mechanisms of necrosis formation. There was applied Ehrlich's ascites tumor (mouse mammary gland adenocarcinoma) at the subcutaneous introduction. In 7-10 days the tumor of 1-1.5 cm diameter is formed under skin at the injection point, and PDT procedure is applied. There were used a chlorine type photosensitizer Radachlorine™ (patents: RF2183956 (2001), GB2389531, US6,969,765 (2005); V.Pivalov et al., SPIE proc., v.5863, pp.186-198, 2005) in form of 0.35% aqueous solution, and 662 nm wavelength diode laser. The drug is injected by intravenously at the dose of 40 mg/kg; the irradiation is executed in 2-2.5 hours at the surface dose of about 200 J/cm². Each of the mice had a photochemical reaction in form of destructive changes at the irradiation region with subsequent development of dry coagulation necrosis. After rejection of the necrosis there occurred epithelization of defect tissues in a tumor place.

Histological investigations were conducted in different follow-up periods, in 5 and 30 min, 1, 3, 6, and 12 hours, 1, 3, 7 and 28 days after irradiation. They included optical microscopy, immune marker analysis, morphometry with measurements of volume density of epithelium, tumor stroma and necroses, vascular bed. The investigations showed that an important role in damaging mechanisms of photodynamic action belongs to hypoxic injuries of tumor. These injuries are mediated by micro vascular disorders and blood circulatory disturbances. They have a stage development: just after PDT there is formed microcirculation angiopasm causing vessel paresis, formation of irreversible stases in capillaries, and diapedetic hemorrhages. Then thromboses joined them, and thrombovasculitis is formed. It is marked mucoid swelling and fibrinoid necrosis of vascular tissue. Progressive vasculitises result in total vessel obliteration and tumor necrosis.

Combination of optical imaging with NIR fluorophore and sonogram in breast cancer diagnosis

K. Liao, T. Yen, G. Lee, Y. Chou, National Chung Hsing Univ. (Taiwan)

The key of breast cancer management is based on the efficient screening and early detection. Presently, mammogram from 2-dimension tissue x-ray absorption is the gold standard of clinical screening, which is with limited contrast and inconclusive in the malignancy of suspicious lesions. More invasive needle biopsy procedure can provide the efficient evidence of malignancy, but it has the risk of metastasis from tumor cell detachment and release in the circulation.

The project will evaluate the potential of the combination imaging tools (optical imaging with near infrared fluorophore, SIDAG, and sonogram) for non-invasive, low facility requirement and low cost breast cancer diagnosis. The average value of optical and echo signals from normal tissue, benign lesion xenografts (extracellular membrane extract from the EHS mouse sarcoma) and malignant tumor xenografts (MCF-7 cell) developed in nude mice will be recorded and mapped for the following procedures:

1. Average threshold value of contrasts among the normal tissue, benign lesion xenograft and malignant tumor xenograft (screening).
2. Size and boundary of tumor tissue (staging of cancer).
3. Size and boundary of tumor tissue before and after chemotherapy (evaluation of treatment).

Model for effects of a broad threshold dose distribution for multi-session of photodynamic therapy

Photodynamic Therapy (PDT) has been used like an alternative local cancer technique. The main limitation of PDT is its limited treatment depth, which causes at bulky tumors the necessity of multiple PDT sessions or the association with surgical procedure. We present a theoretical model to describe the expected effects caused by successive PDT sessions in a bulky tumor, supposing a partial PDT response of a hypothetical tumor and considering the existence of a threshold dose distribution within the tumor tissue represented by a Modified Gaussian distribution and applying a one dimensional light-tissue model. We simulated the tumor response after two PDT applications. Whether there is a continuous distribution of threshold values, a single PDT session does not induce the killing of all cells. The cell fraction left behind promotes a tumor regrowth with different threshold dose distribution. This simple model points out one possible relation between the concept of threshold dose, and the existence of a variety of cells in a tumor mass. We performed an in vitro experiment using Hep-G2 (human liver tumor) cells in a sequence of PDT applications to evaluate the existence of cellular selectivity and to correlate tumor cell variety with threshold distribution. The result showed that the cell death fraction decreases after each PDT application using the same parameters. In addition, such effect
may be one of the reasons why a recurrent cancer lesion shows a higher resistance to PDT treatment.

7886-49, Poster Session

Monitoring HPPH-mediated photodynamic therapy of head and neck cancer with optical spectroscopies

D. J. Rohrbach, W. Mo, N. Rigual, E. Tracy, K. Keymel, M. T. Cooper, H. Baumann, B. W. Henderson, U. Sunar, Roswell Park Cancer Institute (United States)

Head and neck cancer occurs in the oral cavity, oropharynx, larynx and salivary glands. Although treatments such as surgery, chemotherapy and radiation have been improving, there are often unwanted side effects. Photodynamic therapy (PDT) provides an alternative treatment method for head and neck cancer patients. It can help preserve important functions such as speech and swallowing. It can also be used several times if one treatment fails. An important step to improving PDT efficacy is being able to monitor the treatment. Optical spectroscopies provide a non-invasive way to monitor multiple parameters related to treatment effectiveness and patient outcome. We present initial results obtained during a Phase I clinical trial of 2-1[hexyloxoyethyl]-2-devinylpyropheophorbide-a (HPPH)-mediated (PDT). We quantified blood flow, oxygenation, blood volume and HPPH drug photobleaching before and after treatment with diffuse correlation spectroscopy, diffuse reflectance spectroscopy and diffuse fluorescence spectroscopy. Our results show that HPPH-PDT induced significant drug photobleaching, and reduction in blood flow and oxygenation suggesting significant vascular and cellular reaction. These changes were accompanied by cross-linking of the signal transducer and activator of transcription 3 (STAT3), a molecular measure of PDT. These preliminary results suggest optical spectroscopies permit non-invasive monitoring of PDT of head and neck cancer patients in clinical settings.
Conference 7887: Mechanisms for Low-Light Therapy VI
Saturday 22 January 2011 • Part of Proceedings of SPIE Vol. 7887 Mechanisms for Low-Light Therapy VI

7887-01, Session 1
How to teach low-level light therapy (LLLT) in one day
J. D. Carroll, THOR Photomedicine Ltd. (United Kingdom)

For the medical doctor or therapist attempting to study LLLT the myriad of new terminology and concepts could be off-putting. There appear to be an improbably wide range of clinical applications, several possible molecular mechanisms, and many new concepts from physics as properties of light, the various sources and light propagation in tissue. Then there are the irradiation parameters (wavelength, irradiance and pulses) that may or may not exert an effect and their dependance on an appropriate dose (time). Finally there are clinical considerations such as where to apply the beam, how often and how many times, safety, contraindications, health regulations and reimbursement. A training syllabus and story telling roadmap is proposed that is deliverable and understandable in one day.

7887-02, Session 1
To what extent is coherence lost in tissue?
T. L. M. Hode, Immunophotonics, Inc. (United States); P. A. Jenkins, Irradia USA (United States); S. Jordison, Irradia AB (Sweden); L. Hode, Swedish Laser-Medical Society (Sweden)

The importance of coherence in phototherapy has been discussed over the last 30 years. There are primarily two separate aspects that are discussed: First, what is the significance of laser speckles in tissue, and second, to what extent is coherence preserved in tissue? It is known that a speckle pattern is formed when random phased laser light interfere, and if a volume is filled with scattered laser light, a three-dimensional speckle pattern is formed in the volume of tissue that is reached by the scattered coherent light. It is also known that the coherence length decreases as a function of depth in the tissue.

Occasionally, however, it is purported that the coherence is lost as soon as the laser light enters the tissue, and that coherence therefore cannot have any therapeutic significance in phototherapy. The question is important. If lasers are not needed to acquire an optimized therapeutic effect, then light emitting diodes or halogen lamps with band pass filters could just as well be used, and at a lower expense for the practitioner.

In a series of experiment we investigated the extent to which coherence is lost in tissue. We investigated whether the decrease in coherence length is dependent on the coherence length of the illuminating light, and possibly also if the light is polarized. We compared highly coherent light, e.g. from a HeNe laser, and less coherent light, e.g. from a semiconductor possibly also if the light is polarized. We compared highly coherent light, is lost in tissue. We investigated whether the decrease in coherence

7887-03, Session 1
The PASER concept: an explanation of patient amplification of the spontaneous effects of radiation and its relevance to the clinical effectiveness of LLLT
M. Dyson, King’s College London (United Kingdom)

The physiological mechanisms result in patient amplification of the spontaneous effects of radiation (PASER) in response to photon absorption during LLLT involve the neuroendocrine immune and circulatory systems. The release of soluble protein mediators (SPMs) eg cytokines from superficially located immune (inflammatory) and non-inflammatory cells occurs spontaneously as a result of the absorption of photons at suprathreshold levels. When these mediators diffuse to nearby target cells they can affect their activity, ie they have a paracrine effect. If they enter the circulation via superficial lymphatics and blood capillaries such as those in the papillary layer of the dermis, they can be transported around the body and affect distant target cells, ie they have an endocrine effect, amplifying in time and space the response of the patient to photon absorption.

Together with peripheral neural c-fibres, SPMs released from platelets during blood coagulation following injury initiate acute inflammation, an essential part of wound healing. SPMs secreted by inflammatory and noninflammatory cells control the cascade of events that occur during the inflammatory and proliferative phases of healing. Many SPMs including cytokines are produced by the following superficial components of the NEIS:

(a) cells of the skin-associated lymphoid tissue (SALT)
(b) blood-borne immune cells that enter the dermis via cutaneous lymphatics and blood vessels.

These cells, together with the cutaneous neural c-fibers, immune and stem cells in transit through the cutaneous vessels, and some cutaneous noninflammatory cells, are readily accessible to photons and susceptible to their direct, spontaneous effects following absorption. SPMs produced by cells that have absorbed photons can be transported to and affect distant cells that have not absorbed photons. Systemic (indirect and delayed) effects as well as local (direct and spontaneous) effects can therefore be produced in patients exposed to photons.

Investigations into the direct and indirect effects of photons on SALT, other components of the NEIS and the circulatory system relevant to PASER will be reviewed. The relevance to PASER to the enhancement of healing of local and distant, acute and chronic, wounds in patients treated with LLLT will be described.

7887-04, Session 1
Beam measurement problems in LLLT for single sources and cluster arrays
J. D. Carroll, THOR Photomedicine Ltd. (United Kingdom)

Irradiance is an important parameter in LLLT but but few authors appreciate the non uniformity of the light sources and how to measure these correctly. A typical uncorrected diode laser beam is neither round nor homogenous in distribution, many LLLT systems employ multimode laser diodes which produce highly divergent beams on one axis, narrow divergence on another and are far from Gaussian in distribution let alone flat top. Measurement methods are rarely reported in LLLT literature and so are likely inconsistent between authors. To add to this there are no guidelines on how to report the irradiance and energy delivered by cluster arrays. These issues will be illustrated and solutions proposed.

7887-05, Session 1
Signal pathway analysis of the effectiveness of low-level laser irradiation in rheumatoid arthritis
Y. Abiko, Nihon Univ. (Japan)

Rheumatoid arthritis (RA) is an inflammatory joint disorder whose progression leads to the destruction of cartilage and bone. Low-level laser irradiation (LLLI) is currently being evaluated for the treatment of RA, but the molecular mechanism underlying the effectiveness is unclear. Human synovial cells (MH-7A) were challenged with IL-18, treated by linear polarized red light (Super Lize, Tokyo Iken). Collagen-induced arthritis (CIA) in rats caused severe swelling in joints, and LLLI (Ga-As; Panasonic 830 nm) reduced the swelling. RNA was isolated from MH-7A and rat joint tissues and analyzed by DNA microarray. Ingenuity
Pathways Analysis (IPA; Ingenuity System) was used to search for possible biological processes, pathways, and networks. IL-1β induced the release of IL-8 from MH7A and was blocked by Bay11-7085 (inhibitor of IκB phosphorylation), indicating that activation of NF-κB signaling plays an important role in the secretion of IL-8 in RA. Interestingly, the phosphorylation was also inhibited by Super Lizer, suggesting that the suppression of the NF-κB signal might be an important mechanism of the right therapy against RA. Ga-Al-As LLLI reduced the gene expression of key mediators of RA such as CXCL1, 12, and 13 and related receptors including CXCR3. Furthermore, IPA shows that the CCR5 signal pathway plays an important role in the molecular mechanism of RA and effectiveness of LLLI. Thus, genome based gene-expression monitoring provides unprecedented access to elucidate the mechanism of the biostimulatory effects of LLLI.

7887-06, Session 2

Cellular studies with cultured brain cells and slices relevant to low-level laser therapy of traumatic brain injury

S. K. Sharma, G. B. Kharkwal, M. Sajo, Y. Huang, W. Xuan, Q. Wu, M. R. Hamblin, Massachusetts General Hospital (United States)

Recent results from a number of laboratories (including ours) suggest that transcranial low-level laser therapy (LLLT) may be an effective treatment for traumatic brain injury (TBI). A single treatment administered a few hours after TBI significantly improves neurological function as assessed by several behavioral tests. The hypothesis is that LLLT reduces inflammation, acts as a neuroprotectant, and restores neuronal energy metabolism in the brain. We carried out experiments designed to elucidate these mechanistic aspects on cultured cortical neurons, glial cell cultures and organotypic brain slice cultures.

7887-07, Session 2

Glycogen synthase kinase-3β facilitates high-fluence low-power laser irradiation-induced cell apoptosis through acceleration of Bax translocation

L. Huang, S. Wu, D. Xing, South China Normal Univ. (China)

Glycogen synthase kinase-3β (GSK-3β) is a critical activator of cell apoptosis induced by a diverse array of insults. However, the effects of GSK-3β on the human lung adenocarcinoma cell (ASTC-a-1) apoptosis induced by high fluence low-power laser irradiation (HF-LPLI) are not clear. Here, we showed that GSK-3β was constantly translocated from cytoplasm to nucleus during HF-LPLI-induced apoptosis. In addition, we found that co-overexpression of YFP-GSK-3β and CFP-Bax in ASTC-a-1 cells accelerated both Bax translocation and cell apoptosis in compared with the cells expressed CFP-Bax only during HF-LPLI-induced apoptosis, indicating that GSK-3β facilitated ASTC-a-1 cells apoptosis through acceleration Bax translocation. Our results demonstrate that GSK-3β exerts some of its pro-apoptotic effects in ASTC-a-1 cells by regulating the mitochondrial localization of Bax, a key component of the intrinsic apoptotic cascade.

7887-08, Session 2

Cryptococcus neoformans capsule protects cell from oxygen reactive species generated by photodynamic antimicrobial chemotherapy

R. A. Prates, Instituto de Pesquisas Energéticas e Nucleares (Brazil); M. R. Hamblin, Massachusetts General Hospital (United States); I. T. Kato, Instituto de Pesquisas Energéticas e Nucleares (Brazil); B. Burgwyn Fuchs, E. Mylonakis, Massachusetts General Hospital (United States); M. Simões Ribeiro, Instituto de Pesquisas Energéticas e Nucleares (Brazil); G. P. Tegos, The Univ. of New Mexico (United States)

Photodynamic antimicrobial chemotherapy (PACT) is based on the utilization of substances that can photosensitize biological tissues and are capable of being activated in the presence of light. Cryptococcus neoformans is a yeast surrounded by a capsule composed primarily of glucuronoxylomannan that plays an important role in its virulence. This yeast causes infection on skin, lungs and brain that can be associated with neurological sequelae and neurosurgical interventions, and its conventional treatment requires prolonged antifungal therapy, which presents important adverse effects. The aim of this study was to evaluate the protective effect of Cryptococcus neoformans capsule against reactive oxygen species generated by PACT. Cryptococcus neoformans KN99α, which is a strain able to produce capsule, and CAP59 that does not present capsule production were submitted to PACT using methylene blue (MB), rose bengal (RB), and pl−cep6 as photosensitizers (PS). Then microbial inactivation was evaluated by counting colony form units following PACT and confocal laser scanning microscopy (CLSM) illustrated visualization localization as well as the preferential accumulation of PS into the fungal cells. C. neoformans KN99α was more resistant to PACT than CAP59 for all PSs tested. CLSM showed incorporation of MB and RB into the cytoplasm and a preferential uptake in mitochondria. A nuclear accumulation of MB was also observed. Contrarily, pl−cep6 appears accumulated in cell wall and cell membrane and minimal florescence was observed inside the fungal cells. In conclusion, the ability of C. neoformans to form capsule enhances survival following PACT.

7887-09, Session 2

Photodynamic action of LED-light on standard and clinical strains of Staphylococci, processed by brilliant green and titanium dioxide nanoparticles

E. S. Tuchina, V. V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

Brilliant green refers to a group of aniline dyes and is one of the more common antiseptics, therefore it is often used to treat infections of the eye, tongue sores and sinus. The absorption maximum of this dye is at 440 and 650 nm.

Titanium dioxide is a conventional generator of reactive oxygen species and bleaching agent. Some authors have shown its high ability to form singlet oxygen after irradiation with ultraviolet or blue light.

In our experiments it was found that the combination of TiO2-nanoparticles and Brilliant Green at two-wavelength irradiation with blue (405 nm) and red (655 nm) light provides a pronounced antimicrobial effect on standard and clinical strains of Staphylococcus.

Materials and methods: The bacterial strains used in this study were Staphylococcus aureus 209 P (obtained from the Culture Collection (SISC, Russia) and Staphylococcus simulans (выделен из содержимого пазухи больного гайморитом). Cultures were grown on dense brain-heart infusion medium and incubated at 37°C.

Source of red light was LED with a maximum radiance in the range around 625 nm and the emission power density of 33 mW/cm2; the...
source of blue light was LED with a maximum radiance in the range around 405 nm and the emission power density of 31.5 mW/cm². The light exposure was ranged from 5 to 30 min. As photosensitizers 0.00007%-aqueous solution of Brilliant Green and 0.02%-suspension titanium dioxide nanoparticles (TiO₂; 100 nm, Sigma-Aldrich, USA) were used. The method of consecutive cultivations of initial concentration of a microbial suspension of 1000 microbial cells per ml was applied. Solutions of photosensitizers were added to a suspension of the microorganisms, the received mixture was remained in darkness during 10 min. Then the suspension was placed to sections of experimental ditch of volume of 0.2 ml for light exposure. After exposure to light culture was distributed to Petry dishes with a dense nutrient medium. The account of results was provided by calculation of colony forming units (CFU) in 24 hrs after incubation at 37°C. As the control accepted colony forming ability values of bacteria not subjected to an irradiation and not processed by dyes. Each experiment has been made in tenfold frequency. Results: It was established that the test microorganisms after sensitization with brilliant green and titanium dioxide were the most resistant to the red (625 nm) light. Reducing the number of staphylococci did not have a dose-dependent nature and occurred at an average of 50% compared with the control.

It was shown that the clinical strain of S. simulans, after treatment of cells with brilliant green and titanium dioxide, was more resistant to irradiation with blue (405 nm) light in comparison with a standard strain of S. aureus, the cells which were treated similarly. Reducing the number of colonies of S. simulans observed at 6 - 61% by varying the duration of exposure from 5 to 30 minutes, while the decrease in the number of colonies of S. aureus occurred at 36 - 89% at a temporary exhibition. Combined effects of blue and red light led to a sharp reduction in the number of staphylococci. After 30 min irradiation the number of colonies of S. simulans decreased by 93%, and S. aureus - 96% compared with the control.

These results can be explained as follows. Clinical strain of S. simulans was more resistant to the effect, because in general better adapted to changing environmental conditions. Probably in this cells protective mechanisms associated with suppression of active radicals and the restoration of membranes and nucleic acids are more active.

7887-10, Session 2
Oxidative stress of photodynamic antimicrobial chemotherapy inhibits Candida albicans virulence
I. T. Kato, R. A. Prates, Instituto de Pesquisas Energéticas e Nucleares (Brazil); G. P. Tegos, The Univ. of New Mexico (United States) and Massachusetts General Hospital (United States) and Harvard Medical School (United States); M. R. Hamblin, Massachusetts General Hospital (United States) and Harvard Medical School (United States) and Massachusetts Institute of Technology (United States); M. Simões Ribeiro, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

Photodynamic antimicrobial chemotherapy (PACT) is based on the principal that microorganisms will be inactivated using a light source combined to a photosensitizing agent in the presence of oxygen. Oxidative damage of cell components occurs by the action of reactive oxygen species leading to cell death for microbial species. It has been demonstrated that PACT is highly efficient in vitro against a wide range of pathogens, however, there is limited information for its in vivo potential. In addition, it has been demonstrated that sublethal photodynamic inactivation may alter the virulence determinants of microorganisms. In this study, we explored the effect of sublethal photodynamic inactivation to the virulence factors of Candida albicans. Methylene Blue (MB) was used as photosensitizer for sublethal photodynamic challenge on C. albicans associated with a diode laser irradiation (λ=660nm). The parameters of irradiation were selected in causing no reduction of viable cells. The potential effects of PACT on virulence determinants of C. albicans cells were investigated by analysis of germ tube formation and in vivo pathogenicity assays. Systemic infection was induced in mice by the injection of fungal suspension in the lateral caudal vein. C. albicans exposed to sublethal photodynamic inactivation formed significantly less germ tube than untreated cells. In addition, mice infected with C. albicans submitted to sublethal PACT survived for a longer period of time than mice infected with untreated cells. The oxidative damage promoted by sublethal photodynamic inactivation inhibited virulence determinants and reduced in vivo pathogenicity of C. albicans.
Effects of LED phototherapy on bone defects grafted with MTA, bone morphogenetic proteins, and guided bone regeneration in a rodent model: a description of the bone repair by light microscopy


We carried out a histological analysis on surgical bone defects grafted or not with MTA, treated or not with LED, BMPs and GBR. We have used several models to assess the effects of laser on bone. On benefits of the isolated or combined use them on bone healing has been suggested. There is no previous report on their association with LED light. 90 rats were divided into 10 groups. On Groups II and I the defect were filled with the clot. On Group II, were further irradiated. On groups III-VI, defect was filled with MTA + Collagen gel (II); animals of group IV were further irradiated. On groups V and VI, the defects filled with the MTA were covered with a membrane. Animals of Group VI were further irradiated. On Groups VII and VIII a pool of BMPs was added to the MTA and was further irradiated. On groups IX and X, the MTA + BMP graft was covered with a membrane. On group X, the defect was further irradiated. LED (0.5 mm, 150 mW, A: 0.5 cm2, 54s, 3.0W/cm2, 16 J/cm2) was applied at 48 h intervals during 15 days. Specimens were taken, processed, cut and stained with H&E and Sirius red and underwent histological analysis. The results showed that MTA seemed not being affected by LED light. However, its use positively affected healing around the graft. It is concluded that MTA is not affected by the LED light due to its characteristics, but beneficial results with LED usage was found.

The effects of photobiomodulation on healing of bone defects in Streptozotocin induced-diabetic rats

M. D. M. Costa Lino, F. B. Carvalho, M. Ferreira Morais, J. A. Cardoso, A. L. Barbosa Pinheiro, L. M. Pedreira Ramalho, Univ. Federal da Bahia (Brazil)

Previous studies have shown positive effects of Laser Phototherapy (LPT) on the repair of bone defects, but there are only a few that associates bone healing in the presence of a metabolic disorder as Diabetes Mellitus and LPT. The aim of the present investigation was to assess histologically the effect of LPT (AsGaAl, 780nm, 70mW, CW,0.4mm, 16J/cm2 per session) on the repair of surgical defects created in the femur of diabetic and non-diabetic Wistar Albinus rats. Surgical bone defects were created in 60 animals divided into four groups of 15 animals each: Group I (non-diabetic - control); Group II (non-diabetic - LPT); Group III (diabetic); Group IV (diabetic - LPT). The animals on the irradiated group received 16 J/cm2 per session divided into four points around the defect (4 J/cm2), being the first irradiation immediately after surgery and repeated every 48h for 14 days. The animals were killed 15, 21 and 30 days after surgery. The results of the present investigation showed histological evidence of improved amount of collagen fibers at early stages of the bone healing (15 days) and increased amount of well organized bone trabeculae at the end of the experimental period (30 days) on irradiated animals, diabetic and non-diabetic compared to non irradiated ones. It is concluded that LPT has a positive biomodulative effect on the healing process of bone defects, even when diabetes mellitus was present.

Photobiomodulatory effects of He-Ne laser on excision wounds

V. Prabhu, S. B. S. Rao, Manipal Univ. (India); P. Kumar, L. Rao, Manipal University (India); K. K. Mahato, Manipal Univ. (India)

Presently, great importance has been given to Low Level Laser Therapy (LLLT) with the intent of promoting wound healing process. The present study was aimed to investigate the promotive effect of LLLT on full thickness excision wounds in Swiss albino mice using optical fiber probe based light device. Circular wounds of diameter 15 mm were illuminated with single exposure of various laser doses 1, 2, 3, 4, 6, 8 and 10 J/cm2 along with appropriate controls. Further, an optimal dose of 2 J/cm2 was applied to excision wounds at different post-wounding treatment schedules (0, 24 h and 48 h) to explicate the relations between treatment schedule and its tissue regeneration potential. Wound area, mean wound healing time along with hydroxyproline and glucosamine levels from wound ground tissue was assessed to evaluate the resultant photobiostimulatory outcome. Histological analysis was performed on day 10 of post-wounding. A significant increase in hydroxyproline (P< 0.001) and glucosamine levels (P< 0.01) were observed in 2 J/cm2 irradiation group, which was also substantiated by histological findings. In conclusion, the present study demonstrated that the immediate irradiation of 2 J/cm2 dose following wounding hasten the healing process compared to the unilluminated control.

Optimization of treatment parameters for repair of severely injured rabbit peripheral nerves using 980-nm irradiation

J. Anders, X. Wu, H. Moges, Uniformed Services Univ. of the Health Sciences (United States); B. Pryor, LiteCure, LLC (United States)

Background: Severe peripheral nerve injury causes chronic loss of sensation and motor function though microsurgical techniques are used to reconnect the proximal and distal segments. Based on our previous research on light therapy (LT) wavelengths and parameters, 980 nm light was applied post-operatively and transcutaneously at the site of nerve repair, and axonal re-growth was assessed. Methods: White New Zealand rabbits that had their left peroneal nerve exposed, transected, and sutured were randomized into 3 groups 1) control, no light treatment; 2) 2W: LT at 2W output power, 30 second exposure and 3) 4W: LT at 4W output power, 15 second exposure. The beam area at the nerve was 3.8 cm2. The total energy delivered to the skin surface was 60J for both LT groups. LT was performed immediately after surgery and then once daily for 10 consecutive days. Peroneal nerve samples were collected from 1 cm proximal to 5 cm distal to the lesion site. Immunolabeling of axons for PGP9.5 was done to characterize axonal regeneration. Results: At 3 weeks post-injury, there was no significant difference in fluorescent labeling between the control and the laser treatments at 2 cms distal to the injury. At 3 and 4 cms distal to the transection site, there was significantly less labeling in the 4W group than control and 2W groups (p<0.001) while the 2W laser treatment had significantly better regeneration than the control (p=0.08). Conclusions: Significant inhibition of nerve regeneration was found with the 4W parameters. The 2W parameters increased the rate of regeneration.
7887-18, Session 4

Photophrophylactic treatment using low-levels of visible light
D. Barolet, Opusmed Inc. (Canada)

Low levels light therapy (LLLT) has been shown to produce significant therapeutic benefits including wound healing and pain control as well as reduction of swelling and inflammation in a variety of medical conditions. The present paper focuses on a specific application of LLLT designated as photoprophylaxis. Photoprophylaxis is defined as a photophrophylactic treatment modality using low levels of visible light (red and near-infrared light) for the prevention of adverse cutaneous manifestations following trauma. In essence, the method consists of the administration of serial LLLT treatments prior or after trauma to prevent cutaneous damage. The hypothesized underlying mechanisms involve the triggering of natural skin protection machinery. It has been shown that light energy could trigger cell signalling pathways such as p53. Based on this principle, it is expected that LLLT administered prior to an insult to the skin could prevent undesirable consequences like sunburn, hyperpigmentation or scarring. Qualitative and quantitative efficacy as well safety results obtained from clinical trials with LED treatments to prevent sunburn and mechanical trauma (e.g., CO2 laser treatment, surgery) will be presented. The results obtained to date are encouraging and support the use of LED as a photophrophylactic modality.

7887-19, Session 4

Efficacy of continuous wave and pulsed wave transcranial laser therapy (TLT) in the treatment of Alzheimer’s disease (AD) in an amyloid precursor protein transgenic mouse (APP Tg) model
L. H. De Taboada, PhotoThera, Inc. (United States); J. Yu, S. El-Amouri, Medical Univ. of South Carolina (United States); S. Gattoni-Celli, Charleston VA Medical Ctr. (United States); S. Richieri, T. McCarthy, PhotoThera, Inc. (United States); J. Streeter, Banyan Biomarkers, Inc. (United States); M. S. Kindy, Medical Univ. of South Carolina (United States)

Continuous Wave (CW) and Pulsed Wave (PW) TLT was tested for efficacy in a transgenic (Swedish/London) APP mouse model of AD. Starting at 3 months of age, TLT was administered noninvasively using near-infrared (808nm) laser energy applied 3 times/week at various doses for a total of six months, and was compared to a control group (no laser treatment). Administration of TLT was associated with a dose-dependent reduction in amyloid load and the numbers of Ap+ plaques. All TLT doses mitigated the behavioral deficits seen with advanced amyloid deposition and reduced the expression of inflammatory markers in the APP Tg mice. All TLT doses produced an increase in sAPP-α and a decrease in sAPP-β levels consistent with inhibition of the β-secretase activity.

Follow up studies were performed to determine brain tissue mitochondrial oxygen (O2) consumption, adenosine triphosphate (ATP) concentrations, and c-fos protein expression following PW TLT, and compared to a control group (APP Tg with no laser treatment) and wild type (WT) mice. Mitochondrial (isolated) O2 consumption and ATP concentrations in the APP Tg mice were significantly reduced compared to WT mice, but essentially restored in the PW TLT treated APP mice. PW TLT induced a transient increase in c-fos protein expression, while WT and APP Tg mice alone showed little to no c-fos activity.

These studies suggest that TLT stimulates mitochondrial activity helping to maintain neuronal function and is a potential candidate for the treatment of AD.

7887-20, Session 4

Laser treatment in modulation of TMJ inflammation
G. Ross, Private Practice (Canada)

No abstract available.

7887-21, Session 4

Preconditioning and low-level laser therapy in dental practice
A. A. Darbar, R. Darbar, Smile Creations (United Kingdom)

For the last decade we have been applying the principles of Low level laser therapy and have broadened the concept for practical use before and after treatment with surgical lasers. The purpose of this clinical presentation is to demonstrate how this concept has improved treatment outcomes. Cases treated in our dental practice will be demonstrated with the protocols used. A look at the science behind this concept will be examined in an effort to explain the results and to open discussion.

7887-22, Poster Session

Evaluation of the viability of the chemiluminescence as a PDT light source for microbial control
R. C. Mattosinho Ferraz, C. R. Fontana, Univ. de São Paulo (Brazil); E. C. Cabral Correia Lins, Univ. Federal do ABC (Brazil); V. S. Bagnato, C. Kurachi, Univ. de São Paulo (Brazil)

The photodynamic therapy is a combination of using a photosensitizer agent, light and oxygen that can cause oxidative cellular damage. This technique is applied in several cases, including for microbial control. The most extensively studied light sources for this purpose are lasers and LED-based systems. Few studies only treat alternative light sources. Sources which present flexibility, portability and economic advantages are of great interest. In this study, we evaluated the in vitro feasibility for the use of chemiluminescence as a PDT light source to induce Staphylococcus aureus reduction. The photosensitizer concentration varied between 0 and 75µg/ml and the illumination time varied from 60 minutes to 240 minutes. The long exposure time was due to the low irradiance achieved with chemiluminescence reaction at µW/cm² level. The results demonstrated an effective microbial reduction of around 98% for the highest photosensitizer concentration and light dose. These data suggest the potential use of chemiluminescence as a light source for PDT microbial control with advantages in terms of flexibility compared to conventional sources.

7887-23, Poster Session

Comparative study of the effects of low-intensity pulsed Ultra-sound and low level laser therapy on muscle repair
R. Toma, Univ. Federal de São Paulo (Brazil)

Skeletal muscle injury is one of the most common lesions in sport activities and repetitive traumas are also very frequent in athletes. Although the muscle tissue is able to regenerate, this process is considered very slow and some of the injuries affect muscle function, leading to atrophy, contracture, pain and increased likelihood of reinjury. In this context, there is a need of treatments able of accelerating the muscle cell proliferation and prevent fibrosis during the process of healing. The main purpose of the present work was to compare the effects of ultrasound...
and low level laser therapy on muscle repair after a cryolesion by means of histopathological analysis and immunohistochemistry for COX-2. A total of forty male Wistar rats were randomly distributed into 4 groups: control group (CG); animals without any kind of injury; injured control group (IG); muscle lesion without treatment; laser treated group (LG); muscle lesion treated with 830 nm laser and ultra-sound treated group (USG); muscle lesion treated with ultra-sound. The treatments started 24 hours post-surgery and were performed during 6 sessions. The animals exposed to lasertherapy pointed out minor degenerative changes of muscle tissue. In the same way, exposure to ultrasound was able to reduce tissue injuries induced by cryolesion, but less intense than the laser therapy. Strong COX-2 positive cells were found in rats submitted to cryolesion only, whereas COX-2 immunoexpression was lower in laser treated or ultrasound treated groups. In summary, this study reveals that both lasertherapy and ultrasound have positive effects on muscle repair in rats.

7887-25, Poster Session

Efficacy of low-power laser irradiation in the prevention of D-galactose-induced senescence in human dermal fibroblasts

C. Meng, D. Xing, S. Wu, South China Normal Univ. (China)

Low-power laser (He-Ne) irradiation (LPLI) has been found to modulate various biological effects, especially those involved in promoting cell proliferation and metabolic regulation. However, the underlying mechanisms that LPLI prevents human cell senescence remain undefined. Herein, we devised a model enabling cell senescence using D-galactose for two days then treat with or without LPLI(< 15J/cm2), and investigated whether LPLI delays cell senescence in human dermal fibroblasts cells (HDF-a). First in this study, using SA-β-gal staining, compared with control cell we detected a lower frequency of SA-β-gal staining under the treatment of LPLI. Moreover, we found the growth rates of cell with LPLI was higher using CCK-8 analysis. Additionally, we also found LPLI induced HDF-a entered the irreversible G1 arrest measured by flow cytometry system. Therefore, LPLI may promote cell proliferation by stimulating cell-cycle progression and delay human cell senescence. Taken together, Low-power laser irradiation delays HDF-a cells senescence provides new information for the mechanisms of biological effects of LPLI.

7887-26, Poster Session

Low-power laser irradiation inhibits amyloid beta-induced cell apoptosis

H. Zhang, S. Wu, D. Xing, South China Normal Univ. (China)

The deposition and accumulation of amyloid-β-peptide (Aβ) in the brain are considered a pathological hallmark of Alzheimer's disease(AD). Apoptosis is a contributing pathophysiological mechanism of AD. Low-power laser irradiation (LPLI), a non-damage physical therapy, which has been used clinically for decades of years, is shown to promote cell proliferation and prevent apoptosis. Recently, low-power laser irradiation (LPLI) has been applied to moderate AD. In this study, Rat pheochromocytoma (PC12) cells were treated with amyloid beta 25-35 (Aβ25-35) for induction of apoptosis before LPLI treatment. We measured cell viability with CCK-8 according to the manufacture's protocol, the cell viability assays show that low fluence of LPLI (2 J/cm²) could inhibit the cells apoptosis. Then using statistical analysis of proportion of apoptotic cells by flow cytometry based on Annexin V-FITC/PI, the assays also reveal that low fluence of LPLI (2 J/cm²) could inhibit the Aβ-induced cell apoptosis. Taken together, we demonstrated that low fluence of LPLI (2 J/cm²) could inhibit the Aβ-induced cell apoptosis, these results directly point to a therapeutic strategy for the treatment of AD through LPLI.

7887-27, Poster Session

Evaluation of the effect of laser radiation on fibroblast proliferation in repair of skin wounds of rats with iron deficiency anemia


The aim of this study was to assess the effect of low level laser therapy (LLLT) on fibroblast proliferation on repair of skin wounds of rats with Iron deficiency anemia (IDA) by histological analysis by HE staining. Wisters aged 21 days and weight of 50g were fed for 2 weeks with a free and standardized diet (No iron-AIN 93-G) for induction of IDA and distributed into 5 groups: (I)Healthy + wound; (II) IDA + no wound;(III) IDA + wound; (IV) Healthy + Laser; (V)IDA + Laser and sacrificed at 7, 14 and 21 days. GaAIA Laser diode was model TWIN FLEX, 660nm, 40mW, CW, 10J/cm2 (MMOptics, São Carlos, SP). Data were analyzed by ANOVA. Significant differences where seen between groups I and IV (p=0.002) and between III and V (p=0.03). The groups III and V showed 100% of fibroblast proliferation at 7 days of treatment, unlike other groups in the same period showed heterogeneity in the results. At 14 days the groups treated with laser showed intense proliferation regarding the groups not treated. On 21 days, groups IV, V and I showed in most animals more fibroblastic proliferation. In group III, no intense fibroblastic proliferation was shown. Based on this findings it can be concluded that LLLT, within the specified parameters, has a positive effect on the proliferation of fibroblasts on healthy subjects.

7887-28, Poster Session

Influence of laser and LED irradiation on mast cells of cutaneous wounds of rats with iron deficiency anemia


Objective: This paper aimed to study the histological effect of Laser and LED Phototherapy on mast cells of cutaneous wounds of rats with iron deficiency. Methods: Eighteen newborn Wistar rats were used. To induce iron deficiency, the animals were fed with a special pelleted iron-free diet (No iron-AIN 93-G) for 15 days. An excisional wound was created on the dorsum of each animal. The rats were divided into: Group I - Control with anemia + no treatment; Group II - Anemia + Laser; Group III - Anemia + LED; Group IV - Healthy + no treatment; Group V - Healthy + Laser; Group VI - Healthy + LED. Irradiation was performed immediately after surgery using a diode AsGaAl Laser with 1.680nm, 40mW, CW, total dose of 10J/cm² and a RED-LED with 1.700nm, 15mW, CW, total dose of 10J/cm². The animals were killed on day 7. Histological specimens were stained with toluidine blue and underwent histological analysis for mast cell counting. Results: No significant statistic difference was found between groups as to the number of degranulated, non-degranulated or total mast cells. However, greater mean values were found for degranulated mast cells in the Anemia + LED (group III). LED irradiation on healthy specimens (group VI), on the other hand, resulted in a smaller number of degranulated mast cells. Conclusion: Our results lead to conclude that there are no significant differences in the number of mast cells seven days after irradiation with either Laser or LED Phototherapy.
7887-29, Poster Session
Assessment of bone healing on tibial fractures treated with wire osteosynthesis associated or not with infrared laser light and Biphasic ceramic bone graft (HATCP) and guided bone regeneration (GBR): Raman spectroscopy study
F. B. Carvalho, G. T. dos Santos Aciole, J. M. Aciole, Univ. Federal da Bahia (Brazil); L. Silveira, Jr., Camilo Castelo Branco Univ. (Brazil); J. Nunes dos Santos, A. L. Barbosa Pinheiro, Univ. Federal da Bahia (Brazil)

The aim of this study was to evaluate, through Raman spectroscopy, the repair of complete tibial fracture in rabbits fixed with wire osteosynthesis - WO, treated or not with infrared laser light (780nm, 50mW, CW) associated or not to the use of HATCP and GBR. Surgical fractures were created under general anesthesia (Ketamine 0.4ml/Kg IP and Xilazine 0.2ml/Kg IP), on the tibia of 15 rabbits that were divided into 5 groups and maintained on individual cages, at day/night cycle, fed with solid laboratory pelleted diet and had water ad libidum. On groups II, III, IV and V the fracture was fixed with WO. Animals of groups III and V were grafted with hydroxyapatite and GBR technique used. Animals of groups IV and V were irradiated at every other day during two weeks (16/J/cm², 4 x 4J/cm²). Observation time was that of 30 days. After animal death the specimens were kept in liquid nitrogen for further analysis by Raman spectroscopy. Raman spectroscopy showed significant differences between groups (p<0.001). It is concluded that IR laser light was able to accelerate fracture healing and the association with HATCP and GBR resulted on increased deposition of calcium hydroxyapatite.

7887-30, Poster Session
Clinical efficiency of use of TDC with feedback for treatment of brucellosis patients
I. A. Chesnokov, Federal State Unitary Enterprise (Russian Federation); E. P. Lyapina, Saratov State Medical Univ. (Russian Federation); N. A. Bushuev, Federal State Unitary Enterprise (Russian Federation); Y. Eliseev, A. A. Shuldyakov, V. F. Spirin, A. V. Anashchenko, Saratov State Medical Univ. (Russian Federation)

The special author’s procedure of application of low-intensive electromagnetic radiance of the extremely high-frequency range (EMR EHF) was used for a complex therapy of chronic brucellosis patients. Treatment was carried out with the treatment-and-diagnostic complex with a biological feedback (TDC with biological feedback).

The opportunity to individualize parameters of action has allowed to raise efficiency of EHF-therapy, in particular to achieve faster cupping of clinical exhibitions of an inflammation, a vegetative dysfunction, polyneuropathy and to considerably improve quality of life of patients due to pinch of parameters both physical and (in the greater level) mental health. After the EHF-therapy course the decrease of a case rate has been marked for he following diseases: sharp respiratory disease in 1,7 times, aggravations of inflammatory process in the locuses of persistent infection of an ear, a throat and a nose, and also a respiratory organs in 1,7 times in dependence on used procedure. Application of the EHF-therapy is not accompanied by development of the secondary (toxic, allergic) effects.

7887-31, Poster Session
Evaluation of the survival of cutaneous flaps on diabetics rats with or without photostimulation
P. C. Oliveira, N. Ribeiro Santos, J. Nunes dos Santos, A. L. Barbosa Pinheiro, Univ. Federal da Bahia (Brazil)

Aim: The aim of this study was to assess and compare the effects of Laser Phototherapy - LPT on cutaneous flaps on diabetic rats. Animals and Methods: Twelve a Wistar rats were randomized into 3 groups. Diabetes was induced with streptozotocin. Only animals with blood sugar levels of 350mg/100ml were used. Under intraperitoneal general anesthesia-GA cutaneous flap was raised on the dorsum of each animal. A plastic sheet was introduced between the flap and the bed in order to cause blood supply impairment and the flap was then sutured. G1 (diabetic control animals), G2 (diabetic animals irradiated with 680nm) and G3 (diabetic animals irradiated with 790nm). LPT: Red Light (800nm) 30mW; 40mW; 660nm 30mW; 40mW; 790nm; 790nm). LPT: Red Light (680nm) 30mW; 40mW; 790nm; 40mW; 790nm). LPT: Red Light (790nm) 40mW). Non irradiated animals acted as controls. The dose per session was 40J/cm². Laser light was applied transcutaneously and fractioned on 16 contact points at the wound margins (16 x 2.5 J/cm²). Results: Statistical analysis between all groups showed significant differences on the level of acute inflammation between groups 1 and 3 (p=0.04); tissue necrosis between groups 1 and 2 (p=0.03); chronic inflammation (p=0.04); fibroblastic proliferation (p=0.05); and neovascularization (p=0.04).Conclusion: LPT was effective on increasing angiogenesis as seen on irradiated subjects being this more pronounced when IR laser light was used.

7887-32, Poster Session
Evaluation of laser photobiomodulation in repair of cutaneous wounds in rats infected of Staphylococcus aureus
N. Ribeiro Santos, P. C. Oliveira, J. Nunes dos Santos, A. L. Barbosa Pinheiro, Univ. Federal da Bahia (Brazil)

The Laser photobiomodulation acts directly in the healing process through the increase of the cellular proliferation, synthesis of substances and liberation of growth factors. The infection is a constant fight between the mechanism of defence of the organism and the concentration and virulence of the microorganism. This work has as objective evaluates through a histological study the action of the laser in cutaneous wounds infected by Staphylococcus aureus. Sixty mice will be used (Wistar), being accomplished in the back of each animal a wound of 1cm², the animals will be divided in four groups: group control, group laser 680nm; group Laser 790nm; group Laser 790nm + 790nm (7 and 14 days); 10, 20 and 30J/cm². After the treatment, the animals will be sacrificed and submitted to biopsies excisionals of the wounds, for making of the sheets and histological analyses in coloration HE, Picrosirius. The histological results, in general, showed a better healing in the laser groups, in comparison to the controls groups. The results showed higher deposition of collagen fibers, larger amounts of granulation tissue, better inflammatory reaction and revascularization on laser-treated subjects. It is concluded that lasertherapy resulted in a better repair in the groups with 30J/cm² and 680 and 790nm laser light.
The morphology of apoptosis and necrosis of fat cells after photodynamic treatment at a constant temperature in vitro

I. Y. Yanina, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

Photodynamic therapy with temperature control is a new approach for treatment of obesity and cellulite. Cell death can occur under the action of various physical, chemical and biological factors. Depending on the inductor, this is apoptosis or necrosis. These two forms of cell death differ in the biochemical and morphological levels. Biochemical changes occur quickly enough and it raises difficulties of their detection. One of the morphological characteristics of apoptosis is a decrease (contraction) of cells, and necrosis - an increase in the size of the cell (swelling). This attribute simply determined visually using a digital microscope. The aim of our work is to design a computer program to monitor changes in cell size and intercellular structure in frames before and after light treatment and to quantify the experimental data. The program was designed using LabVieiv media, which allowed us to develop the software for interaction with the measuring and control equipment, data collection, processing and displaying the information and results of calculations and simulations for the individual cells and ensembles of cells, and, in general, to automate process.

Silicon photonic microring resonator arrays for scalable and multiplexible bioanalysis

A. L. Washburn, M. S. Luchansky, A. J. Qavi, J. T. Kindt, R. C. Bailey, Univ. of Illinois at Urbana-Champaign (United States)

The ever increasing demands of clinical diagnostics have placed an impetus on developing innovative biomolecular analysis tools that are capable of performing high throughput, multiplexed, and cost-effective measurements from relevant patient samples. In response to this challenge, our group is developing a biosensing platform based upon arrays of silicon-on-insulator (SOI) microring resonators and have demonstrated them in several key detection applications. High Q-factor cavities, batch fabricated on 8" SOI wafers at commercial-scale foundries, support resonant optical modes that are extremely responsive to biomolecular binding-induced changes in the refractive index at or near the ring’s surface. Each sensor is independently functionalized with a target-specific capture agent, transforming an array of uniquely addressable microrings into a scalable and multiplexible bioanalysis platform. Using this approach, we have demonstrated the detection of cancer and inflammation biomarkers at biologically-relevant levels equivalent to conventional protein assays and have shown the ability to simultaneously perform up to 20 independent label-free immunoassays with no corresponding loss in sensitivity or precision. We have also adapted the technology for the multiplexed detection of DNA and RNA disease signatures without the need for sample amplification. In addition to these biosensing results, we will also discuss the detailed empirical characterization of the absolute mass and distance-dependent sensitivity of our microring sensing platform, which will serve as a guide for future device optimization efforts.

Bioconjugation of ultra-high-Q optical microcavities for label-free sensing

H. K. Hunt, A. M. Armani, The Univ. of Southern California (United States)

The development of label-free biosensors with high sensitivity and specificity is of significant interest to the fields of medical diagnostics and environmental monitoring, where rapid and real-time detection of antigens, bacteria, viruses, etc., is necessary. Optical microcavities, which have very low optical loss, are uniquely suited to sensing applications, but previous research efforts in this area have focused attention on the development of the sensor itself. While device sensitivity is dependent on the low optical loss, specificity is an equally important feature. Therefore, it is crucial to develop a high density, covalent surface functionalization process, which also maintains the device’s performance. Here, we demonstrate a facile method to impart specificity to optical microcavities, without adversely impacting their optical performance. In this approach, we selectively functionalize the surface of the silica microtoroids with amine-terminated, silane coupling agents of various lengths using both organic solvent and vapor deposition. The surface chemistry of these devices is demonstrated using X-ray photoelectron spectroscopy, fluorescent, optical and scanning electron microscopy, and contact angle measurements. The devices are characterized quantitatively by microcavity analysis, using tapered optical fibers and narrow linewidth, CW tunable lasers to measure the quality factors in the undercoupled regime, to determine the impact of the surface functionalization methods on the device sensitivity. The resulting devices show high density surface coverage, with no microstructural damage. This work represents one of the first examples of non-physiosorption-based bioconjugation of optical microtoroid resonators, which can be used for the label-free detection of biomolecules.

Fluorescence-enhancement in a polymer-based photonic crystal biosensor

B. Hamza, Y. Liu, J. M. Dawson, West Virginia Univ. (United States)

Detecting labeled or naturally-fluorescent single biomolecules at very low concentrations is of a significant importance for health sciences, agricultural sciences, and counter-terrorism applications. Photonic crystals (PhC) are microfabricated nano-structures of varying dielectric permittivity in one, two, or three dimensions that possess very unique light manipulation properties. These include the ability to prevent specific wavelengths from propagating in specific angles as well as localizing the electromagnetic waves at particular PhC lattice locations. Two novel ultra-sensitive detection modalities using thin-film PhC structures fabricated in semiconducting materials have been achieved. In the active modality, the adsorption of target molecules to the PhC surface causes a refractive index change that is translated into reflectance or transmission peak shifts. The passive modality demonstrated by our group utilizes the PhC structure to observe a ~25-fold fluorescence enhancement at specific resonant defect cavities within the PhC structures. However, integrating these semiconductor-based PhC structures with biocompatible microfluidic channels is a challenging task that can significantly increase the final cost of the sensor system. In this talk, we demonstrate soft lithographic nanofabrication techniques for polymer-based PhC structures that are easily integrated with microfluidic channels to provide a portable means of biosensing using PhC structures. Modeling and optical characterization results of the polymer-based PhC biosensor will also be presented.

Silicon photonic wire evanescent field sensors: sensor arrays and instrumentation


Instruments for detecting molecules and affinity binding analysis are essential components in the tool set used in drug development and fundamental biochemistry research, and eventually may also find applications in food safety and chemical sensing. We are developing a silicon photonic wire evanescent field (PWEF) sensor chip using 260 nm x 450 nm cross-section silicon photonic wire waveguides. The waveguide mode is strongly localized near the silicon surface, so that light interacts strongly with molecules that bind to the waveguide surface. The millimeter long sensor waveguides are folded into tight spiral structures less than 200 micrometers in diameter, which can be arrayed at densities up to ten or more independent sensors per square millimeter. The long propagation length in each sensor element gives a response to molecular binding much better than currently available tools for label free molecular sensing. Sensitivity is commonly used to compare affinity binding sensors. Nevertheless, cost of instrumentation, cost per measurement, ease-of-use, and the number of sensors that can be simultaneously monitored on a sensor array chip are equally important in determining whether an instrument is practical for the end user and hence commercially viable. The objective of our recent work on PWEF sensor array chips and the associated instrumentation is to address all of these issues. This conference paper will review the photonic wire sensor chip
design and layout, on-chip integrated fluidics, optical coupling, and chip interrogation using arrays of grating couplers formed using sub-wavelength patterned structures.

7888-05, Session 1

A novel evanescent field biosensor with an integrated photodetector array

K. L. Lear, R. Yan, D. S. Dandy, N. S. Lynn, R. A. Slayden, L. C. Kingry, Colorado State Univ. (United States)

A label-free, evanescent field biosensor is implemented using SiNx/ SiO2 optical waveguides in a conventional silicon integrated circuit manufacturing process. Rather than surface plasmon, interference, or resonance phenomena, the transduction mechanism relies on leaky-mode coupling to an array of photodetectors buried under the waveguide. Changes in surface refractive index locally modulate photocurrent, motivating naming the device a local evanescent array coupled (LEAC) sensor. Operation has been demonstrated using ~1 nm average thickness protein films as well as tuberculosis antigen and antibody capture probes. Preliminary investigations on virus detection are also reported as part of research to develop a multi-pathogen detection platform.

7888-06, Session 1

Molecular detection via hybrid peptide-semiconductor photonic devices

C. Gergely, E. Estephan, M. Saab, M. Martin, T. Cloitre, Univ. Montpellier 2 (France); C. Larroque, Institut de Recherche en Cancérologie de Montpellier (France); F. J. G. Cuisinier, Univ. Montpellier 1 (France)

The aim of this work was to investigate the possibilities to support device functionality that includes strongly confined and localized light emission and detection processes within nano/micro-structured semiconductors for biosensing applications. The interface between biological molecules and semiconductor surfaces, yet still under-explored is a key issue for improving biomolecular recognition in devices. We report on the use of adhesion peptides, elaborated via combinatorial phage-display libraries for controlled placement of biomolecules, leading to user-tailored hybrid photonic systems for molecular detection. An M13 bacteriophage library has been used to screen 1010 different peptides against various semiconductors to finally isolate specific peptides presenting a high binding capacity for the target surfaces. When used to functionalize porous silicon microcavities (PSM) and GaAs/AlGaAs photonic crystals, we observe (via AFM) the formation of extremely thin (~1nm) peptide layers, hereby preserving the nanostructuration of the crystals. This is important to assure the photonic response of these tiny structures when they are functionalized by a biotinilated peptide layer and then used to capture streptavidin. Molecular detection was monitored via both linear and nonlinear optical measurements. Our linear reflectance detection then used to capture streptavidin. Molecular detection was monitored via both linear and nonlinear optical measurements. Our linear reflectance measurement approach to microcavities in a biosensor and thus high sensitivity and better noise tolerance can be achieved without requiring very high resolution spectroscopic equipment. To test our hypothesis, we have developed a full vectorial finite element model of a silica toroidal micro-cavity immersed in water. Our modeling results show that a toroidal cavity with a major diameter of 70µm and a minor diameter of 6µm can achieve a sensitivity of 28.6 µs/refractive index units (RIU) at 580nm. Therefore a picosecond resolution detector would result in a lower limit to sensitivity of 5 x 10^-8 RIU. Hence we propose a micro-cavity ring down biosensor with high sensitivity which will find wide applications in real time and label free bio-sensing.

7888-07, Session 1

Application of ring-down measurement approach to microcavities for biosensing applications

M. I. Cheema, A. G. Kirk, McGill Univ. (Canada)

Resonant micro-cavities with ultra high quality factors (Q~10^8-10^9) have a great potential for ultra sensitive bio-sensing as a single photon will interact with a sample many times. Until now, most work on micro-cavity biosensors has been based on measurement of the resonant frequency shift induced by binding of biomarkers to a cavity. However, there are two aspects of this approach which impede practical applications: 1) Intensity fluctuations of the light source add noise; and 2) Due to the very high Q of the silica micro-cavity, high field intensities are produced, resulting in large thermal effects at resonance which result in thermally induced shifts in resonant frequency. We hypothesize that these two issues can be overcome by the application of the ring down measurement approach to the micro-cavity in a biosensor and thus high sensitivity and better noise tolerance can be achieved without requiring very high resolution spectroscopic equipment. To test our hypothesis, we have developed a full vectorial finite element model of a silica toroidal micro-cavity immersed in water. Our modeling results show that a toroidal cavity with a major diameter of 70µm and a minor diameter of 6µm can achieve a sensitivity of 28.6 µs/refractive index units (RIU) at 580nm. Therefore a picosecond resolution detector would result in a lower limit to sensitivity of 5 x 10^-8 RIU. Hence we propose a micro-cavity ring down biosensor with high sensitivity which will find wide applications in real time and label free bio-sensing.

7888-08, Session 1

Silicon-based photonic crystal nanocavities for label-free virus detection

S. Pal, A. R. Yadav, Univ. of Rochester (United States); B. L. Miller, Univ. of Rochester Medical Ctr. (United States); P. M. Fauchet, Univ. of Rochester (United States)

In recent years, photonic crystal (PhC) nano/micro cavities have evolved as ultra-sensitive optical biosensing platforms for real-time and label-free detection of analytes. The optical sensing platform exploited in this study is a two-dimensional w1 PhC waveguide created by removing a central array of holes from the crystal periodic structure [1, 2]. A nanocavity when coupled to the w1 waveguide, allows light to be transmitted except at frequencies that correspond to the resonant mode of the cavity. This PhC nanocavity design is extremely sensitive and allows multiplexed detection of analytes on a single biosensing platform. The PhC device was fabricated on silicon-on-insulator (SOI) substrates using standard nanolithography and reactive-ion-etching techniques. FDTD simulations indicate that the device is sensitive to the presence of single nanoparticles in the nanocavity region having radii in the range of 50-100 nm. The fabricated device has been tested with human papillomavirus (HPV) virus-like particles (VLPs) to study the sensor response in virus detection. Preliminary results show an average resonant red-shift of ~3 nm at high VLP concentrations with the device thus indicating successful detection of the VLPs. Future research will involve integration of optical manipulation functionalities into the above PhC design for single virus detection. The authors wish to thank the NSF (CBET-0730469) and the NIH (1 R01A108077-01) for financial support and the Cornell NanoScale Facility (NSF Grant ECS 03-35765) for device fabrication.

References:
PMMA-microcone resonators for biosensing applications
T. Beck, M. Hauser, T. Grossmann, S. Schleede, J. Fischer, H. Kait, C. Vannahme, T. Mappes, Karlsruher Institut für Technologie (Germany)
Micro cavities supporting high-Q whispering gallery modes (WGMs) have been intensively investigated for a wide range of fundamental and applied studies, including e.g. cavity quantum electrodynamics, low-threshold lasing, and chemical/biological sensors. For sensing applications the change of resonance wavelength of a WGM due to a binding event is exploited. These sensors derive their high sensitivity from the temporal photon confinement that is described by the quality factor of the mode, and the spatial confinement that is characterized by the mode volume. Here we report on a new type of WGM-resonators. These micro resonators are made of low-loss, thermoplastic polymer poly (methyl methacrylate) (PMMA). Large numbers of resonators can be directly processed on a single silicon substrate in parallel. A thermal reflow step results in an ultra-smooth surface leading to low scattering losses. Due to high quality factors (above two million) and the low-cost fabrication process, these conical shaped resonators are promising candidates for the development of sensitive and cheap sensors for biological materials like DNA-strands or proteins. Selective functionalization of every single resonator enables parallel detection of different substances on one chip. First results on sensing will be presented.

Confocal Raman microscopy for identification of bacterial species in biofilms
B. D. Beier, Univ. of Rochester (United States); R. G. Quivey, Jr., Univ. of Rochester Medical Ctr. (United States); A. J. Berger, Univ. of Rochester (United States)
Raman spectroscopy is a non-invasive technique capable of providing chemically specific information about a sample. In biomedical applications, the specificity of Raman spectroscopy has been used to discriminate between similar tissue or cell types, from identifying cancerous vs. healthy tissue to distinguishing between species or strains of bacteria. Implemented here through a confocal microscope, Raman spectroscopy has been used to distinguish between biofilm samples of two common oral bacteria species, Streptococcus sanguis and mutans. These species are of particular interest due to their association with dental plaque and periodontal disease. The specificity of Raman spectroscopy has been used here to determine the species composition of intact two-species hydrated biofilms. Preliminary results show that the method can be used to identify the composition of biofilms with high accuracy and precision. The results are in agreement with conventional methods of biofilm analysis, validating the feasibility of using Raman spectroscopy for rapid and non-invasive biofilm analysis.

Field-portable lensfree on-chip microscopy for detection of waterborne parasites
O. Mudanyali, C. Oztoprak, D. K. Tseng, A. Erlinger, A. Ozcan, Univ. of California, Los Angeles (United States)
Screening of water quality is a vital requirement to prevent water-related diseases caused by insufficient sanitation techniques.
Although current water-treatment methods offer effective solutions, highly populated urban areas and resource-limited settings still have this risk, and outbreaks continue to occur. Therefore, ancillary testing tools for rapid and quantitative determination of pathogenic contaminants in field-settings are needed to ensure public well-being.

To provide a solution to this need, here we introduce the use of a lensfree holographic on-chip microscope for detection of waterborne parasites within a mechanically robust, alignment-free and highly sensitive platform that weighs ~46 grams and measures 4.2x4.2x5.8 cm. Providing a numerical-aperture of ~0.2 over a wide field-of-view of ~24mm², this compact and light-weight on-chip microscope utilizes a simple LED and a CMOS sensor-array to record lensfree in-line holograms of the parasites. With minimum user interference, these lensfree holograms are then processed by custom-developed digital signal processing algorithms to rapidly provide microscopic images of the parasites. We successfully tested the performance of this on-chip imaging platform on waterborne pathogens such as Giardia Lamblia and Cryptosporidium Parvum, to achieve a detection limit of ~385 parasites/mL (with <10% mean error) without the use of any pre-concentration steps. Using well established sample preparation steps such as centrifugation and filtration, we can further improve this limit by e.g., ~100X to claim a detection sensitivity of ~5 parasites/mL. These initial results demonstrate the promising potential of this field-portable on-chip imaging tool for rapid screening of contaminants in both fresh and recreational water resources.

7888-14, Session 2

SAF immunodiagnostic system: subpicomolar sensitivity in minutes at low costs

T. Ruckstuhl, C. M. Winterflood, S. Seeger, Univ. of Zürich (Switzerland)

We introduce an inexpensive and easy-to-use immunosassay system for the sensitive detection of analytes within a short time. It comprises of polymer test tubes with incorporated detection optics and a compact fluorescence reader. The tubes are fastened by injection moulding and are designed for single use. The performance of the system is characterized for several relevant analytes in realtime sandwich immunoassays. Due to the exclusive capture of supercritical angle fluorescence (SAF) by the tube optics analyte concentrations below 1 pM can be detected within 15 min without washing steps. In order to prove the feasibility of this device the detection and analysis of interleukin-2 (IL-2) was performed as a simple test procedure. The formation of sandwich complexes at the receptor antibody coated tube bottom were monitored in realtime, allowing the measurement of IL-2 at concentrations as low as 50 pM within two minutes. Lower concentrations were measured using a sensitive readout mode based on photobleaching of the surface-bound fluorescence. For IL-2 the limit of detection within 20 min was 0.27 pM (4.5 pg/ml). Compared to the enzyme-linked immunosorbtant assay (ELISA) our method is much faster, less laborintensive and requires only a fraction of the amount of receptor and detection antibodies. Consequently, the SAF immunodiagnostic system addresses the need for the rapid detection of analytes at low costs. Its ease of use is well-suited for the non-professional operator. Efforts towards extending the device in order to create a parallel multiplexed assay system will also be discussed.

7888-15, Session 2

Portable surface plasmon resonance biosensors for on-site biodetection

J. Homola, Institute of Photonics and Electronics of the ASCR, v.v.i. (Czech Republic)

Surface plasmon resonance (SPR) biosensor technology has become a central tool for study of biomolecules and their interactions. Although the majority of SPR biosensors developed up to now have been designed for laboratory use, in recent years we have witnessed an increasing effort towards development of portable SPR sensing devices for use in the field. Such devices may be of great interest for rapid and sensitive detection of chemical and biological species in various fields such as medical diagnostics, environmental monitoring, food safety and security. In this paper, we present two recently developed SPR sensor platforms for field use. These sensors are based on resonant coupling of light into surface plasmons via diffraction on special diffractive structures and angular and wavelength modulation. The use of diffractive optics allows for construction of low-cost disposable SPR chips which can be readily integrated with functional biomolecular coatings and microfluidics into miniature easy-to-use cartridges. It is demonstrated that the portable SPR sensors can measure refractive index changes as small as 3x10^-7 and thus deliver performance that is fully comparable with the best available laboratory SPR sensors. Examples of bioanalytical applications of these sensors which will be discussed include environmental monitoring (detection of endocrine disrupting compound bisphenol A in wastewater), food safety (detection of antibiotic residues in milk) and medical diagnostics (detection of microRNA for early cancer diagnostics).

7888-16, Session 2

LED-interferometric reflectance imaging sensor for label-free detection of nanoparticles

G. Daaboul, Boston Univ. (United States); P. F. Renda, Graef, MITRE Corp. (United States); A. Yurt, X. Zhang, C. Lopez, J. H. Connor, Boston Univ. (United States); G. M. Hwang, MITRE Corp. (United States); M. S. Unlu, Boston Univ. (United States)

Single nanoparticle detection has received a great deal of attention recently because it could offer ultimate sensitivity for detection of virus and airborne pollutants that can be hazardous to human health. However, current nanoparticle detection technologies require extensive instrumentation and are too delicate to be used in the field. We report on a simple wide-field phase imaging technique, LED-Interferometric Reflectance Imaging Sensor (LED-IRIS), that utilizes on-chip common path interferometry for real-time detection and sizing of low-index particles such as viruses. The on-chip common path interferometer consists of a silicon substrate with a thin silicon dioxide top layer. The surface is serially illuminated with LEDs spanning the visible range and intensity images are recorded using a CCD camera. To size the nanoparticles on the surface the intensity response of the nanoparticles for multiple wavelengths are fit to a forward model based on interpreting the nanoparticles as dipoles on a layered substrate. We demonstrate nanoparticle sizing from 70nm-200nm in diameter and also resolve hundreds of individual H1N1 viruses over a wide area in a single experiment. An immunoasay is being developed for specific-capture and detection of single H1N1 virus using various probes multiplexed on the same substrate. The long-term impact of these results is the future development of a portable optical immunoassay technology which would address the need for high-throughput and ultra-sensitive virus detection in the US as well as in resource limited settings.

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7888-17, Session 2

Aqueous arrayed imaging reflectometry as a sensitive platform for real-time biomolecular interaction analysis

A. R. Yadav, Univ. of Rochester (United States); C. R. Mace, Harvard Univ. (United States); B. L. Miller, Univ. of Rochester Medical Ctr. (United States)

Arrayed Imaging Reflectometry (AIR) has been previously demonstrated as a highly sensitive biosensing technique, with picomolar limits of
detection observed for certain cytokines and growth factors. However, this detection has been on dry chip surfaces that preclude real-time monitoring of interactions. Here, an appropriate substrate for AIR imaging under an aqueous environment has been described, and its ability to detect Ångström level thickness differences has been demonstrated.

The simple substrate format used for dry AIR (a thermally grown oxide film on silicon) is unsuitable for imaging in an aqueous medium due to near grazing angles of incidence required to achieve total destructive interference. Hence, a new substrate was identified that would allow practically realizable incidence angles. This substrate consisted of a two-layer stack on silicon: a silicon nitride film followed by a sputtered oxide film. This resulted in minimum reflectance for 632.8 nm wavelength at an angle of incidence of ~52 degrees. The imaging setup used was essentially the same as that used for dry AIR, modified by the incorporation of a flow cell.

Several chips were patterned with oxide steps such that the background yielded minimum reflectance and the step heights were varied to test the thickness sensitivity of the setup. Easily detectable contrast was observed for single Ångstrom thickness steps imaged under water and several concentrations of sucrose. This substrate can thus be used to monitor biomolecular interactions in real time with a high sensitivity.

7888-18, Session 2

Chemical and biological detection using surface plasmon resonance in metalized ultrathin porous silicon membrane

K. Shome, M. N. Kavalenka, D. Z. Fang, P. M. Fauchet, Univ. of Rochester (United States)

Thin (15 nm) continuous metal films have been deposited onto ultrathin (≤ 30 nm) porous silicon membranes1 to create a novel free standing surface plasmon resonance (SPR) sensor platform. Different deposition geometries have been investigated to increase the generation of SPR and thus the sensitivity of the device. 3D FDTD simulations have been performed to identify and optimize the modes responsible for sensing2. Preliminary investigations have been carried out in sensing and differentiating between iso-propyl alcohol(IPA) and methyl alcohol(MeOH). The SPR transmission peaks for IPA and MeOH blueshift by 10 nm and 27 nm with respect to air. The prospect of this platform for biological sensing will be discussed.

This work was supported by the CEIS Bio Imaging Program of the New York State and by SIMPore Inc.


7888-19, Session 3

Nanofluidic Raman spectroscopy: how combining nanofluidics with SERS can provide new insights into protein aggregation

D. Erickson, Cornell Univ. (United States)

The extracellular and/or intracellular formation of protein aggregates is one of the pathological hallmarks shared by many diseases including ageing-related neurodegeneration and systemic amyloidosis. Trace detection and physicochemical characterization of protein aggregates can have a large impact in understanding and diagnosing diseases at early stages. Towards this end, multiple analytical techniques, including size exclusion chromatography, dynamic light scattering, fluorescence spectroscopy, circular dichroism spectroscopy, and nuclear magnetic resonance spectroscopy, have been employed to detect and characterize protein aggregates. While all these techniques are quite functional, it remains extremely challenging to detect and characterize very low levels of protein aggregates. In this talk I will introduce a novel approach for size-selective concentration and label-free detection of protein aggregates with disease implications. The method is based on the formation of micro/nanofluidic junctions on a nano-structured Raman active substrate. Using this technique we show the concentration dependence of protein aggregation over the low concentration ranges, which cannot be investigated with existing analytical tools. As a performance tests of a device, we investigated two types of protein aggregates, Cu/Zn-superoxide dismutase (SOD1) aggregates and amyloid beta (Aβ) fibrils, which are implicated in representative neurodegenerative diseases, Alzheimer’s disease and amyotrophic lateral sclerosis, respectively.

7888-20, Session 3

Optical and fluidic design for guaranteed trapping and detection of particles in a silicon microfluidic and photonic crystal system

A. Heiniger, P. M. Fauchet, Univ. of Rochester (United States)

Recent work has shown that optical forces on nanoparticles near photonic devices can be exploited for control of particle trajectories. Demonstrated effects include particle transport in a slot waveguide [1] and dynamic trapping and release of a particle in a photonic crystal (PhC) resonator [2]. Such control could lower the minimum concentration of particles that can be detected in PhC resonator biosensors. These devices can detect the presence of single particles, but only if the particle is located near the resonator [3]. The optical gradient force attracts particles to exactly this high intensity region.

However, optical forces are insignificant just a short distance from the resonator, if the particle is moving too fast, or if the field is too weak. The optical force also can cause the particle to be trapped short of the resonator if it is moving too slowly or if the field is too strong. In these cases, the sensor would fail to detect the particle.

We determine the flow rates and optical powers that ensure that a particle, if present, is captured in the resonator. We consider a slotted-ring PhC resonator and use finite element modeling to determine particle trajectories in the presence of fluidic and optical forces. We find that 10 mW of input power captures a particle traveling between 1 and 100 µm/s.


7888-21, Session 3

On-chip optofluidic concentrator

J. E. Baker, R. Sriram, Univ. of Rochester Medical Ctr. (United States); P. M. Fauchet, Univ. of Rochester (United States); B. L. Miller, Univ. of Rochester Medical Ctr. (United States)

Photonic crystal (PC) biosensing platforms have the potential to achieve single-pathogen detection using nanoscale optical resonant cavities. Real-time sample analysis requires the PC sensor to be interfaced with a fluidic environment, but current practical fluidic structures typically have dimensions much larger than the PC sensing cavities. To enhance sensing probability, an on-chip optofluidic structure is being developed to concentrate target material within a narrow sensing region of the microfluidic channel. The device relies on fluid drag forces to propel material along the microfluidic channel. Dielectric material is guided transversely within the microfluidic channel by optical gradient forces due to the evanescent field surrounding a ridge waveguide within the channel. Results of computational modeling and preliminary experimental trials are presented.
7888-22, Session 3

Merging nanophotonics and nanofluidics for active analyte delivery and biosensing

H. Altug, Boston Univ. (United States)

Label free biosensors are offering a rapid way to detect biomarkers and pathogens, and to determine the kinetics of biomolecular interaction. In particular, nanophotonic biosensors based on resonances are taking significant attention for detection of low concentrations of analytes with large multiplexing capabilities and signal-to-noise ratios. However, performances of surface biosensors are often controlled by the analyte delivery rate to the sensing surface instead of sensor’s intrinsic detection capabilities. For biosensors integrated with conventional microfluidic channels, analyte transportation to the sensor surface by diffusion severely limiting the performance. At low concentrations, this limitation known as mass transport limitation causes impractically long detection times. Previous approaches based on stirring and mixing strategies resulted in moderate performance improvements. One of the main conceptual constraints so far is that microfluidics and biosensing are always considered as different parts of a sensor platform rather than a fully merged single entity. In this talk, we demonstrate a new biosensing platform merging nanophotonics and nanofluidics. Unlike conventional approaches where the analytes simply stream pass over the surface, our platform enables active delivery to the sensing surface. Our detection platform is based on suspended nanohole arrays supporting photonic-plasmonic resonances. The nanoholes also act as nanofluidic channels connecting the fluidic chambers on both sides of the sensors. Using our platform, we show 14-fold increase in mass transport rate constant appearing in the exponential term. Such an improvement means superior analyte delivery to the biosensor surface and dramatically improves sensor response time at low concentrations.

7888-23, Session 3

All-fiber optofluidic biosensor

Y. Guo, H. Li, J. Liu, K. Chinna Balareddy, X. Fan, Univ. of Michigan (United States)

Optical fiber provides a unique and versatile platform for developing point-of-care optical sensing systems. Here we propose a novel optofluidic biosensor, which fully utilizes optical fibers to achieve highly-sensitive, label-free biomolecular detections with inherent fluidic channels. This sensor consists of two single mode fibers (SMFs) with reflecting surfaces and a photonic crystal fiber (PCF) vertically sandwiched by them. Firstly, the SMFs act as waveguides for delivering light into and out of an optofluidic device (like PCF); secondly, instead of using the optical properties of the PCF, we take advantage of its inherent multiple fluidic channels and large sensing surface; thirdly, the two reflecting surfaces and the PCF form a Fabry-Perot resonator and its resonance mode is sensitive to the change of the properties in the fluidic channels, which can be used to detect the substances flowing through the fluidic channels or deposit on the channel surface. In the report, we will explore the operating principle of the all-fiber optofluidics biosensor, theoretically and experimentally investigate its feasibility and sensitivity. The all-optical optofluidic sensor is a promising technology platform for multiplexing, highly-sensitive and accurate biomolecular detection.

7888-24, Session 3

Application of field-modulated birefringence and light scattering to biosensing

L. H. Strong, D. B. Hall, C. Edson, G. Varadi, Radiation Monitoring Devices, Inc. (United States)

Superparamagnetic nanoparticles (NPs) coated with surface ligands are shown to be an effective means to impart magnetic field modulation to optical signals from targeted receptor complexes. The resulting temporally modulated signals can be used for a number of important high throughput applications in bio-sensing including: detecting (weaponized) viruses, screening recombinant libraries of proteins, identifying pathogenic conversions of microbes, and monitoring gene amplification. We will compare the results of two dynamic methods of measuring target binding to NPs: birefringence and field modulated light scattering (FMLS). These measurements reflect complementary manifestations of NP alignment (orientation) and de-alignment (relaxation) dynamics. Birefringence originates from the specific crystalline properties of a small subset of paramagnetic NPs (for example, maghemite ) when oriented in a magnetic field. Upon quenching the field, it decays at a rate exhibiting the Debye-Stokes-Einstein rotational relaxation constant of the target-NP complex. Birefringence relaxation reflects the particle dynamics of the mixed suspension of NPs, with signal components weighted with respect to both free and complexed NP size distributions. FMLS relaxation signals, on the other hand, originate predominantly from the inherent optical anisotropy of the target complexes, show little contribution from non-complexed NPs, and provide a more direct and accurate method for determining target receptor concentrations. Several illustrations of the broad range of applications possible using these dynamic measurements and the kind of information to be derived from each detection modality will be discussed.

7888-25, Session 3

Optofluidic biosensing with colorimetric signatures of deterministic aperiodic metal nanoparticle arrays

S. Y. Lee, S. V. Boriskina, Boston Univ. (United States); F. G. Omenetto, Tufts Univ. (United States); B. M. Reinhard, L. D. Negro, Boston Univ. (United States)

In this study, we study colorimetric optical sensing of biological substance within microfluidic channels by investigating distinctive changes of light scattering spectra and colorimetric patterns from nanostructured aperiodic surfaces. Aperiodic arrays consisting of metallic nanoparticles on quartz substrates with minimum interparticle separations ranging from 50nm to 200nm were fabricated using electron beam lithography and 20 micron-high microfluidic PDMS channels were imprinted from a SU8-mold. We have recently shown that the colorimetric fingerprints of aperiodic nanosturctures feature broadband frequency responses with wide angular intensity distributions and are ideally suited as a novel transduction mechanism for optical biosensing in a microfluidic environment. Our previous work has demonstrated that chromium (Cr) Gaussian prime nanoparticle arrays can detect protein monolayers with attomolar sensitivity by monitoring structural modifications of the scattered fields with simple autocorrelation analysis. In this work, DNA molecules flowing in the microfluidic channel are bonding to the nanostructured gold (Au) surfaces and their scattering spectra are experimentally measured under white light illumination with conventional dark-field spectroscopy. Using DNA molecules as our target, we fabricated and optimized different aperiodic structures, which provide different field localization patterns for enhanced biological sensing in a microfluidic environment. Our results demonstrate for the first time that the characteristic colorimetric fingerprints observed in a microfluidic environment can be directly utilized as highly sensitive platform for DNA detection and developed as an inexpensive optical biosensor. The integration of this sensitive technique with microfluidic technology may result in the engineering of novel integrated, multiplexed, optofluidic lab-on-a-chip platforms for bio-chemical detection.
An integrated microfluidic biosensor for the rapid screening of foodborne pathogens by surface plasmon resonance imaging

M. D. Zordan, M. G. Grafton, J. F. Leary, Purdue Univ. (United States)

The rapid detection of foodborne pathogens is of vital importance to keep the food supply rid of contamination. Previously we have demonstrated the design of a hybrid optical device that performs real-time surface plasmon resonance (SPR) and epifluorescence imaging. Additionally we have developed a biosensor array chip that is able to specifically detect the presence of two known pathogens. This biosensor detects the presence of the pathogen strains by the selective capture of whole pathogens by peptide ligands functionalized to the spots of the array. We have incorporated this biosensor array into a self contained PDMS microfluidic chip. The enclosure of the biosensor array by a PDMS microfluidic chip allows for a sample to screened for many strains of pathogens simultaneously in a safe one time use biochip. This disposable optical biochip is inserted into with the hybrid SPR/epifluorescence imaging device to form an integrated system for the detection of foodborne pathogens. Using this integrated system, we can selectively detect the presence of E. coli 0157:H7 or S. enterica in a simultaneously in real-time. Additionally, we have modeled the mechanical properties of the microfluidic biochip in order to manipulate the flow conditions to achieve optimal pathogen capture by the biosensor array. We have developed an integrated system that is able to screen a sample for multiple foodborne pathogens simultaneously in a safe, rapid and label-free manner.

The radix 4 base number system for use in theoretical genetics

B. S. Tice, Advanced Human Design (United States)

A radix 4 base number system will be used as a compression program in the area of theoretical genetics with applications to DNA sequences. Examples from the field of genetics will be presented in the paper.
We demonstrate new ultrahigh speed, swept source/Fourier domain OCT instrumentation at up to 400,000 axial scans per second using short cavity swept laser technologies. Short round trip times of the short cavity enable a significant increase in sweep speeds over conventional swept laser technology. Using a data acquisition clock derived from the optical sweep, the A/D converter can be clocked at variable rate, such that the OCT fringe is automatically sampled to be linear in k (wavenumber). The long coherence length of the new lasers results in superior sensitivity roll-off when compare to spectral/Fourier domain and conventional swept source OCT technology. The laser sweep rate of 100kHz can be doubled by buffering and multiplexing the sweep to achieve 200kHz axial scan rates. Data acquisition rates can be further increased by imaging using two beams, two interferometers, and two balanced detectors in parallel to achieve a 400,000 axial scan rate, which we believe is the fastest acquisition rate performed in vivo for the human retina. A large 12mmx10mm volume consisting of 1100x930 transverse pixels acquired in 2.9 seconds enables comprehensive visualization of the optic disc and fovea within the same acquisition. Acquisition of multiple data sets with orthogonal scan directions enables the generation of motion corrected 3D volumes. The results of this paper suggest that swept source/Fourier domain OCT using short cavity lasers, sweep buffering, multibeam OCT acquisition, and 3D data registration will be important enabling technologies for ultrahigh speed OCT imaging in ophthalmology and other applications.

We demonstrate retinal OCT imaging at up to 1.4M A-scans per second. The system relies on a buffered Fourier domain mode locked (FDML) laser with a sweep range of up to 80nm centered at 1050nm. Hardware spectral shaping is employed to achieve resolutions <10µm in tissue at 685kHz. Shot noise limited detection is achieved by a Michelson interferometer layout that corrects for chromatic imbalances, improving common mode noise rejection. This way sensitivities of 95dB (685kHz) and 92dB (1.4MHz) are obtained with only 1.5mW on the sample. The system provides more than 4x higher speed than previously demonstrated ultra-high speed OCT setups.

We developed AO-OCT by combining an AO retinal scanner and spectral domain OCT with one-micrometer probe. A broadband achromatizer was newly designed for 700 - 1150 nm wavelength band using Zemax (ZEMAX Development Corporation, WA). The performance of the achromatizer was analyzed using an extended Condon equation up to 1200 nm and compared with the previously demonstrated achromatizers. To demonstrate high-speed retinal imaging, we used a prototype InGaAs camera with a line rate of 100,000 lines/s (Hamamatsu Photonics K.K., Hamamatsu, Japan), which provides a speed of 3 volumes/s and an en face image size of 256 x 128 pixels. In addition, to demonstrate high-penetration Doppler imaging, we used an InGaAs camera with a line rate of 47,000 lines/s (Sensors Unlimited, Inc., NJ). As a result, we measured high contrast photoreceptor mosaic and Doppler signals beneath the choriocapillaris. 1-µm AO-OCT is capable of providing a higher contrast and higher resolution photoreceptor mosaic than 1-µm AO-SLO. Responsible for this improvement is the confocality that OCT provides and the addition of a broadband achromatizer. High-penetration Doppler imaging demonstrated blood flow in the choroid, similar to non-AO-OCT. AO-OCT will provide a more precise blood flow measurement.

We have developed a non-invasive photoacoustic ophthalmoscopy (PAOM) for in vivo retinal imaging. PAOM detects the photoacoustic signal induced by pulsed laser light shined onto the retina. By using a stationary ultrasonic transducer in contact with the eyelids and scanning only the laser light across the retina, PAOM provides volumetric imaging of the retinal micro-vasculature and retinal pigment epithelium at a high speed. For B-scan frames containing 256 A-lines, the current PAOM has a frame rate of 93 Hz, which is comparable with state-of-the-art commercial spectral-domain optical coherence tomography (SD-OCT). By integrating PAOM with SD-OCT, we further achieved OCT-guided PAOM, which can provide multi-modal retinal imaging simultaneously. The capabilities of this novel technology were demonstrated by imaging both the microanatomy and microvasculature of the ret retina in vivo.
Ultra-high-speed in-vivo Fourier-domain full-field OCT for the human retina

T. Bonin, M. Hagen-Eggert, G. Franke, Medizinisches Laserzentrum Lübeck GmbH (Germany); P. Koch, Thorlabs GmbH (Germany); G. Hüttmann, Medizinisches Laserzentrum Lübeck GmbH (Germany)

Since the first demonstration of in-vivo imaging of the retina, optical coherence tomography (OCT) has changed the diagnosis in ophthalmology. Today, the fundamental speed limit to retinal imaging is not given by the technology but by the exposure limit to the retina. The limited photon flux, which is allowed to enter the eye, directly links imaging speed and OCT sensitivity, which is given by the number of photons detected in one A-scan if the detection is quantum noise limited.

In this work, in-vivo full field (FF) optical coherence tomography (OCT) images of human retina with 2.6 million A-lines/s are presented by using a rapidly tunable laser source in combination with an ultra-high speed CMOS camera. It is shown that Fourier domain (FD) full field OCT principally provides a way to overcome limitations in imaging speed which are posed by the maximal possible exposure (MPE) of the retina. It combines a simple setup without any moving parts with a high sensitivity by taking advantage of a spatially parallel image acquisition which allows for a significantly higher retinal exposure.

Cross-talk reduction by incoherent illumination was expendable for imaging the low scattering layers of the retina above the retinal pigment epithelium (RPE). With a 100–Hz sweep rate FF-OCT was fast enough to acquire OCT images without motion artifacts. FF-OCT may therefore become an attractive alternative for ultrafast retinal imaging boosting image speed by a lack of moving parts and the use of considerably higher irradiation power.

Fast dispersion-encoded full-range OCT for retinal imaging at 800 nm and 1060 nm

B. Hofer, B. Považay, A. Unterhuber, B. Hermann, Medizinische Univ. Wien (Austria); L. Wang, S. M. Rey, Cardiff Univ. (United Kingdom); G. Matz, Technische Univ. Wien (Austria); W. Drexler, Medizinische Univ. Wien (Austria) and Cardiff Univ. (United Kingdom)

The dispersion mismatch between sample and reference arm in frequency-domain OCT can be used to iteratively suppress complex conjugate artifacts and thereby increase the imaging range. We propose a fast dispersion encoded full range (DEFR) algorithm that detects multiple signal components per iteration. The influence of different dispersion levels on the reconstruction quality is analyzed for in vivo retinal tomograms at 800 nm. Best results have been achieved with about 30 mm SF11, with neglectable resolution decrease due to finite resolution of the spectrometer. Our fast DEFR algorithm achieves an average suppression ratio of 55 dB and converges within 5 to 10 iterations. The processing time on non-dedicated hardware was 5 to 10 seconds for tomograms with 512 depth scans and 4096 sampling points per depth scan. Application of DEFR to the more challenging 1060 nm wavelength region is demonstrated by introducing an additional optical fibre in the sample arm.
In-utero imaging of mouse embryonic development with optical coherence tomography

S. H. Syed, Univ. of Houston (United States); I. V. Larina, M. E. Dickinson, Baylor College of Medicine (United States); K. V. Larin, Univ. of Houston (United States)

Embryonic imaging is the most important tool in understanding and investigating developmental diseases. Mouse embryos have long served as an ideal model for the study of mammalian embryonic developmental processes. However, there are several challenges when performing phenotypic analysis of mouse embryos. Several imaging modalities have provided in utero imaging of mouse embryos such as MRI, MicroCT and Ultrasound. However, most of the imaging methodologies are either required to be made invasive, or have low resolution. Optical Coherence Tomography (OCT) is a promising technique introduced recently to developmental biology. Despite of limited depth penetration when compared to other imaging modalities, OCT can perform live mouse embryonic imaging in utero. In this study we introduce OCT as a novel technique to image live mouse embryos from stage 12.5 through 17.5 days post-coitus (dpc). During these stages of gestation development of limb, brain and eye was explored. This study suggest that OCT can serve as a powerful tool to image mouse embryos with a resolution of ~8 µm and can help in understanding abnormalities in developmental processes caused by mutations, or toxic drugs.

Semi-automatic segmentation of 4D OCT images of the avian embryonic heart using a level sets approach

A. P. Bishop, M. Gargesha, M. W. Jenkins, D. L. Wilson, A. M. Rollins, Case Western Reserve Univ. (United States)

We are developing Optical Coherence Tomography (OCT) technologies and image processing methods for the study of embryonic cardiac structure and function. Here we describe a semi-automatic algorithm for segmenting the compact myocardium of the avian embryonic heart from 4-D OCT imaging data (3D volumes + time) using an iterative Level Sets segmentation technique. In order to validate our image segmentation algorithm, we have employed the popular Dice Similarity Coefficient metric using human-expert segmented contours as ground truth. Rapid segmentation will enable us to fully characterize the function of multiple hearts used in a study by allowing measurements of morphology, hemodynamics and stresses in a timely manner. Our results have been encouraging and hold much promise towards the development of a fully automated segmentation tool for 4D OCT images.

Three-dimensional functional imaging of lung parenchyma using Fourier-domain optical coherence tomography combined with fluorescence microscopy

M. Gärtner, P. Cimalla, L. Knels, S. Meissner, E. Koch, Dresden Univ. of Technology (Germany)

Optical coherence tomography (OCT), as a non-invasive technique for studying tissue morphology, is widely used in vivo studies, requiring high resolution and fast three-dimensional imaging. Based on light scattering it reveals micrometer sized substructures of the samples due to changes in their optical properties and therefore allows quantification of the specimen’s geometry. Utilizing fluorescence microscopy, further information can be obtained from molecular compositions embedded in the investigated object. Fluorescent markers, specifically binding to the substance of interest, reveal the samples chemical structure and give rise to functional studies.

This research presents the application of a combined OCT and laser scanning confocal microscopy (LSCM) system to investigate structural details in lung tissue. OCT reveals the three-dimensional morphology of the alveoli whereas fluorescence detection, arising from the fluorophor Sulforhodamin B (SRB), which is binding to elastin, shows the elastic meshwork of the organ’s extracellular matrix. Different plains of fluorescence can easily be obtained by using a piezo driven objective and exploiting the confocal functionality of the setup. Both techniques, combined in one optical system, not only ease the experimental procedure but also contribute to a thorough description of tissue’s morphology and chemical composition. Especially in the research field of lung protective ventilation strategies there is a need for detailed geometrical and molecular information. The data can be used for mathematical lung modeling and simulation of its dynamics in dependence on airway pressure and tidal volume.

Imaging necrosis in mouse models of muscular dystrophy with three-dimensional optical coherence tomography

B. R. Klyen, T. Shavlakadze, M. D. Grounds, D. D. Sampson, The Univ. of Western Australia (Australia)

Biological tissue from animal models is routinely assessed in pre-clinical experiments of therapies for human muscular dystrophy. This paper reports the use of three-dimensional optical coherence tomography (3D-OCT) in conjunction with Evans blue dye (EBD) for visualizing necrotic lesions in skeletal muscle tissue from exercise-induced damage models of dystrophopathy. 3D-OCT scans were acquired using a time-domain optical coherence tomography system with center wavelength of 1320nm, resolution of ~8µm and ~11µm in the axial and lateral dimensions respectively, and system sensitivity of 104dB. Ex vivo skeletal muscle samples were obtained from the hindlimbs of the mdx mouse model of human Duchenne muscular dystrophy. 24 hours prior to sampling, the mice completed a treadmill-exercise protocol and were injected with EBD, an established method for visualizing muscle fiber permeability. This is an indicator for the onset and presence of necrosis. Imaged samples underwent serial histological sectioning and haematoxylin and eosin (H&E) staining for subsequent analysis and necrotic lesion identification. Skeletal muscles of the hindlimb that displayed an accumulation of EBD were preferentially 3D-OCT imaged. Analysis of these 3D-OCT data sets revealed a characteristic pattern of disrupted fiber structure and lower OCT signal. When compared with the corresponding H&E-histology, these regions were routinely found to correlate with necrotic lesions, areas of muscle fiber breakdown, necrosis and inflammation. The results reported here show that EBD can be used to guide 3D-OCT imaging for the identification of necrotic lesions in small animal imaging studies of human muscular dystrophy.

Imaging of retinal tissue changes during photocoagulation by high-speed OCT

H. H. Müller, L. Ptaszynski, K. Scholt, T. Bonin, M. Bever, G. Hüttmann, R. Brinkmann, R. Birngruber, Medizinisches Laserzentrum Lübeck GmbH (Germany)

Photocoagulation is one of the most successful laser therapies in medicine. By irradiating the retina with a cw-laser, tissue damage is produced, which spans from the RPE to the adjacent parts of the neural retina. Recently, an interest in more selective retinal treatments is
observed. With microsecond irradiation or near threshold coagulation, a damage confined to the RPE is possible.

OCT is the only non-invasive imaging modality, which can visualize the layered structure of the retina in-vivo. Though post operatively OCT images showed changes in tissue morphology due to the photocoagulation, the direct response of the tissue during and shortly after irradiation is still not known.

Aim of this study was to visualize the tissue changes in the retina during photocoagulation by high speed phase sensitive OCT. Experimental laser treatments of the retina of enucleated porcine eyes were followed by high speed phase-sensitive OCT. OCT could visualize the increase of tissue scattering during the photocoagulation in a time-resolved way. Immediate and late tissue changes were visualized with more than 15 µm resolution. OCT may play an important role in understanding the mechanisms of photocoagulation. This may lead to a new treatment strategies.

The measured OCT images were correlated with fundus images and measurements of the temperature development during the coagulation by a new optoacoustic thermometer.

Visualization of vitreoretinal surgical manipulations using intra-operative spectral domain optical coherence tomography

Y. K. Tao, J. P. Ehlers, C. A. Toth, J. A. Izatt, Duke Univ. (United States)

Vitreoretinal surgical visualization by ophthalmic microscopy is limited in its ability to distinguish thin translucent tissues from other retinal substructures. Conventional methods for supplementing poor contrast, such as with increased illumination and application of exogenous contrast agents, have been limited by the risks of toxicity at the retina. Spectral domain optical coherence tomography (SDOCT) has demonstrated strong clinical success in retinal imaging, enabling high-resolution, motion-artifact-free cross-sectional imaging and rapid accumulation of volumetric macular datasets. Current generation SDOCT systems achieve <5 µm axial resolutions in tissue, and have been used to obtain high resolution datasets from patients with neovascular AMD, high risk drusen, and geographic atrophy. Recently, an intraoperative microscope-mounted OCT system (MMOCT) was presented as a method of augmenting a surgical microscope to concurrently acquire high-resolution, high-contrast SDOCT volumetric datasets. Here, we demonstrated the utility of intraoperative MMOCT for the visualization of vitreoretinal surgical procedures. Vitreoretinal surgery was simulated by performing procedures, through an ophthalmic surgical microscope, on cadaveric porcine eyes. The datasets acquired with the MMOCT show both instrument-tissue interaction as well as the ability of OCT to image certain surgical tools, which would directly translate to better surgical visualization and impact the treatment of ocular diseases.

Investigation of retinal blood flow in glaucoma patients by Doppler Fourier-domain optical coherence tomography

Y. Wang, X. Zhang, O. Tan, D. M. Huang, Doheny Eye Institute (United States)

The measurement of ocular blood flow is important in studying the pathophysiology and treatment of several leading causes of blindness. A pilot study was performed to evaluate the total retinal blood flow in glaucoma patient using Fourier domain optical coherence tomography. For normal people, the measured total retinal flow was between 40.8 and 60.2 µl/minute. We found that eyes with glaucoma had decreased retinal blood flow and average flow velocity, while the venous cross sectional areas were essentially the same as normal. The decrease in blood flow was highly correlated with the severity of visual field loss.

Visualization of human retinal microcapillaries with phase contrast high-speed optical coherence tomography

D. Kim, UC Davis Medical Ctr. (United States); J. P. Fingler, California Institute of Technology (United States); J. S. Werner, UC Davis Medical Ctr. (United States); D. M. Schwartz, Univ. of California, San Francisco (United States); S. E. Fraser, California Institute of Technology (United States); R. J. Zawadzki, UC Davis Medical Ctr. (United States)

We present high-speed Fourier-domain optical coherence tomography (Fd-OCT) with the phase variance based motion contrast method for visualizing retinal micro-circulation in vivo. This technique allows reconstruction of a high sensitivity, two-dimensional retinal perfusion map and concurrently volumetric morphology of retinal microvasculature. Histogram-based thresholding processing is implemented to remove residual high contrast (phase) data caused by eye motion. The phase variance based motion contrast procedure generates the projection view of retinal micro-vasculature. In addition, the high speed acquisition rate at 125 kHz A-scans enables reduction of motion artifacts and increase of the scanning area. Several scanning settings with different sampling densities and scanning areas are evaluated to find optimal parameters for in vivo imaging. In order to evaluate this technique, we compare OCT micro-capillary imaging using the phase contrast technique with fluorescein angiography (FA). Additionally, volumetric visualization of blood flow for normal subjects and patients is presented. The phase contrast OCT image has similar blood vessel networks of the retina with the FA image. Increased imaging speed of the system allows shorter acquisition time, resulting in reduction of imaging artifacts caused by involuntary eye motion. This method has significant potential for early diagnosis of vascular retinal diseases and monitoring treatment outcomes.

Wide-field human retina and choroid visualization using swept-source optical coherence tomography at 1060 nm

R. Motaghiannezam, S. E. Fraser, California Institute of Technology (United States)

A wide-field volumetric swept source optical coherence tomography (SS-OCT) for the intraretinal layer and choroidal substructure visualization is presented. We developed a 1060 nm swept laser source with ~30 nm tuning range (1013-1103 nm) and 0.16 nm instantaneous line width at 50 kHz. The axial SS-OCT depth-scanning range and axial resolution were determined to be ~5.1 mm and 9 µm in air (6.5 um in tissue), respectively. The transverse resolution was ~20 µm. Through high-speed SS-OCT imaging of the human retina and choroid, we were able to investigate the optic nerve head and the macula morphology and reconstruct the retinal and choroidal structure without the use of contrast agents. Horizontal tomograms (10 mm, at ~1024-depth scans) acquired in vivo from a healthy volunteer depict the retina and choroidal layers, as well as main choroidal vasculature across different retinal sections. To avoid microsaccade, 3D OCT data sets were collected by acquiring several neighboring B-scans across 1 cm² area within ~3 seconds. SS-OCT depth-integrated enface images were obtained at different depths to differentiate all retinal layer and choroidal substructure. We show that these enface images are able to visualize the superficial blood vessels and network of larger capillaries within ganglion cell (GCL) layer as well as the choroidal vasculature. A spotted appearance was found beneath the RPE (the upper part of the choroid) in the choriocapillaris (CC) enface image. Finally, we performed imaging in 5.7 second over a 500 um² field of view to achieve the optimum transversal oversampling distance and investigate the interconnected capillary network in the retina and CC.
Optimized Doppler optical coherence tomography for choroidal capillary vasculature imaging

G. Liu, W. Qi, L. Yu, Z. Chen, Beckman Laser Institute and Medical Clinic (United States)

In this manuscript, we analyzed the retinal and choroidal blood vasculature in the posterior segment of human eye with optimized optical coherence color Doppler and standard deviation Doppler method. Depth-resolved structure, color Doppler and standard deviation Doppler images were compared. Blood vessel down to capillary level was able to be obtained with the optimized optical coherence color Doppler and standard deviation Doppler method. A novel, simple and fast segmentation algorithm to indentify retinal pigment epithelium (RPE) was proposed and used to segment the retinal and choroidal layer. The algorithm was based on the detected OCT signal intensity difference between different layers. A spectrometer-based Fourier domain optical coherence tomography (OCT) system with central wavelength of 890nm and bandwidth of 150nm was used in this study. The 3 dimensional imaging volume contained 120 sequential two dimensional images with 2048 A-line per image. The total imagine time is 12 seconds and the imaging area is 5x5 mm².

In-vivo human retina imaging with 5-µm axial resolution, at 92 A-scans/s with 1-µm spectral-domain OCT system

S. Hariri, P. Lee, A. Akhlagh Moayed, K. K. Bizheva, Univ. of Waterloo (Canada)

We have outfitted a 1060nm Spectral Domain Optical Coherence Tomography system with a prototype, high speed infrared linear array camera and a custom spectrally reshaped superluminescent diode to achieve 5µm axial resolution at 91,911 A-scans/s/image acquisition rate in-vivo in the human retina. 4dB loss of sensitivity was observed as a result of the reduced integration time (7µs) of the fast camera as compared to similar commercially available cameras with 14µs integration time and 47kHz readout rate. Fewer motion artefacts were observed in the retinal images acquired with the fast camera, while the higher axial resolution along with deeper penetration allowed for improved visualization of fine morphological details such as retinal and choroidal capillaries and the deep choroidal structure.

Multibeam optical coherence tomography system with a single-line sensor for human eye

N. Suehira, H. Yoshida, T. Yuasa, M. Sato, K. Yamada, Canon Inc. (Japan)

We newly developed a multi beam spectral domain optical coherence tomography system. The number of the beams is three, the total line rate is equivalent to 210 A-scans/s and the maximum scan area is a 10mm square on fundus with the beams. Those three beams focus on fundus 3.1mm apart from each other to satisfy ANSI safety standards. The spectrometer consists of a single line sensor for the three beams. We can acquire 3dimensional data after piecing together by calibrations of depth resolutions and roll-off properties. A preliminary result from a healthy human eye is shown.
7889-23, Session 4

Automated stent strut coverage and apposition analysis of in-vivo intra-coronary optical coherence tomography images


Several studies have proved that intravascular OCT is an appropriate imaging modality able to evaluate stent strut apposition and coverage in coronary arteries. Currently image processing is performed manually resulting in a very time consuming and labor intensive procedure.

We propose an algorithm for fully automatic individual stent strut apposition and coverage analysis in coronary arteries. The vessel lumen and stent strut are automatically detected and segmented through analysis of the intensity profiles of the A-scan lines. From these data, apposition and coverage can then be estimated automatically. The algorithm was validated using manual measurement (performed by two trained cardiologists) as a reference. 108 images were taken at random from in-vivo pullbacks from 9 different patient presenting ‘real-life’ situations (i.e. blood residual, small luminal objects and artifacts). High Pearson’s correlation coefficients were found (R = 0.96 - 0.95) between the automated and manual measurements while Bland-Altman statistics showed no significant bias with good limits of agreement. As such, it was shown that the presented algorithm provides a robust and a fast tool to automatically estimate apposition and coverage of stent struts in in-vivo pullbacks. This will be important for the integration of this technology in clinical routine and large clinical trials.

7889-25, Session 4

The influence of balloon on endoscopic OCT imaging: preliminary observations in swine and human esophagus

W. Kang, H. Wang, G. Isenberg, A. Chak, Z. Hu, A. M. Rollins, Case Western Reserve Univ. (United States)

Volumetric esophageal imaging using Fourier-domain optical coherence tomography is under investigation as a secondary Barrett’s Esophagus (BE) surveillance tool to mitigate the sampling error associated with conventional biopsy diagnosis. One key technology was the cathether probe with a balloon attached to support the mucosal wall. However, balloon contact and pressure on the mucosa affects the visualization of the mucosal surface topology, which is one feature identified for specialized intestinal metaplasia diagnosis and for diagnosis of dysplasia within BE. We have also shown that catheter pressure on colonic mucosa significantly alters the tissue appearance. These observations raise the question whether balloon contact and pressure affects image features that are diagnostic of dysplasia within BE. In this paper, we compare the appearance of normal swine esophagus with and without balloon contact and pressure. The center-symmetric auto-correlation (CSAC) texture features, which have been utilized for computer-aided diagnosis of dysplasia, are also calculated. Two-way analysis of variance (ANOVA) shows 5 out of the 6 CSAC features exhibit significant difference between the two balloon-tissue conditions. We also present preliminary images of normal and high grade dysplastic BE in human patients to illustrate the influence of balloon-tissue contact on the surface topology. Our observations suggest the need to further investigate the advantages and disadvantages of balloon-tissue contact and pressure, and how tissue image features in BE are altered.

7889-24, Session 4

An interactive volumetric microscopy and guided biopsy platform for the management of Barrett’s patients

M. J. Suter, H. Yoo, Massachusetts General Hospital (United States) and Harvard Medical School (United States); K. A. Gallagher, Massachusetts General Hospital (United States); J. R. Thiesse-Namati, G. Y. Lauwers, Massachusetts General Hospital (United States) and Harvard Medical School (United States); B. E. Bouma, Massachusetts General Hospital (United States) and Massachusetts Institute of Technology (United States); N. S. Nishioka, Massachusetts General Hospital (United States) and Harvard Medical School (United States); G. J. Tearney, Massachusetts General Hospital (United States) and Massachusetts Institute of Technology (United States)

Balloon-based optical frequency domain imaging (OFDI) of the esophagus may reduce the sampling error associated with conventional screening and surveillance of Barrett’s patients. Previously, it was not possible to register the OFDI images with endoscopy to confirm the OFDI diagnosis or to guide treatment. We have developed an interactive guided biopsy platform to conduct volumetric OFDI and to use the acquired images to select biopsy sites that are subsequently marked so that they are visible by endoscopy. We will discuss our clinical experience conducting OFDI in patients undergoing regular screening and surveillance for Barrett’s, and our recent developments in OFDI guided biopsy.

7889-26, Session 4

Three-dimensional optical coherence tomography for transcapsule optical biopsy of lymph nodes

R. John, A. Ahmad, E. J. Chaney, M. Marjanovic, K. V. Tangella, S. A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Lymph nodes are highly specialized and vital immunological organs in the lymphatic system that house a large number of lymphocytes and macrophages. A proper evaluation of the gross and microscopic features of lymph nodes would reflect any possible pathological condition of those parts of the body that drain to the respective node. Optical coherence tomography (OCT) is an emerging in vivo imaging modality with resolutions as high as 1-5 µm and is capable of providing real-time microscopic images up to 2 mm beneath the tissue surface. OCT has been used previously to image breast cancer tumor margins intraoperatively in human subjects, and recently, studies have shown the potential of OCT for imaging lymph nodes.

In this study, we present ex vivo trans-capule imaging and pathological assessment of intact popliteal lymph nodes from a preclinical tumor model using OCT. Studies reported here clearly demonstrate that OCT is capable of differentiating normal, reactive, and metastatic lymph nodes based on the structural changes in the lymph nodes. The optical scattering and structural changes revealed by OCT from the 3rd day post-injection of tumor cells to the lymphatic system to the 11th day with reference to normal lymph nodes highly correlate with the inflammatory and immunological changes observed in the capsule, prectoral regions, follicles, and germination centers in the histopathological investigations. These results emphasize the significant potential of OCT as a technique for intraoperative real time in situ 3-D optical biopsy of intact lymph nodes, and for the intraoperative staging of cancer.
Exploring the mechanism of radiation-enhanced hepatocellular carcinoma cell invasion by swept-source optical coherence tomography

W. Kuo, W. Y. Cheng, National Taiwan Normal Univ. (Taiwan); C. H. Chou, J. Cheng, National Taiwan Univ. Hospital (Taiwan)

Ionizing radiation is a standard treatment for various human solid tumors. However, several clinical studies showed that a significant proportion of patients undergoing radiotherapy for hepatocellular carcinoma (HCC) develop intraparenchymal and extrahepatic metastasis. Understanding of radiation-induced cancer cell invasiveness and behavior is essential and of great importance for developing suitable treatment strategies to contain cancer spread. Therefore, in this study we evaluated the effectiveness of using swept source optical coherence tomography (SS-OCT) to monitor the enhancement of hepatocellular carcinoma (HCC) cell invasiveness by radiation. SS-OCT images were acquired and recorded to obtain three-dimensional data sets per four hours in 48 hours after irradiating HepG2 cells with 7.5 Gy. The cell migration behavior in three-dimensional tissue models was quantified from images of radiation-induced and sham-irradiated cells.

Analysis of clinical optical coherence tomography image for real-time diagnosis of oral precancer

C. Lee, National Taiwan Univ. (Taiwan); M. Tsai, Chang Gung Univ. (Taiwan); T. Chi, K. Yang, C. Chiang, C. Yang, National Taiwan Univ. (Taiwan)

Squamous cell carcinoma (SCC) usually evolves from oral mucosal lesions with benign epithelial hyperplasia and mild dysplasia (MiD). If an oral lesion evolves into the moderate dysplasia (MoD) or severe dysplasia stages, it will usually develop further into an early-stage SCC and then a well-developed SCC. At the beginning, dysplastic cells start to develop from the bottom of the epithelium (EP) layer. When the dysplastic cell distribution extends to cover the bottom one-third, two-third, full range of the EP layer, the lesion is classified into the precancer conditions of MiD, MoD, and severe dysplasia, respectively. Beyond the stage of severe dysplasia, the boundary between the EP and lamina propria (LP) layers, disappears. The diagnosis of these precancer stages is important because if a therapy is not applied at the stage of MoD, a lesion will eventually develop into SCC. Here, we demonstrate an effective OCT image analysis procedure for illustrating the important features of oral precancer, particularly the stages of MiD and MoD. We first use the similar procedure for analyzing the histology images from biopsy to show the key parameters in the conventional diagnosis method. The results from histology image analysis indicate the reasonable use of standard deviation (SD) for identifying the distribution of dysplastic cells in the EP layer near the EP/LP boundary. Then, the proposed analysis procedure with empirical parameters is applied to OCT images of different mucosa conditions for plotting the EP/LP boundary and evaluating the extended range in the EP layer of dysplastic cell distribution.

High-speed (92 kHz) Fourier-domain optical coherence tomography system in the 1-µm band with real-time data resampling

A. Bradu, S. Van der Jeught, Univ. of Kent (United Kingdom); D. Machow, Sensors Unlimited, Inc., part of Goodrich Corp. (United States); A. G. Podoleanu, Univ. of Kent (United Kingdom)

An Fourier domain and ultra-fast optical coherence tomography system operating in the 1 µm range with real-time data re-sampling is presented for the first time. It utilizes a newly released 1024 pixels line scan InGaAs camera able to acquire data as fast as 91,900 lines per second. To demonstrate the performances of the system, images from a thumb of a volunteer obtained with real-time processing and displaying are shown.

Performance comparison between 8 and 14 bit-depth imaging in polarization-sensitive swept-source optical coherence tomography

Z. H. Lu, D. K. Kasaragoda, S. J. Matcher, The Univ. of Sheffield (United Kingdom)

Recently the effects of reduced bit-depth acquisition on swept-source optical coherence tomography (SS-OCT) image quality have been evaluated by using simulations and empirical studies, showing that image acquisition at 8-bit depth allows high system sensitivity with only a minimal drop in the signal-to-noise ratio compared to higher bit-depth systems. However, these studies could be challenged as the 8-bit data is actually 14-bit analog-to-digital converter (ADC) data numerically truncated to 8-bits. In practice, a real 8-bit ADC could actually possess a true bit-resolution lower than this due to the electronic jitter in the converter etc.

We compare true 8 and 14 bit-depth imaging of SS-OCT and polarization-sensitive SS-OCT (PS-SS-OCT) at 1.3µm wavelength by using two hardware-synchronized high-speed data acquisition (DAQ) boards. The two DAQ boards read exactly the same imaging data for comparison. The measured system sensitivity at 8-bit depth shows a slight drop compared to that at 14-bit depth. Ex- vivo structural and birefringence images of horse tendon indicate no significant differences between images acquired by the two DAQ boards suggesting that 8-bit DAQ boards can be employed to increase imaging speeds and reduce storage in clinical SS-OCT/PS-SS-OCT systems.

One possible disadvantage is a reduced imaging dynamic range which can manifest itself as an increase in image artefacts due to strong Fresnel reflection.

Real-time display Fourier-domain OCT using multithread parallel computing with data vectorization

T. Eom, H. S. Kim, C. Kim, Y. Lee, Gwangju Institute of Science and Technology (Korea, Republic of); E. Choi, Chosun Univ. (Korea, Republic of)

We demonstrate a real-time display of processed OCT images using multi-thread parallel computing with a quad core CPU of a personal computer. In addition, we have improved signal processing performance by improving a data vectorization for the high speed swept source OCT. The data of each A-line are treated as one vector to maximize the data translation rate between the cores of a CPU and the RAM stored 1 frame data. The parallel computing was implemented via OpenMP in the developing platform of Microsoft Visual Studio 2008. To do vector implementation, we have applied Intel’s Integrated Performance Primitives 6.1. A display rate of 29.7 frames/sec for processed OCT image (4096 FFT size x 500 lateral A-scans) is achieved in our system using a swept source with a swept frequency of 52 kHz. The data vectorization was applied to 4096 data points, and a single vector expressed the 4096 data points. The whole data of the 2D OCT image was expressed as only 500 vectors. Finally, we have achieved 2D OCT display rate of 29.9 frames/sec and 2D Doppler OCT display rate of 7.7 frames/sec. These values correspond data processing times for OCT and Doppler OCT of 23.8 msec and 91.4 msec without regarding data acquisition time.

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7889-88, Poster Session

Adaptive optics assisted Fourier-domain optical coherence tomography with balanced detection

A. Meadway, A. Bradu, Univ. of Kent (United Kingdom); M. W. Hathaway, OPKO Health, Inc. (United States); S. Van der Jeught, Univ. of Kent (United Kingdom); R. B. Rosen, The New York Eye and Ear Infirmary (United States); A. G. Podoleanu, Univ. of Kent (United Kingdom)

Optical coherence tomography (OCT) is a technique that can generate 3D images of a sample with both good depth penetration and high depth resolution. These two properties mean that it is ideal for non-invasive imaging of biological samples. In one modality, time domain OCT (TD-OCT), balanced detection has proved to be a useful technique in improving the signal to noise ratio by reducing excess photon noise and removing image artifacts due to DC terms in the signal. Whilst balanced detection is widely used in TD-OCT, little consideration has been given to it in the other modality, Fourier domain OCT (FD-OCT). We present a system that uses balanced detection to improve image quality, whilst also being optimized by the use of adaptive optics. We show that balanced detection for FD-OCT can reduce artifacts due to the auto-correlation terms. We will assess any improvement in the signal to noise ratio and we will present in-vivo images of the retina.

7889-89, Poster Session

Design and realization of a spectroscopic optical coherence tomography system for medical applications

P. Steiner, C. Meier, V. M. Koch, Berner Fachhochschule (Switzerland); M. Stampanoni, ETH Zurich (Switzerland)

In the presented work, a Fourier-Domain Spectroscopic-OCT system was designed and realized using a simple spectrometer based on off-the-shelf parts and a low-cost, state-of-the-art broadband S-LED light source with three spectrally shifted S-LED modules.

In recent years, modern medicine has shown an emerging need for imaging techniques that are easy to use, non-invasive and therefore painless and safe for the patient but still provide the highest possible resolution both spatially and in time. An expansion of OCT called spectroscopic OCT, extracts information about spectral attenuation from the sample signal in order to provide a tool for characterizing tissue or substances under investigation.

With the system realized and described in this manuscript, spatial resolutions of 13 μm axial and a lateral resolution of approximately 20 μm were achieved. The setup was tested and evaluated towards its ability to measure physical parameters such as blood oxygen saturation quantitatively in vivo. For this reason different sample configurations including multilayer setups and scattering layers were used. It was possible to prove the ability of the presented system to resolve differences in absorption coefficients of approximately 0.15 cm⁻¹.

Additionally, we present the theoretical model and experimental verification of interferences between autocorrelation terms and the signal carrying crosscorrelation terms. Due to the signal post-processing in SOCT, autocorrelation peaks can strongly affect the measurements and lead to beating effects, circumventing a meaningful determination of absorption coefficients. A background subtraction method, minimizing the artifacts caused by the interferences of autocorrelation and crosscorrelation terms is presented and verified.

7889-90, Poster Session

Low-power real-time signal processing engine for optical coherence tomography systems using multicore digital signal processor

M. Ali, R. Parlapalli, Texas Instruments Inc. (United States); R. John, S. A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Frequency domain Optical Coherence Tomography systems are very signal processing intensive. In order to meet the real time requirements of such systems, it is common to employ multi-core general purpose processors (GPP) or graphics processing units (GPU). These processing engines provide large compute capability at the expense of power wattage required to run these processors. The multi-core digital signal processors (DSP) are uniquely designed to perform heavy duty signal processing functions at the fraction of the power needed for GPP or GPUs. In fact, multi-core DSPs provide the best performance per milliwatt. In this paper, we demonstrate how typical signal paths in OCT systems can be efficiently implemented on a multi-core DSP. We start the processing from the samples out of the line scan camera (for spectral domain systems) or the analog to digital converter (for swept source systems). The samples are then passed through the signal processing chain including background subtraction, re-sampling using cubic interpolation, fast Fourier transform (FFT), magnitude computation and log compression. The final image is displayed on an external display. We have used a Texas Instruments C64x+ multi-core DSP. The signal processing functions are all optimized for this architecture. The inherent parallel nature of scanline based operation in OCT systems allows efficient distribution of processing across the multiple cores. We show that for 2048 points per input scanline, existing multi core DSPs can achieve up to 100 Klines per second of real time operation making them a desirable choice for OCT signal processing.

7889-91, Poster Session

Common path FDOCT based on multiple reflections within the sample arm

N. Krstajic, S. J. Matcher, R. A. Hogg, The Univ. of Sheffield (United Kingdom)

We present a common path Fourier domain optical coherence tomography (FDOCT) setup where the reference arm is based on multiple reflections within the sample arm. We present sensitivity analysis of this setup and images of in vivo skin. We believe the idea is of interest for endoscopy applications.

7889-92, Poster Session

Experimental investigation of wavelength dependence of penetration depth and imaging contrast for ultra-high-resolution optical coherence tomography

S. Ishida, N. Nishizawa, Nagoya Univ. (Japan); K. Itoh, Osaka Univ. (Japan)

Optical coherence tomography (OCT) is a non invasive optical imaging technology for micron-scale cross-sectional imaging of biological tissue and materials. OCT has been actively used in ophthalmology, dermatology, and so on. Until now, ultrahigh longitudinal resolution was achieved in several center wavelength regions. Although OCT has many advantages in medical equipments, low penetration depth is a serious limitation for other applications. Therefore it is important for OCT to innovate the technology that can achieve the ultrahigh resolution and the
high penetration depth at the same time. To realize these characteristics, it is effective to choose the proper wavelength to maximize the light penetration and enhance the image contrast at deeper depths. Recently, we have demonstrated ultrahigh resolution and high penetration depth OCT by use of all-fiber based Gaussian shaped supercontinuum source at 1.7 um center wavelength. Gaussian-like supercontinuum with 300 nm bandwidth at center wavelength of 1.7 um was generated by ultrashort pulse Er doped fiber laser based system. In this paper, using this 1.7 um, 0.8 um and 1.3 um SC sources, we have investigated the wavelength dependence of ultrahigh resolution OCT in terms of penetration depth. Longitudinal resolutions at each wavelength region are 3.6 um, 7.9 um, and 6.0 um in air, respectively. The obtained sensitivity was 90 dB for all wavelength regions. We confirmed the difference of imaging contrast and penetration depth with hamster’s cheek pouch and pig esophagus. As the wavelength was increased, the magnitude of penetration depth was increased for these samples.

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**7889-93, Poster Session**

**Geometric phase-shifted full-field optical coherence tomography for rapid 3D imaging of biological samples**

W. Zheng, National Univ. of Singapore (Singapore)

Full-field optical coherence tomography (FFOCT) is an emerging non-invasive, label-free, interferometric technique for 3D imaging of biomedical objects with micron-scale resolutions. To obtain en face OCT images, the conventional phase-shifting technique that involves moving a mirror to change the optical path difference is often used. But with the use of a broadband source in FFOCT, the phase shifts of different spectral components are not the same. To solve this problem, in this study, we utilize the ferroelectric liquid crystal (FLC)-controlled geometric phase shifting technique together with polarization optics to achieve an achromatic phase shift for rapid FFOCT imaging. We demonstrate this novel FFOCT technique by imaging biological samples such as onion epidermis and HeLa cells.

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**7889-94, Poster Session**

**Phantoms for intravascular or endoscopic optical coherence tomography**

C. Bisaillon, S. Vergnole, M. L. Dufour, G. Lamouche, National Research Council Canada (Canada)

No abstract available

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**7889-95, Poster Session**

**Ultra-high-resolution optical coherence tomography imaging of lung structure using Gaussian-shaped supercontinuum sources**

N. Nishizawa, S. Ishida, Nagoya Univ. (Japan); T. Ohta, K. Itoh, Osaka Univ. (Japan); M. Kitatsuji, H. Oshima, HOYA Corp. (Japan); Y. Hasegawa, M. Matsushima, T. Kawabe, Nagoya Univ. (Japan)

Optical coherence tomography (OCT) is an emerging technology for non-invasive cross-sectional imaging of biological tissue and material with um resolution. In the field of pulmonary medicine, non-invasive high resolution cross-sectional imaging is desired for investigation of diseases in lung. So far, a few works have been reported about OCT imaging of lung. Since the lung consists of alveoli separated by thin wall, ultrahigh resolution (UHR) OCT is supposed to be effective for the imaging of fine structure in lung tissue.

In this work, ex vivo cross-sectional imaging of isolated rat and hamster lungs was demonstrated using UHR-OCT. A 120 nm-wide, high-power, Gaussian-like supercontinuum (SC) was generated at wavelength of 0.8 um in air and 2.1 um in tissue was obtained. The achieved sensitivity was 105 dB. Using this system, ex vivo UHR-OCT imaging of isolated rat and hamster lungs was demonstrated for the first time. The structures of the trachea, visceral pleura, and alveoli were observed clearly. When saline was instilled into the lung, the penetration depth was improved, and clear images of the fine structure of the lung, including alveoli, were observed owing to the index matching effect.

We have also demonstrated the UHR-OCT imaging of lung tissue using 1.3 um and 1.7 um SC sources. As the results, owing to the precise structures of lung tissues, the finest images were observed with 0.8 um UHR-OCT system.
FDML laser based on polygon-scanner filter, the scanning rate is limited by the length of the delay fiber and the speed of the filter. Buffered FDML is an efficient way to break the limitation and improve the speed[7]. In this paper, we report a FDML laser based on a Fox-Smith cavity[8] which is a novel buffered structure. The laser is centered at 1315nm with a full range of 80nm. The power of the laser is near 10mw at a 45kHz repetition rate.

Figure 1 shows the configuration of the swept source with the Fox-Smith cavity. The 50:50 optical coupler is the core device of the Fox-Smith cavity. The left ports have the same structure which consist of the delay fiber and reflective mirror. The length of delay fiber 1 is about 4.5 km, and the length of delay fiber 2 is half that of fiber 1. The semiconductor optical amplifier(SOA) whose central wavelength locates at 1310nm is the gain medium of the laser. The tunable filter is composed of a collimator, a polygon and a grating. The grating has a density of 900lines per millimeter and work at Lithrow condition. The polygon has 72 facets. The circulator, SOA and the filter form the other part of the laser cavity. The laser outputs from the remaining port of the coupler.

The output of the laser is shown in Figure 2 and Figure 3. Figure 2 shows the output from an oscilloscope. The repetition rate of the pulse is about 45kHz, which is double the scanning rate of the polygon due to the fact that the output from different ports have different delay times. The equal amplitude can be achieved by adjusting the polarization in delay fiber 2. Figure 3 shows the spectrum centered at 1315nm with a tunable range of 80nm.

Reference:

L. An, R. K. Wang, Oregon Health & Science Univ. (United States)

We demonstrate an ultra high speed (92 kHz A-line rate) one micron spectral domain ultra high sensitive optical micro-angiography system for in vivo imaging of blood perfusions within both retina and choroid. A one-micron ASE module was used as the illuminating light source, which provides enhanced penetration depth for the system as compared to the conventional 800 nm system. The system runs at 200 frames per second, taking ~5 seconds to acquire one 3D data set, covering ~3x3 mm2 on the retina. We show that the system can extract detailed capillary level ocular perfusion maps at different depth-layers within both retina and choroid. The promising results give an excellent agreement with the standard text book, showing great potential in clinical application.

7889-103, Poster Session

One-micron double-beam Doppler optical coherence angiography

F. Jaillon, S. Makita, Univ. of Tsukuba (Japan); M. Miura, Tokyo Medical Univ. Kasumigaura Hospital (Japan); Y. Yasuno, Univ. of Tsukuba (Japan)

Knowledge of choroidal blood flow is necessary to monitor and diagnose retinal diseases. We here present preliminary results of an optical setup that provides high sensitive Doppler optical coherence angiography (DOCA) for choroidal regions. First, high sensitivity of low speed flows is achieved by utilizing two beams that are delayed in time. This time delay is larger than the CCD acquisition rate. Therefore compared to conventional Doppler SD-OCT method, sensitivity to low speed flows is enhanced. In the current system, we can measure velocities that are at least 40 times smaller than phase-resolved SD-OCT. Second, to obtain deeper penetration into the choroid, the presented system operates at one micron wavelength. Indeed, with respect to 800 nm wavelength, one micron wavelength allows lower light absorption by melanin that occurs in the retinal pigment epithelium. Moreover, light scattering is also reduced by using longer wavelength. To demonstrate high penetration and flow sensitivity of double beam DOCA, retinal 3D volumes of fovea and optical nerve head have been acquired with this system. Besides Doppler signal from retinal layers, strong Doppler variance signal originating from choroidal regions can be easily visualized. Therefore in addition to flow velocities accessible with conventional phase resolved SD-OCT, low velocity flows inside choroid regions are measurable with double beam DOCA. In conclusion, these preliminary results of one-micron double beam DOCA are encouraging in its ability to monitor low-speed blood flow inside choroid.

7889-104, Poster Session

Effect of Doppler optical coherence tomography algorithms on blood vessel diameter and relative blood flow estimates

J. M. Tokayer, The Univ. of Southern California (United States); D. M. Huang, Casey Eye Institute (United States)

In vivo measurement of blood flow in the retina has been made possible with the advent of Fourier domain optical coherence tomography (OCT). Doppler OCT has seen many advances in recent years in algorithms used for quantifying blood flow. We compare the relative retinal blood flow estimates as measured by the standard phase-resolved (PR) algorithm and the more recent moving-scatterer-sensitive (MSS) algorithm as a function of vessel size. We find that the PR-to-MSS flow ratio significantly decreases with decreasing vessel diameter. We also develop a simulation to approximate the scattering from blood cells in tissue and compare the relative blood flow estimates. The flow ratio measured with simulation closely matches that found in vivo. Our simulation predicts that whereas PR overestimates the flow, MSS overestimates it. Also, the simulated vessel diameter measurements for both PR and MSS closely agree with previously reported comparisons made using phantom systems. Our simulation may help to correct for algorithm bias in in vivo retinal flow estimates.

7889-105, Poster Session

In-vivo ultra-high-resolution human retinal imaging by dual-channel full-field optical coherence tomography

M. Akiba, Topcon Corp. (United States); C. Reisman, Z. Wang, Y. Fukushima, K. Chan, Topcon Medical Systems, Inc. (United States)

Full-field optical coherence tomography (FF-OCT) capable of non-scanning horizontal cross-sectional imaging has been demonstrated for cellular-level human retinal imaging. The system is based on an interference microscope illuminated by a broadband light source. A dual-channel two-dimensional detection technique incorporated with a pair of CCD cameras has been employed, where a pair of interferometric images with a phase shift of pi are simultaneously captured. Basically, taking a square of the absolute value from two CCD outputs yields FF-OCT image.

The subject was a healthy volunteer's eye. A long-working distance water immersion objective was employed where a phosphor buffered saline was used as an immersion media. The light source was a super continuum source whose output was reshaped to a quasi-Gaussian shape, centered around 800 nm with 80 nm bandwidth. To minimize the interference fringe washout due to eyeball motion, a short duration illumination (1 ms) method was employed. Human eye under measurement was flood-illuminated in an area of ~650 um in

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diameter. Axial and transverse resolutions were 3 µm and 6 µm at retina, respectively. From a series of FF-OCT images, nerve fiber bundles could be observed when the reference position of the interferometer was set to the anterior retina. Meanwhile, by setting the reference position to the posterior retina, a retinal cone mosaic could be observed. Our preliminary results give some evidences that FF-OCT may become a useful tool for high-resolution retinal imaging.

7889-106, Poster Session
Dynamic analysis of a small artery of a human finger by optical coherence tomography
M. Kuwabara, N. Takahashi, D. Takada, M. Ohmi, M. Haruna, Osaka Univ. (Japan)
OCT is highly potential for development of a new field of dynamic skin physiology, as recently reported by the authors in this paper. We demonstrate dynamic analysis of a small artery of a human finger by the SS-OCT. Among the vascular system, only the small artery has two physiological functions both for the elastic artery (like main and middle arteries) and for muscle-controlled one (like arterioles). It, therefore, is important for dynamic analysis of blood flow and circulation. In the time-sequential OCT images obtained with 25 frames/s, it is found that the small artery makes a sharp response to sound stress for contraction and expansion while it continues pulsation in synchronization with the heartbeats. This result indicates that the small artery exhibits clearly the two physiological functions for blood flow and circulation. In response to sound stress, blood flow is controlled effectively by thickness change of the tunica media which consists of five to six layers of smooth muscles. It is thus found that the thickness of the tunica media changes remarkably in response to external stress, reflecting activity of the sympathetic nerve. The dynamic OCT of the small artery presented here will allow us not only to understand the mechanism of blood flow control and also to detect abnormal physiological functions in the whole vascular system.

7889-107, Poster Session
Imaging vibration of the cochlear partition of an excised guinea pig cochlea using phase-sensitive Fourier-domain optical coherence tomography
N. Choudhury, Y. Zeng, Oregon Health & Science Univ. (United States); A. Fridberger, Karolinska Institutet (Sweden); F. Chen, D. Zha, A. L. Nuttall, R. K. Wang, Oregon Health & Science Univ. (United States)
Current research in hearing mechanics is concerned with how sound propagates though the cochlea and how sound stimulates the vibration of various structures of the cochlea, namely, basilar membrane (BM), reticular lamina (RL), outer hair cells(OKHC) and tectorial membrane (TM). The cochlea is a complex structure that vibrates in a complex way, for example, when BM vibrates it imparts a shear stress on stereocilia of the OHC, which extend from RL to TM. The OHCs are in turn active elements that provide a positive feedback at the resonant frequency to enhance the vibration of the organ of organ of corti and hence the gain of our hearing. In order to better understand these mechanisms it is essential to have a device capable of making measurements of these various structures of the cochlea. Most of the literature on measuring the vibration of cochlea is dependent on using long coherence length laser light sources. The cochlea structure is around 100 µm thick, which is much smaller than a typical laser’s coherence length. In these studies the optical cross-sectioning was determined by the N.A. of the imaging system, and since the hole made on the bony wall to visualize the cochlea is very small (< 500 µm), the resulting effective N.A. of these systems were very low. So it is impossible to obtain the localization needed to measure vibrations from different surfaces. One of the ways to overcome the localization problem is to use highly reflective beads to increase the signal from a local position, but it is not feasible to put beads on every surface of interest. In order to overcome the problems of localization of vibration measurements, we have previously reported the use of time-domain optical coherence tomography to measure the vibration of different surfaces of cochlea10,11 in vivo. However, one of the drawbacks of the system was that it made a point by point measurement of the vibrations and hence getting a 2-d map of vibration was time-consuming, which is a big disadvantage of any in vivo measurement. In this report we present a phase-sensitive Fourier domain optical coherence tomography method to obtain vibration image of the guinea pig cochlea. In order to test the feasibility of our system to image vibration from a guinea pig cochlea, we imaged an excised cochlea that was stimulated with sound of various frequencies. The results clearly show the ability of our system to detect vibrations from various structures and position of the cochlea.

7889-108, Poster Session
Imaging of the intact mouse cochlea by spectral-domain optical coherence tomography
S. S. Gao, Rice Univ. (United States); A. Xia, Stanford Univ. (United States); T. Yuan, P. Raphael, Baylor College of Medicine (United States); R. L. Shelton, B. E. Applegate, Texas A&M Univ. (United States); J. S. Ogahalai, Baylor College of Medicine (United States)
Current medical imaging modalities, such as MRI and CT, do not provide high enough resolution to detect minor changes in the cochlea. We sought to develop the technique of optical coherence tomography (OCT) to image the cochlea noninvasively and within its native environment. We used spectral domain OCT with 950 nm as the center wavelength and a bandwidth of ~100 nm to image freshly excised normal mouse cochlea different developmental ages. The OCT system has an axial resolution of 4 µm (in air) and a lateral resolution estimated at ~10 µm. When we imaged normal adult mouse cochlea through the round window membrane, Reissner’s membrane, the basilar membrane, the tectorial membrane, the spiral ligament, the spiral limbus, and the modiolus could be clearly identified. When we imaged intact adult cochleae, we were able to image through ~130 µm of bone and tissue to see up to a depth of ~600 µm, and all of the previously identified structures were still visible. Imaging of early postnatal mice during the timeline of cochlear development permitted visualization of many of the expected structural differences from adult cochleae. Therefore, we conclude that spectral domain OCT is an effective technique for noninvasive imaging of the murine cochlea.

7889-110, Poster Session
Morphometry of the myopic optic-nerve head using Fourier-domain optical coherence tomography
S. Lee, M. Young, Simon Fraser Univ. (Canada); E. Lebed, P. J. Mackenzie, The Univ. of British Columbia (Canada); M. F. Beg, M. V. Sarunic, Simon Fraser Univ. (Canada)
Recent advances in Fourier Domain Optical Coherence Tomography (FD OCT) technology have facilitated acquisition of high resolution volumetric images for clinical ophthalmic research. In this report, we investigate volumetric imaging of the Optic Nerve Head (ONH) in humans using a laboratory grade 830nm FDOCT system. We introduce the development of a computational model of the ONH morphology using the Bruch’s Membrane Opening (BMO) as a reference plane in order to study physiological changes which may be associated with susceptibility to glaucoma. The morphometric analysis is focused on myopic subjects, where the anterior surface of the lamina cribrosa can be visualized.
7889-111, Poster Session

**Fast retinal layer identification algorithm for optical coherence tomography imaging**

T. Fabritius, Univ. of Oulu (Finland); S. Makita, Y. Yasuno, Univ. of Tsukuba (Japan); M. Miura, Tokyo Medical Univ. Kasumigaura Hospital (Japan); R. A. Myllylä, Univ. of Oulu (Finland)

Fast method for identifying the internal limiting membrane (ILM) and retinal pigment epithelium (RPE) from optical coherence tomography images is demonstrated. Main ambition of this work was to reduce a needed data processing time to perform layer identification. A basic idea to avoid unnecessary increment of calculation time is to down-sample strongly the original data set to reduce a number of pixels to be processed. In ILM segmentation, the obtained data cube is filtered with two different kinds of parameters and two estimates for the position of ILM is determined. A simple smoothness value is determined for both estimates and the better estimate is used for future processing. A smaller portion of pixels around estimated ILM are extracted from the down sampled data and filtered again and new estimation for ILM position is determined. That procedure is repeated with smaller portion of pixels around ILM and with different filtering parameters. After five iterations, the final ILM depth position matrix is obtained. The principle of RPE segmentation is very much similar with ILM identification. Only the used filtering and processing parameters are changed. RPE segmentation required 4 iteration rounds to achieve satisfying results. Algorithm was tested with eight data sets measured from healthy and diseased eyes with good reliability. Over 98% of each scans had smaller segmentation error than 5 pixels (Optic disc area is not included). Total required data processing time (ILM and RPE segmentation) for data volume with (400x1024x140) pixels was less than 9 sec.

7889-113, Poster Session

**Au nanoring as contrast agent of optical coherence tomography and its photothermal effect**

C. Lee, H. Tseng, S. Wu, T. Chi, K. Yang, J. Wang, Y. Kiang, C. Yang, National Taiwan Univ. (Taiwan); M. Tsai, Chang Gung Univ. (Taiwan)

For biomedical applications, the widely used Au nanoparticles (NPs) include nanorods, nanoshells, and nanocages. However, most of the localized surface plasmon (LSP) resonance wavelengths of those reported Au NPs are shorter than 1000 nm. From the viewpoint of optical imaging application, particularly of optical coherence tomography (OCT), although a shorter wavelength around 800 nm can lead to higher imaging resolution, the use of a light source of a longer wavelength around 1300 nm can result in deeper tissue penetration due to weaker tissue scattering. Reliable Au NPs of other geometries for longer-wavelength and stronger LSP resonance are needed for further developments of targeted optical imaging and photothermal therapy. For this purpose, Au nanoring (NRI) shows the advantage of long-wavelength LSP resonance. The fabrication of Au NRIs on a substrate has been reported based on the methods of colloidal lithography and secondary sputtering of metal. However, the delivery of such Au NRIs into tissues or cells for optical imaging, therapy, and other biomedical applications has not yet been reported. In this paper, we report the fabrication of Au NRIs on sapphire substrate using the methods of colloidal lithography and secondary sputtering of Au and the development of a process for NRI lift-off from the substrate. The aqueous solution of Au NRIs is prepared for measuring the LSP resonance properties. The Au NRIs are then delivered into pig adipose samples for OCT scanning to demonstrate the enhanced absorption and scattering behaviors of LSP resonance.

7889-112, Poster Session

**Magnetic carbon nanotubes as contrast agents for pulsed magneto-motive optical coherence tomography**

J. Koo, Y. Song, Y. Oh, J. Oh, Pukyong National Univ. (Korea, Republic of); J. Kim, Kyungpook National Univ. (Korea, Republic of); J. Oh, Pukyong National Univ. (Korea, Republic of)

We demonstrated novel optical coherence tomography contrast agent, indocyamine green dye attached to the surface of the single walled carbon nanotube(SWNT-ICG) filling with iron content for molecular optical imaging. This contrast agent was used to conjugate αvβ3 integrins with cyclic Arg-Gly-Asp(RGD) peptides to investigate the tumor angiogenesis. Efficient targeting of integrin positive Hela cancer cell was achieved with RGD peptides and cytotoxicity of nanotubes was investigated using MTT Assay.

Spectral domain optical coherence tomography (SD-OCT) was used this study to image for pulsed magneto-motive (PMM) technique by applying high strength magnetic pulse to activate the Fe content inside specific targeted nanotubes. We used custom-built pulse generator with insulated gate bipolar transistor (IGBT) circuit, producing variable pulse duration (1-10 ms) and repetition frequency (1-10Hz).

Pulsed systems gave a markedly 10 times higher OCT signal in the tumor cell than previously reported MM technique and significantly increased OCT signal due to ICG dye.

Pulsed magneto motive OCT technique targeted Fe-SWNT-ICG-RGD nanoparticles may contribute to overcome depth limitations of noninvasive optical cancer imaging with great extent resolution and high sensitivity.

7889-114, Poster Session

**Tracking Au nanoring delivery into biotissue with optical coherence tomography**

C. Lee, H. Tseng, C. Lee, H. Chou, S. Wu, T. Chi, K. Yang, J. Wang, Y. Kiang, C. Yang, National Taiwan Univ. (Taiwan); M. Tsai, Chang Gung Univ. (Taiwan)

Through a bio-conjugation process, targeted multi-function operation can be implemented with metal nanoparticle (NP) delivery into bio-tissue. For realizing those applications, metal NP distribution in a bio-tissue becomes an important issue. To develop more effective localized surface plasmon resonance (LSPR)-related diagnosis and therapy techniques, investigations of metal NP distribution and their transport process in a bio-tissue sample are needed. Due to its interference (coherence) detection nature, optical coherence tomography (OCT) is a suitable approach for monitoring the LSPR of Au NPs. At LSPR, coherent scattering and absorption of LSP resonance. The Au NRIs are then delivered into pig adipose samples for OCT scanning to demonstrate the enhanced absorption and scattering behaviors of LSP resonance.
**7889-115, Poster Session**

**Polarization sensitive optical coherence tomography at 1050 nm using an all-fiber interferometer and a Fourier-domain mode-locked swept source**

S. Marschall, Technical Univ. of Denmark (Denmark); T. Torzicky, C. Blatter, M. Bonesi, Medizinische Univ. Wien (Austria); P. E. Andersen, Technical Univ. of Denmark (Denmark); M. Pircher, R. A. Lettgeb, C. K. Hitzenberger, Medizinische Univ. Wien (Austria)

We are developing a polarization sensitive (PS) swept source optical coherence tomography (OCT) system at 1050 nm for retinal imaging. The interferometer is based on polarization maintaining fiber-optic components, minimizing the need of alignment. Because the sample is probed with circularly polarized light, both polarization states are detected simultaneously.

Light in the 1050 nm range is beneficial for deep penetration into the sub-retinal layers, because it exhibits less absorption in the vitreous than light at higher wavelengths and less attenuation by the retinal pigment epithelium than light at 800 nm or in the visible range.

As light source, we use a previously developed Fourier domain mode-locked laser providing 30 mW of polarized light with 75 nm tuning range at a repetition rate of 135 kHz. With this high A-scan rate, rapid acquisition of 2D and 3D data sets is possible, reducing artifacts caused by sample motion.

We are currently setting up the interferometer for in-vivo retinal imaging. First test images recorded during the assembly demonstrate that light source and imaging setup are fully operational. Ultimately we aim for the visualization of the polarization properties of the deeper retinal and sub-retinal layers, such as the birefringence of the sclera. This will pave the way to future studies on pathological changes detectable with PS-OCT.

**7889-116, Poster Session**

**Digital phase stabilization for improving sensitivity and degree of polarization accuracy in polarization sensitive optical coherence tomography**

J. W. Jacobs, S. J. Matcher, The Univ. of Sheffield (United Kingdom)

In a recent publication, Tomlins and Wang pointed out an SNR improvement that could be gained in optical coherence tomography (OCT), by altering the averaging scheme used. Specifically they noticed that, given a large number of noisy OCT A-scans, it is preferable if possible to perform the ensemble-averaging over the A-scans and then extract the OCT envelope rather than extract the envelope from each noisy A-scan and then average. In this paper we demonstrate that a similar argument can be applied to the calculation of the degree of polarization (DOP) using polarization-sensitive OCT. However, the advantage now is that direct A-scan averaging can reduce the systematic error in DOP calculation that occurs in the presence of noise due to noise-bin terms. It is therefore interesting to study the accuracy with which DOP can be measured in a tissue phantom and how this might be improved.

**7889-117, Poster Session**

**Polarization sensitive and Mueller matrix OCT measurements and data analysis**

M. P. Raelle, M. M. Amaral, N. Dias Vieira, Jr., A. Zanardi de Freitas, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

The objective of this work was to study the interaction of polarized light with scattering sample. To perform the measurements two OCT setups were mounted, one of them was a polarization sensitive frequency domain OCT system (PS-OCT), the kind of system allow to perform birefringence images as depth sample function, which can be used in medical diagnostics, industry or even to material science. The second one was implemented to determine the sample’s Mueller Matrix (MM-OCT). The Mueller Matrix is a mathematical object, represented by a 4x4 matrix, that describes how an element transform the incident light polarization. Both systems used a Ti:Sapphire mode-locked laser with 30 nm FWHM, one Czerny-Turner spectrometer with 0.027 nm of spectral resolution and a XY scanning system. The sample chose in this work was a common adhesive tape, mainly for the following two reasons: it presents birefringence and has a periodic structure. Software to control and process the data was developed in LabVIEW environment. The PS-OCT system allowed us to determine the birefringence of the Scotch-tape (dn / dλ = 0.03(26)x10^-4), value that encountered agreement with a bibliographical reference. The MM-OCT was capable of determine the adhesive-tape Mueller Matrix, then the matrix were decomposed linearly with another homemade software. This decomposing intended to separate the original matrix as a linear combination of four other matrices, to try to figure out different optical properties of the sample.

**7889-118, Poster Session**

**Modulated deconvolution for resolution improvement in Fourier-domain optical coherence tomography**

E. Bousi, I. Charalambous, C. Pitris, Univ. of Cyprus (Cyprus)

A novel technique for Fourier Domain Optical Coherence Tomography (FD-OCT) axial resolution improvement is presented. The technique is based on the deconvolution of a modulated OCT signal. A resolution improvement by a factor of ~ 7 is achieved without the need for a broader bandwidth light source. This method exploits a combination of two basic principles: the appearance of beating in the FFT of the signal, when adding two slightly shifted interferograms, and the resolution improvement of OCT images by deconvolution of the A-Scan with the modulated source autocorrelation function. In FD-OCT, the real part of the FFT of the signal is modulated by a frequency which depends on the shift of the interferogram. By adding two interferograms, which are slightly shifted relative to each other, beating will appear in the real part of the FFT and thus in the A-Scans. Deconvolution of the resulting A-Scans, using appropriately modulated kernels, results in a narrower resolution width when the amount of shifting is appropriately selected.

**7889-119, Poster Session**

**Use of creep compounding to reduce speckle in optical coherence tomography images**

B. F. Kennedy, A. Curatolo, The Univ. of Western Australia (Australia); T. R. Hillman, Massachusetts Institute of Technology (United States); F. Blume, D. D. Sampson, The Univ. of Western Australia (Australia)

We present a novel technique to reduce speckle in optical coherence tomography images. This technique uses creep deformation in a sample under constant load to introduce decorrelation between successive B-scans. Results are presented for silicone and fibrin phantoms and also from excised chicken breast tissue. Correction for deformation-induced spatial distortions between pairs of B-scans is achieved through geometrical co-registration using an affine transformation. A maximum reduction in contrast ratio of 1.8 was calculated for chicken breast.
7889-120, Poster Session

Quantitative comparison of despeckling and frame-averaging approaches to processing retinal OCT tomograms
J. A. Eichel, D. Lee, A. Wang, P. W. Fieguth, D. A. Clausi, K. K. Bizheva, Univ. of Waterloo (Canada)

Quantitative analysis was used to compare the performance of two speckle denoising approaches, algorithmic despeckling and frame averaging, as applied to retinal OCT images. Human retinal tomograms were acquired from healthy subjects with a research grade 1060nm spectral domain UHR-OCT system with 5μm axial resolution in the retina. Single cross-sectional retinal tomograms were processed with a novel speckle denoising algorithm and compared with frame averaged retinal images acquired at the same location. Image quality metrics such as the image SNR, contrast-to-noise ratio (CNR), edge preservation and equivalent number of looks (ENL) were evaluated for both cases. The effect of retinal motion artefacts and the camera integration time on the quality of the processed retinal tomograms were also investigated.

7889-121, Poster Session

Using phase gradient autofocus (PGA) algorithm for restoration OCT images with diffraction limited resolution
A. A. Moiseev, G. V. Gelikonov, P. A. Shilyagin, V. M. Gelikonov, Institute of Applied Physics (Russian Federation)

Improving the lateral resolution of an Optical Coherence Tomography (OCT) is an important problem. In case of sharp focused scanning beam is used in OCT system, the lateral resolution of an obtained image will dramatically decrease in the layers, out from focal plane. Since we want to observe an inner structure of an random scattering media, the problem of recovering OCT images with diffraction limited resolution seems to be a blind deconvolution problem. In this paper we show the ability of use well known in Synthetic Aperture Radar (SAR) applications Phase Gradient Autofocus (PGA) for recovering OCT images. Recovered images have diffraction limited resolution (~5 μm) in all observed volume. Both numerical simulated and experimental results are presented.

7889-122, Poster Session

Study on effective probe depth of optical coherence system by Monte Carlo simulation
Y. Qing, W. Zhou, C. Zhang, J. Tian, Nankai Univ. (China)

Monte Carlo model for optical coherence tomography is useful. In the model, the photons are divided into class I and class II. The intensity of Class I and class II signal at different probing depth for different coherence length are analyzed by using this model for a pencil beam. Result shows that effective probe depth can affect the axial resolution. A Monte Carlo model for optical coherence tomography system with a focusing Gaussian beam is setup. Using this model, we simulate the intensity of Class I and Class II signal at different probing depth for different radius and focusing depth of the beam. Results show that increasing focusing depth and decreasing beam radius can finitely increase effective probing depth. When effective probing depths is fixing, we can achieve optimal signal intensity by altering beam radius and focusing depth.

7889-29, Session 5

Ultra-high-speed functional optical coherence tomography by spatial frequency multiplexing
T. Schmoll, E. Götzinger, C. K. Hitzenberger, R. A. Leitgeb, Medizinische Univ. Wien (Austria)

We present a single spectrometer functional spectral domain optical coherence tomography system, which allows for encoding additional information within the spatial frequencies. The method is based on a differentiation between orthogonal polarization channels through spatial modulation introduced by an electro-optic modulator. We present two applications for this concept. Ultra-high-speed retinal polarization sensitive optical coherence tomography (PSOCT) and Resonant Doppler OCT with a single spectrometer. First we performed PSOCT measurements of the human optical nerve head region at an acquisition speed of 160,000 A-scans/s. And secondly instantaneous resonant Doppler OCT. Flow tomograms with improved velocity information compared to standard phase Doppler OCT tomograms are shown and a reduction in acquisition time of one third compared to traditional resonant Doppler was achieved.

7889-30, Session 5

Ultra-high-speed fiber-based polarization sensitive optical coherence tomography

We demonstrate an ultra high speed fiber based polarization sensitive spectral domain optical coherence tomography system, using two ultra high speed CMOS line scan cameras. With this system an A-scan rate of up to 200 kHz is possible. The system is based on polarization maintaining fibers and retrieves the backscattered intensity, birefringence and optic axis orientation with only one A-scan per measurement location. To demonstrate the performance of our system we present images of the fovea and the optic nerve head of a healthy human retina. Additionally we show images of intensity and retardation averaged over 30 B-scans.

7889-31, Session 5

Optic axis determination by fiber-based polarization-sensitive swept-source optical coherence tomography
Z. Lu, D. K. Kasaragoda, S. J. Matcher, The Univ. of Sheffield (United Kingdom)

Fiber-based swept-source PS-OCT continuous source-polarization modulation (PS-SS-OCT) is used to determine 3-D optic axis of birefringent biological tissues. 3-D orientation of collagen fibers in horse tendon with different polar and azimuthal angles is measured using a dual-plane variable-incidence-angle (VIA) retardance measurements and the results are generally in good agreement with the expected values within about 8% of the nominal values. Single-plane VIA-PS-OCT is also explored which requires measurement of the absolute fast-axis orientation. A theoretical and experimental analysis of the effect of the sampling fiber on determination of optic axis orientation using a proposed definition based on the orientation of the eigenpolarization ellipse experimentally confirms that this algorithm only works correctly for some settings of the sampling fiber. A proposed algorithm based on the angle between Stokes vectors on the Poincare sphere is confirmed to work for all settings of the sampling fiber.
**7889-32, Session 5**

**Polarization-sensitive optical coherence tomography imaging at 1300 nm using a Fourier-domain mode locked laser**

M. Bonesi, M. Pircher, E. Götzinger, S. Zotter, T. Torzicky, Medizinische Univ. Wien (Austria); C. M. Eigenwillig, B. R. Biedermann, W. Wieser, R. A. Huber, Ludwig-Maximiannis-Univ. München (Germany); C. K. Hitzenberger, Medizinische Univ. Wien (Austria)

We present a polarization-maintaining fiber-based high resolution Fourier domain polarization sensitive optical coherence tomography (PS-OCT) system based on a Fourier domain mode-locked (FDML) laser source with central wavelength of 1300 nm and tunable sweep range up to 130 nm. The imaging principles are inspired from our previously reported bulk optics spectral domain (SD) PS-OCT - the sample is illuminated by circularly polarized light and measures an arbitrary elliptical polarization state - and applied into a polarization-maintaining fiber-based system. We demonstrate the capability of the system to retrieve relectivity, retardation and birefringent axis orientation by using only one single input polarization state of the probing radiation. The use of a broad bandwidth laser source improves depth resolution and reduces speckle sizes, yielding enhanced polarization imaging. System design is described and PS-OCT images of human skin (fingertip), fingernail and eye angle chamber in vivo are reported to demonstrate system performance.

**7889-33, Session 5**

**Optical rheology of porcine sclera by polarization-sensitive optical coherence tomography**

M. Yamanari, K. Ishii, Tsukuba Univ. (Japan); M. Miura, Tokyo Medical Univ. Kasumigaura Hospital (Japan); T. Oshika, Y. Yasuno, Tsukuba Univ. (Japan)

In recent years, polarization-sensitive optical coherence tomography (PS-OCT) has been successfully employed to investigate the birefringence property of tissues. However, the relationship between the birefringence and other physical properties of the tissue has not been well investigated yet. In this presentation, we investigate the relationship between the rheological property and birefringence of porcine sclera.

Seven porcine eyes were dissected to obtain scleral blocks. The stripe of the sclera with a width of 4 mm and a length of 15 mm or longer along latitude lines of the eyeball was mounted between two clamps attached to a force gauge and a motorized translation stage. En face slope map of the phase retardation of the sclera was measured by PS-OCT without applying force to the sample. To measure Young’s modulus of the sclera, the sclera was extended linearly at 8.05 mm/min, and stress of the sclera was measured. Young’s modulus was calculated from a derivative of stress-strain curve.

Our analysis showed that the measured slope of the phase retardation and Young’s modulus had strong correlation with statistical significance. To the best of our knowledge, this is the first time to show a relationship between elasticity and birefringence of sclera. This finding would be important to investigate mechanisms of myopia and glaucoma in future.

**7889-34, Session 5**

**Spectral domain polarization sensitive optical coherence tomography at 1.55 µm: novel developments and applications for dynamic studies in materials science**

D. Stiffer, Johannes Kepler Univ. Linz (Austria); E. Leiss-Holzinger, RECENDT GmbH (Austria); B. Heise, Z. Major, P. Hierzenberger, G. Eder, Johannes Kepler Univ. Linz (Austria); M. Pircher, E. Götzinger, B. Baumann, C. K. Hitzenberger, Medizinische Univ. Wien (Austria)

It will be demonstrated, how research in optical coherence tomography (OCT) for biomedical diagnostics successfully triggered research in the field of experimental mechanics, in non-destructive material characterization and testing: diverse studies performed with a specifically designed, compact and robust spectral domain polarization sensitive OCT setup at 1.55 µm will be presented for dynamic investigations of technical materials, like bulk polymers and composites under various conditions. Furthermore, it is expected, that developments made in these technical fields have the potential to find their application in the biomedical counterparts, like advanced mathematical methods for the 2D analysis of complex fringe structures.

**7889-35, Session 6**

**Label-free 3D optical imaging of microcirculation within sentinel lymph node in vivo**

Y. Jung, Z. Zhi, R. K. Wang, Oregon Health & Science Univ. (United States)

Sentinel lymph node (SLN) is the first lymph node to drain wastes originated from cancerous tissue. There is a need for an in vivo imaging method that can image the intact SLN in order to further our understanding of its normal as well as abnormal functions. We report the use of ultrahigh sensitive optical microangiography (UHS-OMAG) to image functional microvascular and lymphatic vessel networks that innervate the intact lymph node in mice in vivo. The promising results show a potential role of UHS-OMAG in the future understanding and diagnosis of the SLN involvement in cancer development.

**7889-36, Session 6**

**Doppler velocity detection limitations in spectrometer and swept-source Fourier-domain optical coherence tomography**

H. C. Hendargo, R. P. McNabb, A. Z. Dhall, J. A. Izatt, Duke Univ. (United States)

Doppler optical coherence tomography has demonstrated important potential uses in medical imaging and diagnostics, particularly in the field of ophthalmology. Recent advances in Doppler and variance techniques have enabled high sensitivity for imaging regions of biological flow to measure blood flow velocities and vascular perfusion. In recent years, the sensitivity and imaging speed benefits of Fourier domain OCT have become apparent. Spectrometer-based and wavelength-swept implementations have both undergone rapid development, and a wide variety of Doppler acquisition protocols and signal processing approaches have emerged. Comparative analysis of the potential benefits and limitations for the various configurations would be useful for matching technology capabilities to specific clinical problems. Here we take a first step in such a comparative analysis by presenting theoretical predictions and experimental results characterizing the lower and upper observable velocity limits in high speed spectrometer-based versus swept-source Doppler OCT. The effects of fringe washout from imaging high speed flows are discussed. Experimental demonstration of this effect in spectrometer based systems reveals an advantage of swept-source implementations of OCT for imaging fast moving scatterers.
Label-free in-vivo optical micro-angiography imaging of cerebral capillary blood flow within meninges and cortex in mice with the skull left intact

Y. Jia, R. K. Wang, Oregon Health & Science Univ. (United States)

Abnormal microcirculation within meninges is common in many neurological diseases. There is a need for an imaging method that is capable of visualizing functional meningeal microcirculations alone, preferably decoupled from the cortical blood flow. Optical microangiography (OMAG) is a recently developed label-free imaging method capable of producing 3D images of dynamic blood perfusion within micro-circulatory tissue beds at an imaging depth up to -2 mm, with an unprecedented imaging sensitivity to the blood flow at ~4 µm/s. In this study, we demonstrate the utility of OMAG in imaging the detailed blood flow distributions, at a capillary level resolution, within meninges and cortex in mice with the cranium left intact. The results indicate that OMAG can be a valuable tool for the study of meningeal circulations.

Volumetric Doppler imaging of murine brain using spectral and time-domain optical coherence tomography

D. Bukowska, I. Grulkowski, Nicolaus Copernicus Univ. (Poland); G. Wilczynski, Nencki Institute of Experimental Biology (Poland); M. Szkulmowski, S. Tamborski, Nicolaus Copernicus Univ. (Poland); J. Włodarczyk, Nencki Institute of Experimental Biology (Poland); A. A. Kowalczyk, M. Wojtkowski, Nicolaus Copernicus Univ. (Poland)

Stroke belongs to the neural disorders and appears as rapidly developing loss of brain functions due to a disturbance in the blood supply to the neural cells. Hence, it is very important to diagnose early stroke and to monitor the effects of therapeutic interventions. Structural and functional brain imaging represents a significant challenge for better understanding of brain disease pathogenesis. In-vivo imaging using near infrared light (e.g. microscopy, laser Doppler imaging, laser speckle imaging, optical angiography) provides measurement of a range of functional contrasts such as blood flow or concentration of oxygen in blood. However, aforementioned techniques are limited by resolution, imaging speed or penetration depth. Generally Optical Coherence Tomography provides high-resolution and high-speed imaging sufficient for in-vivo studies. Moreover, the method enables non-invasive imaging of biological systems without the removal of tissue specimens. Therefore, OCT is a valuable tool for in-vivo brain imaging. As the technique, which has micrometer resolution and makes possible real time 3-D imaging, it is suitable for visualization of brain disease pathogenesis particularly in the case of strokes. Here, we have demonstrated the applicability of Spectral Optical Tomography (SOCT) in structural and functional brain studies applied to small animals.

Lateral resonant Doppler imaging for quantitative flow extraction in spectral-domain optical coherence tomography

J. Walther, P. Cimalla, E. Koch, Technische Univ. Dresden (Germany)

In spectral domain Doppler OCT, any transverse motion component of the obliquely moving sample relative to the incident sample beam causes a damping of the correlation between subsequent backscattering signals or even the loss of it making a phase-resolved Doppler flow analysis difficult because of the strong mean error of the Doppler phase shift. To circumvent this effect, a new method for resonant Doppler flow imaging and quantification in spectral domain OCT is proposed where the scanner movement velocity is approximately matched to the transverse velocity component of the oblique sample motion similar to a tracking shot where the camera is moved with respect to the sample. As a result, the backscattering signals corresponding to the moving sample will be highly correlated whereas those of static sample structures and slowly moving scatterers will be less correlated and damped depending on the scanner velocity. Advantageously, for the exact flow velocity quantification the new Doppler relationship of phase shift Δ and sample velocity v has not to be applied and the linear relation of the classic Doppler model can still be used. Due to the variable scan velocity of the galvanometer scanner, within its specifications, arbitrary flow velocities can be measured by adapting the scan protocol. In the present work, first results of the lateral resonant Doppler imaging are shown for an in vitro 1% Intralipid flow phantom study with a Doppler angle between the x-direction and sample velocity of 2.2° and a maximum flow velocity of 64 mm/s.

Ultra-high-resolution and ultra-high-sensitive optical micro-angiography based on supercontinuum light source

Z. Zhi, L. An, J. Qin, R. K. Wang, Oregon Health & Science Univ. (United States)

We demonstrate for the first time an ultrahigh resolution and ultrahigh sensitive optical micro-angiography (UHS-OMAG) system that is realized by a supercontinuum light source and an ultrafast CMOS camera. The broad band light source with a central wavelength at ~800nm, emitted from the supercontinuum light source, provides a ~2µm coherence gate for the system. With the fast CMOS camera employed in the spectrometer operating at ~70 KHz line rate, we demonstrate that the detailed blood vessel networks, including capillaries, buried within the tissue bed can be visualized. We present the results obtained from the human finger nail fold and the mouse ear flap. The excellent system imaging performance shows a great potential of our system in the future biological imaging application.

Multimodal optical coherence/photo-acoustic tomography of skin

A. P. Alex, Cardiff Univ. (United Kingdom); E. Z. Zhang, Univ. College London (United Kingdom); B. Považay, Medizinische Univ. Wien (Austria); J. G. Laufer, Univ. College London (United Kingdom); B. Hofer, Medizinische Univ. Wien (Austria); C. Glittenberg, Ludwig Boltzmann Institut (Austria); B. Herrmann, Medizinische Univ. Wien (Austria); P. C. Beard, Univ. College London (United Kingdom); W. Drexler, Medizinische Univ. Wien (Austria)

Optical coherence tomography (OCT) is an emerging non-invasive in vivo biomedical imaging technique capable of generating three dimensional images of tissue microstructure based on localized backscattering of the sample. While, photo-acoustic tomography (PAT) is a non-invasive in vivo imaging modality capable of visualizing structural and functional information of soft tissues based on its optical absorption properties. Hemoglobin is the most important source of naturally occurring contrast for PAT. The intrinsic contrast provided by refractive index variations for PAT is not capable of differentiating various microstructures with similar scattering properties, whereas PAT provides highly localized spectroscopic contrast without any background signals. Hence, a novel non-invasive in vivo multimodal OCT/PAT system capable of
obtaining structural and functional information simultaneously has been demonstrated in skin. A 1060 nm OCT system acquiring 47k depth-scans/s with ~7 µm axial and ~ 20 µm transverse resolutions has been incorporated into a backward-mode PA system based on a planar, optically-transparent Fabry-Perot interferometer (FPI) sensor. For PAT, the excitation wavelength was set to 670 nm and a focused laser beam at 1550 nm was used as the sensor interrogation beam. OCT and PAT images were obtained sequentially from the skin of a hairless mouse and the imaging times were ~10 s and ~4 minutes respectively. The co-registered images were combined to form the final 3D image. This multimodal OCT/PAT approach enabled visualization of vasculature as deep as 3 mm and was clearly able to distinguish blood vessels from similarly scattering micro-morphological features seen in OCT.

7889-42, Session 7
Integrated en-face optical coherence endomicroscopy and two-photon fluorescence endomicroscopy for simultaneous multimodal imaging
J. Xi, Y. Zhang, L. Huo, Y. Chen, X. Li, The Johns Hopkins Univ. (United States)
A 1310-nm optical coherence endomicroscopy (OCEM) system and an 800-nm two-photon fluorescence endomicroscopy system were combined into one single configuration. A dichroic mirror was used to make the two wavelength lights share the same path. Both imaging modalities shared the same miniature imaging probe consisting of a DCF, a PZT scanner and a compound lens, suitable for simultaneous acquisition of en face OCEM and TPF images. The core of the DCF delivered 800 nm excitation light for TPF and 1310 nm light for OCEM while the inner cladding of DCF collected the TPF signal. Two-dimensional beam scanning was realized by resonantly scanning a fiber-optic cantilever with a PZT. We obtained en face OCEM images with axial and lateral resolutions of 9.6 µm in tissue and 1.8 µm, respectively, co-registered with TPF images with axial and lateral resolutions of 7.6 and 1.4 µm, respectively. Preliminary results show promising capability of providing simultaneous structural and molecular images.

7889-43, Session 7
Integrated optical coherence tomography: ultrasound system and miniaturized probes for intravascular imaging
J. Yin, Univ. of California, Irvine (United States); X. Li, The Univ. of Southern California (United States); J. Jing, Beckman Laser Institute and Medical Clinic (United States); C. Hu, Q. Zhou, K. K. Shung, The Univ. of Southern California (United States); Z. Chen, Beckman Laser Institute and Medical Clinic (United States)
We report on the development of a multimodal optical coherence tomography (OCT) - ultrasound (US) system and miniaturized OCT-US probes for intravascular imaging. Both OCT optical components and a US transducer were integrated into a single probe, enabling both OCT and US imaging at the same time. Two different types of miniaturized OCT-US probes using a single element and a focused ring transducer, respectively, were designed with a maximum outer diameter of 1.2 mm, which are suitable for in vivo intravascular imaging. The integrated OCT-US imaging system adopted a two-channel data acquisition card to digitize both OCT and US signals. Simultaneous OCT and US data processing and image display were also achieved using our home-developed software. In vitro OCT and US imaging of rabbit aorta was performed using this multimodal imaging system, which demonstrated the feasibility of the OCT-US system in intravascular imaging and its potential in detection of atherosclerotic plaques.

7889-44, Session 7
Piezo-electric transducer-based miniature catheter for ultra-high-speed endoscopic optical coherence tomography
T. Tsai, Massachusetts Institute of Technology (United States); B. M. Potsaid, Massachusetts Institute of Technology (United States) and Thorlabs Inc. (United States); M. F. Kraus, Massachusetts Institute of Technology (United States) and Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); J. J. Liu, C. Zhou, Massachusetts Institute of Technology (United States); J. Horngger, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); J. G. Fujimoto, Massachusetts Institute of Technology (United States)

We developed a piezo-electric transducer (PZT) based miniature catheter with an outer diameter of 3 mm for ultrahigh speed endoscopic optical coherence tomography (OCT) using Fourier domain modelocked (FDML) laser at a 240kHz axial scan rate. The miniaturized PZT bender actuates a fiber to provide high scanning speed. The side-viewing probe can be pulled back for a long distance to acquire three-dimensional (3D) dataset covering a large area on the specimen. Operating with a high speed data acquisition (DAQ) system, OCT imaging with 4mm imaging range, 9.6um axial resolution, 20um lateral resolution, and frame rate of 480 frames per second (fps) is demonstrated.

7889-45, Session 7
Semiresonant Lissajous scan of a fiber-cantilever scanning endoscope catheter for stable OCT imaging
S. Moon, S. Lee, M. Rubinstein, B. J. Wong, Z. Chen, Beckman Laser Institute and Medical Clinic (United States)

Semi-resonant Lissajous scan with a compact fiber-cantilever scanning endoscope catheter for stable OCT imaging is presented as a new operation scheme for stable OCT endomicroscopy imaging. The resonance frequency was decreased to 63 Hz in a favorable scan frequency range by attaching a weight to the cantilever. To avoid scan instability, driving complexity, and nonlinear effects, the scanner was driven at semi-resonance frequencies out of the resonance peak, but within a frequency range of partial resonance. This technique was evaluated for stable 3D OCT imaging by driving the two axes with slightly different scan frequencies that produce an area-filling Lissajous pattern.

7889-46, Session 7
3D FD-OCT imaging with resonant fiber optic scanning endoscope in a rectangular field of view
J. Xi, L. Huo, Y. Zhang, X. Li, The Johns Hopkins Univ. (United States)

Instead of using the previous spiral scanning pattern for the resonant fiber-optic scanning endoscope, Lissajous scanning pattern was deployed by driving the X and Y electrodes of a tubular lead zirconate titanate (PZT) actuator with sinusoidal wave of slightly different frequencies. Rectangular field of view was realized for 3D optical coherence tomography (OCT) imaging with a resonant fiber-optic scanning endoscope of 2.4-mm diameter in combination with a 40-kHz swept source OCT system.
Adaptive-optics optical coherence tomography tailored for clinical use

K. Sudo, Utsunomiya Univ. (Japan); S. Makita, K. Kurokawa, M. Yamanari, Y. Yasuno, Univ. of Tsukuba (Japan); T. Yatagai, B. Cense, Utsunomiya Univ. (Japan)

We designed and built an AO-OCT system that is tailored for clinical use, to fill the gap between laboratory AO-OCT and standard clinical OCT. An analysis in Zemax demonstrated that compared to a laboratory AO-OCT setup with a 1.5° x 1.5° isoplanatic patch and an effective imaging depth of ~100 µm, a significantly larger isoplanatic patch of 10° x 10° is achieved over a depth that covers the full retina, making this AO-OCT design much more practical for clinical use. As significantly fewer optical components are used than in a classical AO-OCT design, the optical loss is minimal. Compared to the ~1.5 mm aperture of standard OCT, photons reported from the retina are collected over a 3.4 mm aperture, resulting in an estimated ~10 dB increase in SNR, while an improvement in resolution and speckle size of a factor of 2 is achieved. We plan to bring this instrument into the clinic, and demonstrate that AO-OCT is feasible in a clinical environment, where it can be used for detailed investigation of retinal pathology.

Structural and functional imaging with extended focus dark-field OCT at 1300 nm

C. Blatter, Medizinische Univ. Wien (Austria); R. A. Huber, Ludwig-Maximilians-Univ. München (Germany); R. A. Leitgeb, Medizinische Univ. Wien (Austria)

We present an extended focus FDOCT setup with FDML swept source centered at 1310 nm. A high lateral resolution while maintaining a large depth range necessary to take full advantage of the FDOCT parallel measurement is of great importance in biomedical imaging. An optical setup is presented proposing Bessel beam illumination of the sample enabling a fine lateral resolution over a large depth range. A lateral resolution of 4 µm has been achieved extending over a range of 250 µm instead of confocally 15 µm. In order to increase the global sensitivity the illumination and detection beam are decoupled. By efficient spatial separation between the illumination and the Gaussian detection dark-field imaging is realized. Dark field imaging is useful to attenuate strong reflections containing no or few information about the sample. More important, the otherwise small dynamic range of an 8 bit DAQ card can be efficiently exploited. In-vivo measurements of the human skin were performed to demonstrate the gain in lateral resolution while preserving the imaging depth.

The system is then applied to functional imaging of the human skin in-vivo. The calculation of the speckle variance between successive intensity B-Scans offers a nice way allowing clear visualization of micro-vascularization. Comprehensive images of skin capillary network are presented. Functional testing of skin micro-vascular response to cold water is demonstrated.

Megahertz, high-performance 1050-nm Fourier-domain mode locked laser for OCT: low-cost cavity design using oligo-mode fiber

T. Klein, W. Wieser, C. M. Eigenwillig, B. R. Biedermann, R. A. Huber, Ludwig-Maximilians-Univ. München (Germany)

We present a novel Megahertz Fourier-domain mode locked (FDML) laser operating around 1050 nm that overcomes most problems previously encountered with FDML at this wavelength: (a) low power, (b) insufficient sweep range (c) chromatic polarization rotation, (d) high cost of optical delay line. A tuning range of up to 80 nm almost exactly centered at the water absorption minimum for optimum axial resolution, up to 1.4 MHz sweep rate and an output power in excess of 50 mW can be easily achieved. A comprehensive technical description and characterization is provided and a retinal OCT imaging example is shown.

Self-regenerative FDML for OCT imaging

L. Huo, K. Murari, J. Xi, X. Li, The Johns Hopkins Univ. (United States)

In this study, we propose self-regenerative Fourier-domain mode locking which does not require an external function generator for its operation. A feedback circuit was used to utilize the fundamental frequency of the FDML loop itself for producing an auto-starting, sustainable oscillation. As a prototype, a self-regenerative FDML laser was demonstrated at 40 kHz with stable wavelength sweeps generated for OCT imaging.

OCT imaging with wavelength-swept active mode locking fiber laser

H. Lee, C. Kim, M. Jeong, Pusan National Univ. (Korea, Republic of); J. Lee, J. Shin, The Univ. of Seoul (Korea, Republic of)

OCT image is demonstrated with a wavelength-swept active mode locking (AML) fiber laser. Unlike conventional wavelength-swept Fourier domain mode locking (FDML) lasers, this novel laser does not require a tunable wavelength selecting filter in the cavity and, thus, ultra-fast sweeping rate can be achieved over 500 kHz.

1060-nm swept-source laser and system for high-speed direct and Doppler OCT imaging

B. Johnson, W. Atia, M. Kuznetsov, B. Wells, N. Larson, R. A. Murdza, D. Flanders, AXSUN Technologies Inc. (United States)

The performance of a 1060 nm wavelength swept source laser and associated electronic systems is described for high-speed OCT applications. The system operates at 100,000 A-scans per second and digitizes at a peak rate of 330 MS/s. This low-RIN source enables a shot-noise limited system sensitivity of 102 dB for 2.5 mW sample illumination power. Conventional OCT imaging and phase-sensitive/Doppler imaging are demonstrated.

Filterless swept-source based on dispersion tuning for high-speed optical coherence tomography

C. Chong, K. Totsuka, T. Suzuki, K. Isamoto, Santec Corp. (Japan); Y. Takubo, S. Yamashita, The Univ. of Tokyo (Japan)

Swept source optical coherence tomography (SS-OCT) system is becoming a promising candidate for next generation OCT equipment. In practice, higher speed and lower cost should be realized at the same time for commercialization. Most of previously developed swept sources are based on external or extended cavity laser source that use tunable filters such as piezo-driven or MEMS actuated Fabry-Perot filter, or polygon scanner with diffraction grating for wavelength swept operation.
These approaches have fundamental limitation in the swept rate by its mechanical design and also complicate the laser cavity structure. In this paper, we propose a novel wavelength-tunable fiber laser based on dispersion tuning for SS-OCT. Electrical modulation of mode-lock frequency changes the optical wavelength using the chromatic dispersion in the laser cavity. Here we use a length of dispersion compensation fiber (DCF) for dispersion element in the cavity, with no other filter element. Swept rate is only determined by the electrical circuitry because there’s no need of tunable filter inside thus simplifying the laser structure. We realized the fast sweep over 100 nm at the swept rate as high as 300 kHz. This method can compensate the linearity of the sweep easily, and also synchronizes the signal sampling with pulse train of the mode-locked laser, thus eliminates the need of k-trigger. We also suggest the use of gratings pair for alternative dispersion element for further simplification and improvement of the performance.

7889-54, Session 8

A mechanical-free 150-kHz repetition swept light source incorporated a KTN electro-optic deflector

S. Yagi, NTT Photonics Labs. (Japan); K. Naganuma, NTT Advanced Technology Corp. (Japan); T. Imai, Y. Shibata, S. Ishibashi, NTT Photonics Labs. (Japan); Y. Sasaki, NTT Advanced Technology Corporation (Japan); M. Sasaura, NTT Photonics Labs. (Japan); K. Fujiiura, NTT Advanced Technology Corporation (Japan); K. Kato, NTT Photonics Labs. (Japan)

Recently the swept-source OCT draw much attention for the high-speed data acquisition that enables advanced volume rendering as well as avoidance of a blur caused by living body movements. For its light source, we are developing an external cavity LD incorporating an electro-optic deflector based on a KTN single crystal, which uniquely exhibits a fast and a fairly large light deflection based on injected carriers. Built laser consists of an 1.3-µm SOA module having 10% reflection coupled with an optical fiber on its one side, and on the other, through the KTN deflector, a grazing-incident 300 l/mm grating followed by an end reflector. This scheme can keep the beam thin at the deflector, while maintaining grating's resolving power. We observed static linewidth < 0.1 nm, whereas a wavelength scanning range of 110-nm when an up-to 150-kHz ±200 V sinusoidal voltage is applied to the KTN deflector.

These results have multiple impacts. First, our swept source involves no mechanical resonance at all, implying future possibility of still faster scan (in a separate study, deflector itself worked up to 400 kHz), and above all, a wavenumber-linear scanning by designing the waveform of driving voltage. Second, it is clearly shown that the number of wavelength points for the swept source is not limited by the resolving power of the stand-alone KTN deflector, which is about 35 in our case. We are attributing this to scattered light and its fast acquisition rate, this method allows observation of the evolution of the ion concentration in different cell compartments over time with a high time resolution.

dIOCM is sensitive to variations of the index of refraction inside the specimen. The tremendous [Ca2+] increase due to stimulation of a SMC results in a strongly enhanced signal in the tomogram. As a hypothesis that needs to be verified, we assume that variations of [Ca2+] within the cell affect the local index of refraction. In conclusion, the high sensitivity of dIOCM to weak backscatter signals is well suited for functional cell imaging where small fluctuations need to be detected.

7889-58, Session 9

In-vivo, real-time full-field optical coherence tomography of the rat brain

J. R. H. Binding, Ecole Supérieure de Physique et de Chimie Industrielles (France) and Ecole Normale Supérieure (France) and Max-Planck-Institut für medizinische Forschung (Germany); J. Ben Arous, Ecole Normale Supérieure (France); S. Gigan, C. A. Boccara, Ecole Supérieure de Physique et de Chimie Industrielles (France); J. Leger, L. Bourdieu, Ecole Normale Supérieure (France)

Compared to other OCT approaches, full-field OCT has been recognized as a high-resolution method able to provide micron size resolution in 3D on ex vivo samples. Using a fast, large full well capacity InGaAs camera we demonstrate the ability of FF-OCT to image the brain cortex of living rats with an imaging frequency of 33Hz and a numerical aperture of 0.8. The main features that appear are individual myelin fibers and the blood flow in the vessels where the movement of individual red blood cells as well as leukocytes can be followed. Confocal imaging of rat brain slices with immuno-stained myelin confirms in vitro that we indeed observe myelin fibers, which to our knowledge has not been demonstrated before in-vivo in depths beyond 100µm. It is known that myelin plays an important role in many nervous system diseases and we hope that our imaging tools can play a role in the monitoring of myelination.

Imaging to several hundred microns is only possible at this high numerical aperture using reference arm length optimization to...
compensate defocus induced by the refractive index mismatch between brain and immersion water. From the optimal defocus values at different imaging depths the refractive index of the rat somatosensory cortex is determined to be n= 1.3522 ± 0.003 (s.t.d.) at lambda=1100nm, with no significant dependence on imaging depth or animal age between 3 and 12 weeks. The mean free path in the tissue has been estimated from the defocus-corrected signal decay to be around 200μm.

7889-59, Session 9

Interferometric synthetic aperture microscopy with aberration correction for enabling virtual adaptive optics

S. G. Adie, B. W. Graf, B. Darbarsyah, S. A. Boppart, S. Carney, Univ. of Illinois at Urbana-Champaign (United States)

Interferometric synthetic aperture microscopy (ISAM) reconstructs the scattering potential of a sample with spatially invariant resolution, based on the incident beam profile, the beam scan pattern, the physical model of light sample interaction, and subsequent light collection by the system. In practice, aberrations may influence the beam profile, particularly at higher NA, when ISAM is expected to provide maximum benefit over optical coherence microscopy. The description of the incident beam as a perfect Gaussian provides a good approximation at low NA, however with increasing NA, beam and sample-induced aberrations are expected to play a more significant role. Hardware-based aberration correction, such as the use of objective lens collars, immersion fluids with varying refractive indices, or deformable mirrors generally apply correction for a fixed depth, which limits their practical use for high-NA tomographic imaging. Thus it is of interest to determine the effects of aberrations on ISAM reconstruction. In this paper, we present the forward model and system point-spread function that incorporates aberrations, as represented by the Zernike polynomial basis. Preliminary simulations and experimental results suggest that aberrations can be computationally corrected during object reconstruction, enabling ISAM to function as a new virtual adaptive optics method. By computationally correcting for aberrations in the system hardware, and possibly in the samples, ISAM has the potential to enable a method for virtual adaptive optics, without having to implement extensive hardware modifications to coherence imaging systems.

7889-60, Session 9

Numerical movement correction for swept-source full-field optical coherence tomography

D. Hillmann, Thorlabs GmbH (Germany); T. Bonin, G. Franke, M. Hagen-Eggett, Univ. zu Lübeck (Germany); P. Koch, Thorlabs GmbH (Germany); G. Hüttmann, Univ. zu Lübeck (Germany)

Compared to standard scanning Optical Coherence Tomography (OCT) in Swept Source Full Field Optical Coherence Tomography (FF-OCT) a major problem is the increased measurement and integration time needed for acquiring a single volume or a single depth scan, respectively. For in vivo measurements the sample in many cases moves during image acquisition, resulting in blurring of the images and thus rendering FF-OCT with its current acquisition speeds unsuitable for many applications. We developed a suitable model describing the effects of movements on Swept Source Full-Field OCT measurements. Additionally, we suggest an algorithm for measuring and correcting movements of the sample without additional experimental requirements. The movement measurement method is based on Short Time Fourier Transformations and uses the fact that the complete image is present in each part of the spectral data, though with reduced resolution. The correction is based on suitable multiplications of the spectral data with complex phase factors prior to the Fourier transformation, reverting the effects of the movements.

7889-61, Session 9

Spatial-domain low-coherence quantitative phase microscopy for cancer diagnosis

P. Wang, R. K. Bista, Univ. of Pittsburgh (United States); R. Bhargava, Univ. of Pittsburgh Medical Ctr. (United States); R. E. Brand, Y. Liu, Univ. of Pittsburgh (United States)

Quantitative phase microscopy has emerged as an important technique for the investigation of cell structures and dynamics due to the ultra-sensitivity of light interference effect to detect nanoscale structural alterations. However, the clinical applications of quantitative phase microscopy, especially cancer diagnosis, have not been extensively explored. We present a novel microscopy technique, spatial-domain low-coherence quantitative phase microscopy (SL-QPM) for quantitative phase imaging of sub-cellular structures with sub-nanometer sensitivity. SL-QPM utilizes a low spatial-coherence from a thermal light source and produces a speckle-free, nanoscale-sensitive quantitative phase map of scattering objects. With this technique, for the first time to our knowledge, we quantified the refractive index of the cell nuclei on the original unmodified histology specimens. We showed that the refractive index of the cell nucleus is significantly increased in malignant cells from patients with invasive breast cancer compared to those of the normal cells from healthy patients undergoing reduction mammoplasty. Most importantly, we found that the histologically normal-looking cells from cancer patients exhibit significantly higher average refractive indices in the cell nuclei compared to those from healthy controls. Our results suggest the potential of the accurate assessment of nuclear refractive index for detecting cancer from histologically-normal tissue, which may have a significant impact on the development of novel strategies for diagnosis, risk stratification and prognosis of breast cancer patients. Because this technique is simple, sensitive, does not require special tissue processing, and can be applied to archived specimens, it can be disseminated to all clinical settings.

7889-62, Session 9

Hybrid MT-OCM imaging platform for in vivo tissue imaging

A. Isomäki, L. Thrane, H. E. Larsen, Technical Univ. of Denmark (Denmark); K. Koenig, Univ. des Saarlandes (Germany); P. E. Andersen, Technical Univ. of Denmark (Denmark)

We report on a combined multiphoton tomography (MT) and optical coherence microscopy (OCM) imaging platform. The combination of the two optical imaging modalities allows for multi-modal cellular and molecular diagnostic imaging. The system is based on a commercial multiphoton tomograph (Dermainspect, Jenlab GmbH) which is modified to accommodate an OCM unit. Two different OCM unit designs are considered here. The first one is using a separate broadband light source at 1.3 micron wavelength range. The second approach takes advantage of sub-12 fs pulses from a Ti:sapphire laser. Ultrafast broadband pulses enable high resolution optical biopsy with simultaneous MT and OCM image acquisition.

Department of Dermatology, Roskilde Hospital (DD/RH) and DTU Fotonik have recently conducted a large optical coherence tomography (OCT) study including more than 100 patients with non-melanoma skin cancer. One of the issues studied was the applicability and precision of OCT for tumour thickness measurement. The results were promising: OCT is reproducible and easily applicable. However, OCT seems to have a tendency to overestimate tumour thickness. Furthermore, the level of resolution did not allow for differential diagnosis between various types of skin cancers like basal cell carcinomas, squamous cell carcinomas, and the premalignant actinic keratosis. We suggest that the improved resolution that comes with the MT-OCM imaging platform will overcome these obstacles and increase the accuracy for non-invasive diagnosis of non-melanoma skin cancer. Thus, the combination of MT and OCM is seen as a promising method for optical biopsy in early detection of skin cancer.
We present images of the human optic nerve head in vivo. Our approach is similar to previously reported heterodyne SD-OCT techniques employing electro-optic or acousto-optic modulators, but we instead use a carefully configured optical delay line (ODL) to apply the required phase modulation. The ODL is similar in design to a rapidly scanning optical delay line and provides precise control of the phase modulation frequency. This technique confers numerous and substantial advantages over other heterodyne techniques: it is easier and less expensive to implement; it provides a means for hardware dispersion compensation; and, most significantly, the phase modulation is applied linearly in wavenumber, precluding the need for additional resampling or demodulation. To demonstrate this approach, we constructed an SS-OCT system that can be used with either a standard reference arm or an ODL. Using the ODL, we demonstrated suppression of the complex conjugate artifact limited only by the noise floor of the system, implying a suppression ratio of at least 63dB. The usable depth range of the heterodyne SS-OCT system is thus nearly doubled to over 9mm.

We describe a novel technique for resolving this artifact in heterodyne swept source OCT (SS-OCT), essentially doubling the usable depth range of FDOCT systems. Our approach is to acquire additional phase information to overcome this problem by using a newly developed spectrometer and applying the required phase modulation before hitting the object. Therefore any sample motion is easy and less expensive to implement; it provides a means for hardware dispersion compensation; and, most significantly, the phase modulation is applied linearly in wavenumber, precluding the need for additional resampling or demodulation. To demonstrate this approach, we constructed an SS-OCT system that can be used with either a standard reference arm or an ODL. Using the ODL, we demonstrated suppression of the complex conjugate artifact limited only by the noise floor of the system, implying a suppression ratio of at least 63dB. The usable depth range of the heterodyne SS-OCT system is thus nearly doubled to over 9mm.

We outline a method for digital dispersion compensation using polynomial fit of an ultrabroad-bandwidth PS-OCT system using a single camera spectrometer. In order to enjoy the benefits of this instrument, we report the construction of an ultrabroad-bandwidth PS-OCT system using a single camera spectrometer. In order to enjoy the benefits of this instrument, we outline a method for digital dispersion compensation using polynomial fit parameters derived from a reference object, which removes the necessity for special camera alignment. We find that there are three non-negligible types of dispersion to consider: 1) the aforementioned camera pixel-to-wavenumber nonlinearity, 2) the refractive index dispersion in the sample itself, and 3) the dispersion imbalance between the arms of the OCT interferometer. We find that dispersion types 1 and 2 have the same functional effect and can be combined into one compensation step. During analysis of the reference object, the relative scaling and positioning of the two polarization images is adjusted to align the scatterers. We show how our technique provides high-resolution PS-OCT with precise alignment between the orthogonal polarization images, in our demanding high-bandwidth application. Retardance images of chicken muscle are observed to have suppression of artifacts when scatterer alignment procedures are employed.
7889-67, Session 10

Segmented scanning protocols for speckle contrast reduction in spectral OCT images

Spectral Optical Coherence Tomography (SOCT) is a rapidly developing modality that allows for noninvasive cross-sectional imaging of weakly scattering semitransparent samples with high sensitivity, high speed and high resolution. Resolution is limited by spectral properties of the light source used, while the latter depends on the light beam spot size on the sample, which by itself depends on focusing optics of the system and central wavelength of the light. Another factor depending on the light source properties, that limit resolution in both axial and lateral direction - the speckle pattern. The speckle pattern can be misleading while interpreting tomographic images. In retinal imaging the pattern can mimic small structural details like capillaries or photoreceptors. The most popular approach to speckle pattern reduction uses averaging of several tomograms acquired at almost the same location of the sample. The main issue of this approach are the unpredictable shifts of the object, that in some cases can be too large leading to tomograms being taken from distant parts of the object and strong loss of the lateral resolution. Because of that, sophisticated algorithms are required to properly superpose acquired tomograms before averaging or expensive and complicated eye-tracking devices need to be incorporated into the setup. In this contribution we propose an efficient speckle contrast reduction technique that uses Ascans averaging combined with specialized scanning protocols. Therefore, it is almost free of tomogram superposition problems and does not need any sophisticated and time consuming algorithms to generate speckle-free image.

7889-68, Session 10

Optimal processing of nonlinearity in swept-source and spectral-domain optical coherence tomography
S. Vergnole, D. Levesque, National Research Council Canada (Canada); K. K. Bizheva, Univ. of Waterloo (Canada); G. Lamouche, National Research Council Canada (Canada)

We demonstrate the efficiency of the convolution using an optimized Kaiser-Bessel window to resample non-linear data in wavenumber for Fourier-domain optical coherence tomography (OCT). Extending our initial demonstration based on experimental swept-source OCT data, we now apply this method to experimental and simulated data for both swept-source OCT and spectral domain OCT. It shows that the new optimized method is efficient for handling all the non-linearities in wavenumber that one can encounter in Fourier-domain OCT. The efficiency of the method is evaluated through comparison with resampling methods using interpolation and with an accurate but time consuming nonlinear Fourier transform.

7889-69, Session 11

Generating multiple-depths en-face images in optical coherence tomography
A. Bradu, L. P. Neagu, J. A. Rogers, A. G. Podoleanu, Univ. of Kent (United Kingdom)

A novel low coherence interferometer configuration is presented, equipped in each arm with an adjustable optical path length ring. By compensating the losses in the rings using semiconductor optical amplifiers, interference of low coherence light after traversing the two rings 18 times is obtained. This configuration can be employed to produce simultaneous en-face OCT images from different depths.

7889-70, Session 11

Ultra-high-speed real-time 4D display system installed in ultra-high-speed parallel OCT system at a volume rate of 12 volumes/sec
K. Ohbayashi, Kitasato Univ. School of Medicine (Japan); D. Choi, H. Hiro-Oka, Kitasato Univ. (Japan); A. Kubota, T. Ohno, R. Ikeda, System House Co., Ltd. (Japan); K. Shimizu, Kitasato Univ. (Japan)

Optical coherence tomography (OCT) has become promising diagnostic tool in many medical fields. Also for internal organs, such as the digestive and respiratory systems, extensive OCT developments have been achieved by many research groups. In the case of endoscope, area of object is large and the endoscope image must be refreshed as the probe scans the different position of organs in real time. In our previous work, we have demonstrated ultra-high speed OCT at an A-scan rate of 60 MHz. Although we could successfully image dynamical volumetric change of tomographic 3D images as a function of time (4D imaging), the processing was retro-capturing of only about 2.5 seconds time duration. In order to extend the capability of the ultra-high speed OCT, we installed new FPGA based ultra-fast real time processing system to realize real time 4D OCT imaging of tissues. With the system, volumetric 4D OCT images are displayed at a volume rate of 12 volumes/second following the displacement of the probe instantaneously.

7889-71, Session 11

An approach for Megahertz OCT: streak mode Fourier-domain optical coherence tomography
R. Wang, Clemson Univ. (United States); X. Yuan, Nankai Univ. (China); R. L. Goodwin, Univ. of South Carolina (United States); R. R. Markwald, Medical Univ. of South Carolina (United States); B. Z. Gao, Clemson Univ. (United States)

In a Fourier Domain OCT (FD-OCT), the OCT spectrum is recorded by a line-scan camera. Currently, state-of-art area-scan camera can achieve higher data acquisition rate than linescan camera can. Area-scan camera can achieve 7500 Mega pixels per second (Fastcam SAS, Photron), much quicker than 560 Mega pixels per second of a line-scan camera (SPL4096-140k, Basler Vision Tech).can. Thus, high speed area-scan camera makes a Megahertz OCT possible. In a reported area-scan camera based parallel SOCT, an area-scan camera was used to record the spectrum of the OCT signal. However, both of the signal to noise ratio (SNR) and the spatial resolution are seriously decreased by crosstalk among different spatial image spots since there is no confocal gate to screen the multiply scattered photons.

Here, we report a technique, which uses an area-scan camera to record the interference spectrum. Traditional point-scanning is remained in this streak-mode FDOCT so that the small aperture of the single-mode fiber functions as a confocal gate and screens multiply scattered photons very well. While the sample beam is scanning the specimen laterally, the interference spectrum is physically scanned on the area camera using a streak scanner. Therefore, pixels of the camera are illuminated by the spectrum of OCT signal row by row, corresponding to each A-scan at different lateral position. Preliminary data was obtained and compared with imaging result from a traditional FD-OCT. This technique is highly potential for multi-Megahertz OCT imaging. A patent application has been filed.
Triggered optical coherence tomography for dynamic imaging of rapid periodic motion

E. W. Chang, Boston Univ. (United States); J. B. Kobler, S. Yun, Massachusetts General Hospital (United States)

Abstract: Human physiology involves periodic motion of tissue, such as heartbeat for circulation and vocal fold vibration for voice production. The ability to visualize the tissue microstructure and deformation of organs during motion would be useful for functional analysis and disease diagnosis. However, limited frame rates of current beam-scanning OCT systems make it challenging to image rapidly moving tissues without introducing image artifacts. Here, we demonstrate a dynamic OCT technique based on triggered beam scanning and A-line acquisition synchronized with motion sensor signals. Subsequent image reconstruction produces a sequence of high-resolution 3D images of the sample over a full cycle of vibration. We demonstrate its potential to provide cross-sectional, quantitative information of dynamic oscillation of vocal folds and biomaterials at frequencies up to 150 Hz and amplitudes of >1 mm.

Dual-band refractive low-coherence interferometry in the spectral domain for dispersion measurements

J. Liebermann, C. Brückner, Technische Univ. Ilmenau (Germany); B. Grajciar, Medizinische Univ. Wien (Austria); J. Haueisen, Technische Univ. Ilmenau (Germany); A. F. Fercher, Medizinische Univ. Wien (Austria)

We present a spectral domain refractive low coherence interferometry technique (SD-RLCI). In our system a novel extreme broadband Super Continuum laser source acts as a white light source which provides a spectral range from 460-2400 nm and a maximal output power of 3W. To measure the visual (VIS) and near infrared (NIR) range of the spectrum simultaneously a dual spectrometer system allows to determine broadband dispersion data and moreover acquiring the data with significant higher speed. The visual range is detected with a silicon (Si) spectrometer (spectral responsivity of 350-1100 nm) and the near infrared part of the spectrum is recorded by using an indium gallium arsenide (InGaAs) based spectrometer (spectral responsivity of 900-1780 nm). The setup was verified obtaining the second order dispersion of distilled water. The dispersion characteristic of a sample can be used for quantifying its material composition because the spectral phase delay is sensitive to the material's refractive index. Therefore we will use this system for measuring the dispersion sensitivities of important tissue substances like glucose, protein, uric acid, urea, cholesterol or triglycerides in order to determine the analyte concentrations within mixtures. Obtained results will be compared to our previous time domain measurements. Besides that, a temperature compensation will be included to control and record temperature dependent dispersion changes.

1.7-micron optical coherence tomography for enhanced image contrast

E. W. Chang, Boston Univ. (United States); S. Yun, Massachusetts General Hospital (United States)

The imaging depth in OCT is limited by the attenuation of ballistic light due to scattering and absorption in biological samples. Multiply scattered photons accumulate over propagation distance, and further reduce penetration depths by blurring images and degrading spatial resolution at larger depths in a sample. Although backscattering is the major contrast mechanism in OCT, reduced scattering with longer wavelengths may actually lead to an increase in the penetration depth and sharper images at greater depths. We have improved the previously reported 1.7-µm OFDI system both in axial resolution (24 µm to 12 µm) and sensitivity (100 dB to 110 dB), now matching those of the state-of-the-art 1.3 µm OCT systems. We show evidence that the use of long wavelength OCT at 1.7 µm improves image contrast in certain samples when compared to the equivalent 1.3 µm system.

Phase unwrapping with PMF Sagnac loop filter in swept-source OCT

J. S. Park, C. Kim, M. Jeong, Pusan National Univ. (Korea, Republic of)

In general, all phase imaging techniques suffer from 2π ambiguities because the phase images contain 2π discontinuities when the measured phase varies over the period of 2π radians. Since the 2π ambiguity may limit the measurement range less than the half of source wavelength, a phase unwrapping algorithm is necessary to measure a large displacement.

We propose a novel phase unwrapping method of the phase-sensitive OCT imaging with wavelength-swept laser source. To remove a 2π ambiguity, multiple Gaussian subsets of broad swept-source range are easily induced using a polarization-maintaining fiber (PMF) Sagnac loop filter, which can control the center wavelength, subset spacing and the number of subset inside the broad range. Two subsets are generated to measure interference separately and calculate a longer beat wavelength. Each center wavelength is 1519.952 nm, 1571.952 nm and the spacing of each subset is 52 nm. As each subset can measure phase variations separately and combine each phase map again, we can calculate a height profile over a few micrometers without phase jumps in phase-sensitive OCT. Phase noise level is reduced from the case of single wavelength because two subsets have Gaussian shape spectra. It is expected to measure the higher sensitivity image by widening the center spacing of each subset.

Single-shot full-complex spectrum spectrometer-based OCT

P. A. Shilyagin, V. M. Gelikonov, G. V. Gelikonov, Institute of Applied Physics (Russian Federation)

Spectral domain optical coherence tomography (SD-OCT) is based on measuring of optical spectrum of sum of two interfering waves: the reference one and the backscattered from the object one. Because of the obtained spectrum is the real function of optical frequency, the reconstructed by Fourier transformation image has mirror-symmetrical structure relative to zero of path-difference. Some methods of eliminating of the mirror artifacts, obtained for SD-OCT, are based on consecutive obtaining of spectral components with different phase shift between reference and object waves. The simultaneous obtaining of full complex spectrum makes possible eliminating the influence of Doppler phase shifts of moving scatterers in the object. The simultaneous obtaining of quadrature interference components in spectrometer-based OCT was proposed by using polarization optics.

The first interferometer for simultaneously observing of interference with different phase shifts between interfering waves was described in 1926 by Kennedy, R. J.. The relative phase difference between two parts of one beam was obtained by a short step. Unfortunately, using of this setup for observing broad spectra, which is necessary for OCT, will result in increasing of mirror artifacts.

We propose polarization optics free SD-OCT setup with achromatic phase shifting and simultaneous obtaining of quadrature interference components. The components are obtained in air-spaced non-
polarization interferometer by partition of reference beam onto two parts and using an achromatic phase shifter. Several setups are described and compared.

7889-77, Session 12
Magnetomotive optical coherence microscopy for cell dynamics and biomechanics
X. Liang, B. W. Graf, R. John, H. Ding, Univ. of Illinois at Urbana-Champaign (United States); H. Song, Purdue Univ. (United States); G. Popescu, Univ. of Illinois at Urbana-Champaign (United States); A. Wei, Purdue Univ. (United States); S. A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Magnetomotive microscopy techniques are introduced to investigate cell dynamics and biomechanics. These techniques are based on magnetomotion of nanotransducers attached to the cells and optical coherence imaging techniques. In this study, nanotransducers include magnetic nanoparticles (MNPs) and colloidal gold nanostars, while the optical coherence imaging techniques include integrated optical coherence (OCM) and multiphoton (MPM) microscopy, and diffraction phase microscopy (DPM). Samples used in this study include silicone phantoms and macrophage cells. MPM visualizes nanostars based on two-photon luminescence, while magnetomotive OCM is demonstrated on a silicone phantom, for probing the mechanical properties of the phantom. DPM is used to image single cells at a lower frequency magnetic excitation, and with its Fourier transform light scattering (FTLS) analysis, oscillation amplitude is obtained, indicating the relative biomechanical properties of macrophage cells. These magnetomotive microscopy methods can be used to image and measure cell dynamics and biomechanical properties. The ability to measure and understand biomechanical properties of cells and their microenvironments, especially for tumor cells, is of great importance and may provide insight for diagnostic and subsequently therapeutic interventions.

7889-78, Session 12
Visco-elastic time-dependent strain as a contrast mechanism in optical coherence tomography
B. F. Kennedy, F. Blume, R. A. McLaughlin, The Univ. of Western Australia (Australia); R. E. Day, Royal Perth Hospital (Australia); D. D. Sampson, The Univ. of Western Australia (Australia)

We present a novel approach to provide additional contrast in optical coherence tomography (OCT). This technique is based on measurement of the viscoelastic time-dependent displacement of samples under a constant load using a spectral-domain OCT system. We present results from tissue-mimicking phantoms with both high fibrin and low silicone viscoelasticity. Results are also presented from chicken breast and demonstrate the potential of this technique to distinguish tissue types based on their viscoelastic response. For validation, we also performed measurements using a standard materials testing system to measure the bulk motion of the samples under constant load.

7889-79, Session 12
Gold nanoparticles as cellular contrast agents in spectroscopic optical coherence tomography
J. Yi, K. C. L. Black, P. B. Messersmith, X. Li, Northwestern Univ. (United States)

Due to surface plasma resonance (SPR) effect, gold nanoparticles (GNPs) exhibited extremely high scattering and absorption efficiency which makes a great candidate for contrast agents in OCT. The SPR spectrum is highly tunable throughout the visible and NIR range based on the composition and geometry of the NPs.[1] We demonstrate that GNPs can serve as cellular contrast agents in SOCT. Binding of GNPs and cells was confirmed by bright field microscopic images and enhanced cellular signal in OCT images. The SOCT spectra were analyzed and a cellular spectral contrast mechanism was proposed and illustrated based on spectral slope.

7889-80, Session 12
Assessing hemoglobin concentration using spectroscopic optical coherence tomography for feasibility of tissue diagnostics
F. E. Robles, A. P. Wax, Duke Univ. (United States)

Tissue hemoglobin (Hb) concentration and oxygen saturation levels are important biomarkers for various diseases, including cancer. Here, we investigate the feasibility of measuring these parameters for tissue using spectroscopic optical coherence tomography (SOCT). Depth resolved spectroscopic analysis is achieved using the dual window method. The calculated spectra are analyzed using Beer's law and a linear least squares fitting method. A novel analytical model is presented, based on a subtractive Kramers-Kroning relation and the non-linear phase of the OCT signal to determine absorber concentration in turbid samples without a priori information. Experimental demonstration of the approach is presented using a frequency domain OCT system with detection spanning the visible region of the spectrum (450 nm to 700 nm). Scattering and non-scattering, oxygenated Hb phantoms are analyzed, as well as deoxygenated Hb phantoms. The results show that Hb concentrations as low as ~1 g/L at 1 mm in depth can be retrieved, indicating that measurements may be obtained for both normal and cancerous tissue (concentration of ~2 g/L and ~6 g/L, respectively). However, we find that in order to accurately measure oxygen saturation levels, a concentration of at least ~4 g/L is required; and hence, this parameter may not be accurately assessed in normal tissue using this approach. The results based on this novel analytical method show that accurate Hb concentrations can be achieved in turbid samples; furthermore, using this analysis, we demonstrate that the scattering coefficient may also be measured.

7889-81, Session 12
Assessment of tissue optical clearing as a function of glucose concentration using optical coherence tomography
N. Sudheendran, Univ. of Houston (United States); V. V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); K. V. Lin, Univ. of Houston (United States)

In imaging of biological samples using optical techniques, optical clearing methods can compensate for the lack of light penetration due to embedded highly turbid structures. The addition of optical clearing agents into turbid media increases the optical homogeneity of the sample, allowing for an increase in light penetration to lower depths within the tissue. Using optical coherence tomography (OCT), this study investigated the extent of optical clearing in porcine skin by utilizing various concentrations of glucose solution. A gold-plated mirror was fixed beneath the tissue and percentage clearing was determined by measuring the change in intensity of the reflected light from the mirror over time. A ratio of percentage clearing per tissue thickness for 10%, 30%, and 50% glucose was determined to be to be (4.7 ± 1.6)% mm-1 (n = 6), (10.6 ± 2.0)% mm-1 (n = 7) and (21.8 ± 2.2)% mm-1 (n = 5), respectively. Although the extent of optical clearing in porcine skin was more significant for 50% glucose, the osmotic stress on the sample can cause considerable morphology change, thus a suitable concentration must be chosen for particular circumstances.
Diffusion-sensitive Fourier-domain optical coherence tomography

M. Hagen-Eggert, Medizinisches Laserzentrum Lübeck GmbH (Germany); D. Hillmann, P. Koch, Thorlabs GmbH (Germany); G. Hüttmann, Univ. zu Lübeck (Germany)

Diffusion-sensitive OCT (DS-OCT) is presented as a new functional extension to OCT.

Fluctuations of signal intensity and phase, which are caused by Brownian motion are analysed by autocorrelation function similar to dynamic light scattering measurements.

Based on an ultrafast Fourier-domain OCT system, DS-OCT can determine quantitatively diffusion properties, like the hydrodynamic diameter or the diffusion-constant of colloidal suspensions with high depth resolution at particle sizes ranging from 20 nm to a few microns.

The experimental setup consists of the Thorlabs spectral radar OCT system Hyperion equipped with a high power SLD from Superlum. As measurement probe a fiber with a FC-PC connector, for which the polished fiber end worked as the reference mirror, or specially designed miniature interferometer were used.

Performance of DS-OCT is demonstrated with polystyrene particle suspensions and compared to measurements of a conventional DLS device. Furthermore, the capability of making spatially resolved measurements with micrometer resolution will be demonstrated. Therefore we used samples of spatially separated particle suspensions and mixtures of different particles, where the separation over time was investigated successfully. Exclusively, first two-dimensional tomographic diffusion contrast images will be presented and compared with common OCT-images.

Applications for DS-OCT may be found in the measurement of particle size distributions of inhomogeneous samples, time dependent changes of particle compositions or diffusion properties at boundary surfaces. Additionally, the method has the capability to become a useful benefit in clinical diagnostics, especially in ophthalmology, where the molecular compositions and pathological changes of anterior eye components could be detected.

Concentration dependent scattering coefficients of intralipid from 600 to 1600 nm

D. J. Faber, N. Bosschaart, Univ. van Amsterdam (Netherlands); V. M. Kodach, J. Kalkman, T. G. van Leeuwen, Academisch Medisch Ctr. (Netherlands)

The contribution of multiple and dependent scattering effects to the OCT-measured scattering coefficient µs was investigated using 600, 800, 1300 and 1600 nm OCT systems. For single, independent scattering µs increases linearly with (low) scatterer concentration C. For higher C, both effects cause an increasing underestimation of µs with increasing C; albeit that multiple scattering is expected to be more pronounced at lower wavelengths and dependent scattering is expected to be more pronounced at higher wavelengths. We determined µs as function of Intralipid concentration. The onset sublinear increase of µs with concentration was found to take place at C ~ 2.5% - 5% for all wavelengths. Results are compared with Mie theory and literature and are found to agree well. The clear presence of these effects at the higher wavelengths (with low µs) suggest that concentration dependent scattering effects play a thus far overlooked role in quantitative µs measurements using OCT.

Measurements of wavelength dependent scattering and backscattering coefficients by low-coherence spectroscopy

N. Bosschaart, D. J. Faber, T. G. van Leeuwen, M. C. Aalders, Jr., Univ. van Amsterdam (Netherlands)

Developing new, or improving existing optical techniques for medical therapeutics and diagnostics often depends on exact knowledge of the optical properties of the tissue. This knowledge may be used as input for light tissue interaction models e.g. to calculate the optical contrast between healthy and diseased tissue. Quantitative measurements of the scattering properties of tissue are therefore invaluable in biomedical optics. However, despite the existence of many spectroscopic methods it is still a challenge to do non-invasive, quantitative measurements of the scattering properties in vivo over a large wavelength range.

We recently introduced Low Coherence Spectroscopy (LCS) as a spectroscopic method for quantitative and localized measurements of absorption coefficients. In this study, we will show the additional ability of LCS to measure the scattering coefficient µs and the backscattering coefficient µb. In advantage over other spectroscopic methods, LCS measures µs (not µs') and µb, within a very confined volume and over a large wavelength range (480-680 nm) with a relatively high spectral resolution (8 nm).

We measured µs and µb on aqueous suspensions of different sized polystyrene spheres with scattering coefficients ranging from 1 to 3 mm⁻¹. The LCS measurements of µs agree within 0.3 mm⁻¹ with Mie theory and the measured µb correctly follow the wavelength dependent oscillations from Mie theory.

Further investigation is needed on samples with higher µs, within the range of tissue scattering. In conclusion, we can state that LCS is a promising technique for non-invasive, quantitative, localized in vivo measurements of tissue scattering properties.
Tactile sensation imaging system for inclusion mechanical property characterization

J. Lee, N. Garcia-Acosta, K. Te, C. Won, Temple Univ. (United States)

Elasticity of the inclusion is an important characteristic in determining malignancy of a tumor. We investigate a portable tactile sensation imaging system that will provide mechanical properties of the inclusion for cancer screening applications. The system is based on the total internal reflection principle. The sensing probe part of the system is a flexible and transparent elastomer (polymethylsiloxane). This is used as a waveguide. We inject light with a carefully calculated light incidence angle. The light will totally reflect if nothing is touching the sensing probe. If an object such as a tumor is embedded in the tissue and when this sensing probe is compressed against the tissue, the light will diffuse. We take images of these diffused lights. Then we analyze these images to obtain the mechanical properties of the inclusion. In particular, we will determine the size, shape, shear modulus, and Young’s modulus of the inclusion using image processing techniques. Using different mechanical properties increase the probability of detecting malignant tumors. Novel imaging processing is an integral part of this system. The other novelties of the system include portability, cost effectiveness, and reliability. Three custom made phantoms that match the mechanical properties of human tissues and tumors will be used to test the system. A small animal study results will also be presented. We envision these characteristics being used in screening for the malignant tumors.

Near-infrared fluorescent imaging to assess lymphatic function improvement after advanced pneumatic compression device treatment of lymphedema

K. E. Adams, B. Niccum, G. Dickinson, M. Bautista, J. C. Rasmussen, I. Tan, C. D. Darne, M. B. Aldrich, M. V. Marshall, L. A. Smith, E. A. Maus, C. E. Fife, R. Guilloyd, The Univ. of Texas Health Science Ctr. at Houston (United States); S. Hoy, Tactile Systems Technology, Inc. (United States); E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

In the United States, lymphedema, a chronic swelling caused by the buildup of lymphatic fluid, most often affects the arms or legs of cancer survivors. Utilization of pneumatic compression device (PCD) therapy to manage lymphedema is limited due to the inability to directly measure response to treatment, and thus a lack of direct evidence of improved lymphatic function. This pilot study employed an investigational imaging technique utilizing indocyanine green (ICG) and near-infrared (NIR) fluorescence imaging to evaluate the lymphatic function response to advanced PCD therapy in 6 normal, control subjects, 6 unilateral arm, and 6 unilateral leg lymphedema subjects. We measured lymphatic contractile functional parameters, such as apparent propulsion velocity and frequency, and lymphatic vessel recruitment from NIR fluorescence imaging conducted before, during, and after advanced PCD therapy; which involves treatment of the lymph-draining basins, then the affected limb. Lymphatic function improved in both arms of the control subjects and untreated, asymptomatic arms of lymphedema subjects, as indicated by increased frequency of lymphatic contractile activity and/or vessel recruitment. In the lymphedema subjects, lymphatic transport improved in 4 out of the 6 subjects after treatment of the affected arms, with improvement defined as proximal movement of ICG after PCD therapy. Analysis of the leg imaging data is underway.

PCDs can stimulate lymphatic function and may be an effective method to manage lymphedema. NIR fluorescence lymphatic imaging may be useful to evaluate response to therapy and may provide direct evidence of therapeutic efficacy.

Effect of background trends removal on noise power spectrum measurements in digital x-ray imaging

Z. Zhou, F. Gao, H. Zhao, S. Gong, K. Jiang, Tianjin Univ. (China)

Noise characterization through estimation of the noise power spectrum (NPS) is a central component of the evaluation of digital X-ray systems. Extensive works have been conducted to achieve accurate and precise measurement of NPS. One approach to improve the accuracy of the NPS measurement is to reduce the statistical variance of the NPS results. However, this method is based on the assumption that the noise in a radiographic image is arising from stochastic (random) processes. In the practical data, the artifacts always superimpose on the stochastic noise as low-frequency background trends and prevent us from achieving accurate NPS. In this study, NPS measurement was implemented and compared before and after background trends removal, the results showed that background detrending reduced the variance of the low-frequency spectral components, hence improving the accuracy of NPS measurement. Our results also showed that involving more samples for ensemble averaging had little effect in reducing the variance of the low-frequency spectral components. All results implied that it is necessary and feasible to get better NPS estimate by appropriate background detrending.

Basic principles of design and functioning of multifunctional laser diagnostic system for non-invasive medical spectrophotometry

D. A. Rogatkin, MONOKI (Russian Federation); S. G. Sokolovski, K. A. Fedorova, Univ. of Dundee (United Kingdom); V. V. Sidorov, SPE LAZMA Ltd. (Russian Federation); N. Z. Stewart, E. U. Rafailov, Univ. of Dundee (United Kingdom)

Last decades marked by great progress in vivo (in situ) studies of optical properties of biological tissues in health and pathology resulted in design of multifunctional non-invasive diagnostics in medicine. The devising of a general engineering theory of multifunctional diagnostic systems, which implement on the same medical facilities various methods of non-invasive medical spectrophotometry such as fluorescence diagnostics, absorption spectroscopy and laser Doppler flowmetry is promising goals in modern biomedical engineering. Main specific characteristic in the design of multifunctional laser non-invasive diagnostic system (MLNDS) is the presence of a large number of problem-oriented computational and interpretative algorithms, which to a greater extent determine the appearance and the functionality of the systems as a whole.

Aiming scientific-engineering formalization of object description of the problem at example of MLNDS structure-functional model of generalized MLNDS as well as it united aim-function was developed and formulated. And finally a key role of the system software in terms of architecture
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construction of MLNDS on the stages of ideological-technical designing has been proved. The basic principles of block-modules composition of MLNDS’ hardware were suggested as well.

7890-54, Poster Session

New non-invasive transcutaneous bilirubin meters for neonatal jaundice using blue-green laser diodes and LEDs

M. Hamza, Mansoura Univ. (Egypt); M. H. Sayed El-Ahl, Tabarak Children’s Hospital (Egypt) and Military Medical Academy (Egypt); A. M. Hamza, National Research Ctr. (Egypt); A. M. Hamza, Tabarak Children’s Hospital (Egypt)

In this paper the authors introduce the theory and design of new noninvasive devices for transcutaneous bilirubin monitoring in neonatal jaundice using blue-green laser diodes and LEDs. The measurements depend upon illuminating the skin of the neonate with radiation of different wavelengths. The choices of wavelengths follow the principles of optical bilirubinometry. The differential absorption detection systems of the new diagnostic tools are designed to make use of the selective absorption characteristics of bilirubin taking into consideration the presence of other chromophores in the skin and blood of the neonate. Our new compact, small size and low cost noninvasive transcutaneous bilirubin meters provide more accurate instruments for either screening or monitoring of serum bilirubin concentrations in a diverse population of neonates. Our new diagnostic tools can accurately detect jaundice in its early stages in order to prevent kernicterus in newborn infants. When properly diagnosed, severe elevation of serum bilirubin can be prevented and effectively treated, preventing brain injury due to hyperbilirubinemia. The detailed description and operating characteristics of our new diagnostic tools are presented.

7890-55, Poster Session

A novel optical probe for pH sensing in gastro-esophageal apparatus

F. Baldini, G. Ghini, A. Giannetti, F. Senesi, C. Trono, Istituto di Fisica Applicata Nello Carrara (Italy)

Monitoring gastric pH for long periods, usually 24 h, may be essential in analyzing the physiological pattern of acidity, in obtaining information on changes in activity during peptic ulcer disease, and in assessing the effect of antisecretory drugs. Gastro-esophageal reflux, which causes a pH decrease in the esophagus content from pH 7 even down to pH 2, can determine esophagitis with possible strictures and Barrett's esophagus.

One of the difficulties of the optical measurement of pH in the gastro-esophageal apparatus lies in the required extended working range from 1 to 8 pH units. Contrary to all acid-base indicators characterized by working ranges limited to 2-3 pH units, methyl red is characterized by a wide working range which fits with the clinical requirements, after its covalent immobilization on controlled pore glass (CPG). A novel probe design, suitable for gastro-esophageal applications, allows to optimize the performances of the coated CPG. This leads to a very simple configuration characterized by a very fast response time.

7890-56, Poster Session

Automatic alignment of a high-performance interferometric medical imaging device

A. T. Cenko, B. B. Behr, Univ. of Waterloo (Canada); P. B. Christensen, Tornado Medical Systems (Canada); A. R. Hajian, Univ. of Waterloo (Canada); J. Hendriks, Tornado Medical Systems (Canada); J. T. Meade, Univ. of Waterloo (Canada); F. D. Sweeney, P. van der Vecht, Tornado Medical Systems (Canada)

For optimal performance of a high-precision optical system, careful and stable alignment is necessary. To achieve robust alignment in a commercial system performance tradeoffs or significant redesigns are often made. We have developed subsystems that allow us to automatically monitor and control the optical system alignment, allowing us to minimize the changes necessary between high-performance research systems and practical commercial designs. In addition, this might allow ruggedization of systems that would be too unstable otherwise.

We have implemented such an alignment system in a high-performance medical interferometric imaging device with a focus on maintaining high throughput and allowing for significant system customization. The system is able to maintain near-optimal alignment without any user interaction over a large thermal range and can compensate for misalignments during initial system construction or resulting from shock events. With careful planning, the cost of such a system can be kept reasonably low and it requires minimal interruption to a normal user’s workflow.

We will discuss the basic principles and necessary considerations for the implementation of such a system, using the developed system as a case study. Similar technology can be used in many optical devices and is especially relevant if access by a trained technician is difficult or costly.

7890-57, Poster Session

Reflectance spectroscopy analysis of oral cavity for mucosal lesions detection

Y. Chen, C. Yeh, National Chung Cheng Univ. (Taiwan); C. Chiang, National Taiwan Univ. (Taiwan); M. Tsai, Chang Gung Univ. (Taiwan); H. Wang, National Chung Cheng Univ. (Taiwan)

In recent years, using spectroscopy as a criterion for diagnosis of oral diseases has become an important part of the development in Biophotonics technology. We make spectrum measurements and analysis of enterovirus-induced angina, especially for mucosal lesions, normal and inflamed tissues. Spectroscopy can provide the information about the parameter changes of light-tissue interaction caused by the morphological and biochemical changes related to the progression of diseases in tissue, thus it can be an approach to distinguish suspected cases. We also analyze the color difference between mucosal lesions and inflamed tissues to study the possibility of symptom changes, so that doctors can identify this angina from other oral diseases easier.

7890-58, Poster Session

A novel magnetically tracked OCT needle probe for improved imaging of deep tissue

B. Y. Yeo, R. A. McLaughlin, R. W. Kirk, D. D. Sampson, The Univ. of Western Australia (Australia)

Optical coherence tomography (OCT) needle probes allow high resolution imaging of structures deep within tissue. However, the manual insertion and placement of a needle probe presents new difficulties in OCT image formation. As is currently done with biopsy needles, the clinician will modify the speed and trajectory angle of the needle probe during insertion to precisely guide the needle through the tissue being scanned. Accurate reconstruction of the image requires some form of needle tracking to account for these changes. Previous work has proposed the use of an optical tracking system, but this requires a clear line of sight from the optical sensor to external detectors, which may be impractical in a complex surgical setting. Image processing approaches have also been proposed to correct for changes in needle insertion speed. However, such algorithms are unable to correct for changes in trajectory angle.
A magnetic tracking system has the potential to overcome these problems. We have developed a proof-of-principle system combining an OCT needle probe with a magnetic tracker to achieve accurate needle tracking. The side-facing OCT probe is encased in a 23-gauge stainless steel hypodermic needle, with a sensor mounted on the base of the needle. During system development, we identified the discrete resolution of the tracking system to be a significant limitation to system accuracy. To address this, we adopted a spatial interpolation scheme calculated over multiple successive position measurements and demonstrated significantly improved accuracy in image reconstruction. The system has been demonstrated on phantom and tissue samples.

7890-01, Session 1

A potential individual cell malignancy indicator: focal length

W. Wang, K. L. Lear, Colorado State Univ. (United States)

Analysis of cells' focal lengths acquired with optofluidic intracavity spectroscopy (OFIS) showed that they can be used as a cell malignancy indicator with a p-value of 0.001. The label-free technique of OFIS has differentiated cancerous and non-cancerous cells based on the distinctive transverse mode patterns of their characteristic transmission spectra in the optofluidic cavities. The transverse mode pattern of HSA cells, the moderately uniform spacing of the transverse mode peaks, and the decreasing maximum transmission at higher frequencies within each free spectral range, motivated the analysis on the apertured optical resonator using paraxial Gaussian beam method that treats a high index object, such as a cell, as an ideal thin lens of radius a, and then incorporates the diffraction less associated with different order of transverse mode.

With this method, the focal lengths of each individual cell, fcem, were extracted from the transverse mode features of their experimental OFIS spectra. A statistical analysis of two sample Student’s t-test revealed that the classification based on fcem values between the cancerous HSA cells and baseline monocytes had a p-value of 1.25E-04, while the one between cancerous lymphoma and monocytes was p=1.29E-03. And the p-value between the fcem of HSA cells and lymphoma was 4.95E-06, indicating that the cell's focal length could also be used to differentiate different types of cancer cells.

Analysis and comparison of cells’ focal lengths showed that they can be used as a cell malignancy indicator with p-value of 0.001, or even as low as 2.5E-07.

7890-02, Session 1

Monitoring SERS-based contrast agents in atherosclerosis experimental models

L. Machtoub, Innsbruck Medical Univ. (Austria)

There have been enormous progresses in developing a class of multimodal contrast agents which combine MRI with optical imaging. Contrast agent targeting can provide enhanced diagnostic information, allowing differentiation between variable and stable atherosclerotic plaques. Recently an intensive efforts have been working on the development of contrast agents that can improve the ability to detect and characterize atherosclerosis in clinical and preclinical applications. Earlier studies on hyperlipidemic rabbits using in vivo MRI have shown accumulation of USPIOs in plaques with a high macrophage content that induces magnetic resonance (MR) signal changes correlated to the absolute iron content in the aortic arch. A potent new class of nanoparticles contrast agents have recently drawn much attention for its wide diverse diagnostic and potential therapeutical applications particularly in monitoring the inflammatory responses. In our previous studies we have investigated USPIO contrast agents uptakes in hepatic and spleen tissues taken from NZW rabbits. The scope of this work encompasses application of an emerging hybrid imaging modality, SERS-based nonlinear optical microscopy, in investigating atherosclerosis experimental models. In this work experiments are performed on contrast treated tissue sections taken from aortic arch of atherosclerotic animal model. Marked contrast enhancement has been observed in the treated aortic sections compared with the untreated control. The obtained images are compared with immunohistochemistry. The work presented can be promising for future studies on in vivo detection of macrophages in human plaques and early detection of atherosclerotic diseases.

7890-03, Session 1

Stand-alone device for point-of-care applications

F. Baldini, Istituto di Fisica Applicata Nello Carrara (Italy); L. Bolzoni, Datamed S.r.L. (Italy); G. Ghini, A. Giannetti, Istituto di Fisica Applicata Nello Carrara (Italy); G. Porro, Datamed S.r.L. (Italy); C. Trono, Istituto di Fisica Applicata Nello Carrara (Italy)

A fluorescence-based stand-alone platform was developed for the detection of bioanalytes for sepsis diagnosis in Point of Care applications, in particular procalcitonin (PCT) and C-reactive protein (CRP). The heart of the platform is a polymethylmethacrylate (PMMA) chip for the simultaneous monitoring of the analytes of interest. The chip, produced by injection moulding, is constituted by 13 microchannels through which the sample flows. The sensing layer where the immunochemical reaction takes place is located on the upper part of each microchannel. A laser line collinear to the microchannel, excites the sensing layer and a large fraction of the emitted fluorescence by the sensing layer is guided along the thickness of the PMMA cover up to its end-face where it is collected by a plastic optical fibre and sent to a photodetector. A motorised translation stage allows the automatic scanning of the 13 different microchannels. A syringe pump and a microfluidic manifold, both embedded into the system, drive the sample under test through the different microchannels. Sandwich assay were implemented for the determination of PCT and CRP. Limit of detections of 0.8 microg/L and 2 microg/L were achieved for PCT and CRP, respectively.

7890-04, Session 1

Bioluminescent microbead assay for serum protease: ‘ome’ activity

F. Chuang, NSF Ctr. for Biophotonics Science and Technology (United States)

Serum proteases are involved in the induction and progression of a broad spectrum of diseases, including atherosclerosis, stroke, microbial infections, neurodegenerative disease, and many forms of cancer. While it makes sense to identify proteases in the blood for early detection and monitoring of these diseases, most in vitro diagnostic methods use antibodies to detect the total protein content, irrespective of protease activity. Furthermore, the concentration of serum proteases in the bloodstream can be exceedingly low, posing a technical challenge for conventional immunoassays; and finally, multiple proteases are involved in different ways with multiple diseases. It is unlikely that any one protease can serve as a necessary and sufficient marker for a given disease. We propose a new biophotonic instrument that obtains a complete profile of protease enzyme activity in a clinical sample of blood in less than one hour. The platform combines three innovative technologies: (1) a bioluminescent assay in which light is specifically produced in direct proportion to serum protease activity; (2) a combinatorial technique to efficiently produce infinitely large oligopeptide libraries conjugated to polystyrene microbeads; and (3) a customised microfluidic chamber to load, test, and display a multiplex microbead array. The microbeads are engineered to each carry an amino acid sequence-specific substrate that, if reactive with a suitable serum protease, releases an aminoluciferin compound that produces light with the addition of luciferase. By arranging the beads in a microarray,
the total profile of serum protease activity can be determined by simultaneously measuring the light produced around each microbead.

7890-05, Session 1
A compact and light-weight differential interference contrast (DIC) microscope for telemedicine applications
S. O. Isikman, C. Oh, D. K. Tseng, O. Mudanyali, A. Ozcan, Univ. of California, Los Angeles (United States)

Differential interference contrast (DIC) microscopy, also commonly known as Nomarski microscopy, allows high-contrast imaging of phase objects such as cells and micro-organisms, which are poorly visualized under bright-field microscopes owing to their low absorption and scattering cross-sections. Unfortunately, relatively large size and complexity of conventional DIC microscopes partially hinder their widespread use for telemedicine applications especially in resource-limited settings, where compact, light-weight and cost-effective microscopes are urgently needed.

To address this need, here we demonstrate a lensfree on-chip DIC microscope, which weighs ~50grams and measures ~4.2x4.2x5.8cm, achieving sub-cellular resolution over a large field-of-view (~24mm2). In this lensfree holographic imaging modality, a simple LED coupled to a large aperture (~50-100um) is utilized for massively parallel illumination of micro-objects over ~24mm2. Two birefringent crystals (~0.2mm thick), assembled at 90° with respect to each other, are placed right after the specimen to shear the wavefronts into two orthogonally polarized waves, which also ensures zero net phase-bias regardless of the LED wavelength employed for illumination. The interference of these two orthogonal waves after passing through a thin plastic polarizer creates DIC enhanced lensfree in-line holograms of the micro-objects on a sensor array. Digital reconstruction of such recorded holograms allows rapid DIC imaging of the micro-objects on the chip in ~1sec, with a resolution of ~1.2-2.0um over ~24mm2. With its lensfree and mechanically robust architecture that does not require any sensitive alignment, this compact and light-weight DIC microscope offers a promising tool for telemedicine microscopy in resource limited settings to combat various global health challenges.

7890-06, Session 1
Comprehensive blood testing using quantitative phase imaging
M. A. H. Mir, H. Ding, K. V. Tangella, G. Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Quantitative phase imaging on red blood cells (RBCs) has recently been demonstrated as a useful clinical tool for morphological analysis of patient blood [1-2]. Although this technique provides several novel three dimensional morphological parameters and unprecedented accuracy in clinical morphological analysis of blood smears, it relies on a priori knowledge of the mean cell hemoglobin concentration (MHC) in a given sample. This decreases the reliability of single cell morphological analysis since the optical phase shift through any specimen, at a given wavelength is a function of both thickness and refractive index or hemoglobin concentration. Here we present a new blood smear analysis tool that exploits the dispersion properties of hemoglobin through quantitative phase imaging at two separate wavelengths. This is accomplished by employing the recently demonstrated instantaneous spatial light interference microscopy technique (iSLIM) [3] which allows for simultaneous phase measurements at red, green and blue wavelengths. Since we now have two equations and two unknowns, we can calculate both topographic and hemoglobin concentration maps of RBC smears. This advance means that quantitative phase imaging may now be used as an independent and comprehensive blood smear analysis, while maintaining its speed, accuracy, low cost, simplicity and flexibility.


7890-07, Session 2
Measuring joint-cartilage thickness using reflectance spectroscopy noninvasively and in real time
M. Canpolat, Akdeniz Univ. (Turkey); T. Denkceken, C. Karagol, A. T. Aydin, Akdeniz Univ (Turkey)

Joint cartilage thickness has been estimated using spatially resolved steady-state reflectance spectroscopy noninvasively and in-real time. The system was consists of a miniature UV-VIS spectrometer, a halogen tungsten light source, and optical fiber probe with six 400um diameter fibers. The first fiber was used to deliver the light to the cartilage and other five were used to detect back reflected diffused light from cartilage. Distances from detector fibers from the source fiber were 0.8 mm, 1.6 mm, 2.4 mm, 3.2 mm and 4 mm. Spectra of back-reflected diffused light were taken on the 40 calf’s patella cartilages. The samples were grouped into four; the first group was the control group and cartilages were not damaged, in the 2nd, 3rd and 4th groups cartilage thickness was reduced approximately 25%, 50% and 100% respectively. A correlation between the cartilage thicknesses and hemoglobin absorption of the light in the wavelength range of 500 nm- 600 nm for source-detector pairs has been found. The system with an optical fiber probe less than 4 mm diameter has potential for the assessment of the cartilage thickness through an arthroscopic channel without giving damage to the cartilage in real-time.

7890-08, Session 2
In vivo monitoring of vessel density pattern in skin phantoms for the application of early sign of shock detection by using diffuse reflectance spectroscopy
R. V. Kanawade, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); G. Sayko, Friedrich-Alexander Univ. Erlangen-Nürnberg (Germany); M. Schmidt, A. Douplik, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany)

Broadband diffuse reflectance spectroscopy is a reliable non-invasive or minimally invasive medical diagnostic modality potentially used for measurements of tissue reduced/-oxy hemoglobin. Medical shock is a common cause of trauma patient’s death in Intensive Care Units (ICU). Still the mortality due to shock is unacceptably high, because of the monitoring/ diagnostic techniques limitations and short time span of shock development. In this paper we introduce a method of monitoring of the vessel density pattern, using diffuse reflectance measurement. The setup contains a spatially resolved optical fiber probe coupled to supersensitive spectrometer and high power light source. The measurement parameters comprising reduced-/ oxyhemoglobin and oxygen saturation are exploited for vessel density spatial pattern detection. Real time monitoring of the vessel density spatial pattern will help to detect the early sign of shock development in intensive care units.
Investigation of source detector separation optimization for an implantable perfusion and oxygenation sensor for liver blood vessels

J. S. Baba, Oak Ridge National Lab. (United States)

We are developing an optical based blood perfusion sensing system to monitoring transplant liver organ vital signals during the critical 7-10 day period post-transplantation. The blood perfusion sensing probe, which is based on 3 LED light sources and a photodiode detector, measures sample diffuse reflectance at 735, 805 and 940 nm wavelengths. In this work, the optimal source-to-detector spacing for maximizing perfusion signal measurement was determined for liver blood vessels. This was accomplished by perfusing dye combinations mimicking the optical properties of different blood oxygenation states through excised porcine liver hepatic arteries and portal veins while collecting data for various perfusion probe source-to-detector spacings. The presented results from this ex vivo study indicate a decrease in the optical signal with increasing distance. They also reveal an optimal range for perfusion probe source-to-detector spacing that allows for sufficient perfusion signal modulation depth with maximized signal to noise ratio (SNR). The findings from this study will be implemented in probe placement and configuration for upcoming in vivo animal studies.

A compact, cost-effective diffuse reflectance spectroscopic imaging system for quantitative tissue absorption and scattering

J. Y. Lo, B. Yu, H. Fu, Duke Univ. (United States); T. F. Kuech, Univ. of Wisconsin-Madison (United States); N. Ramanujam, Duke Univ. (United States)

Wide-field, quantitative spectroscopic imaging of tissue absorption and scattering can have tremendous impact in many clinical situations, such as pre-cancer and cancer diagnosis, tumor margin assessment, and observing tumor response to therapy. We have previously developed a fiber-based quantitative diffuse reflectance spectral imaging system that has reasonable sensitivity and specificity in delineating tissue types in the application of breast tumor margin assessment. However, this system has drawbacks in size, cost, coverage, and clinical practicality and utility. With new illumination and detection strategies, we have redesigned a compact, cost-effective spectroscopic imaging system for quantifying tissue absorption and scattering. The system uses a broadband source with bandpass filters and a light guide for illumination and a 4x4 array of inexpensive silicon photodiodes for detection. The detectors are placed directly in contact with the sample and no sophisticated detection components, such as the CCD and spectrograph, are required. This initial coarse 16-channel imaging prototype has similar performance characteristics as our previous fiber-based system and has been tested in liquid phantoms over a wide of optical properties simulating human breast tissue. The compact device is able to extract optical properties with high accuracy. Unlike its previous fiber-based counterpart, this new system design also allows for simpler scaling and cheaper expansion into more channels and higher pixel density for a more comprehensive surveillance of a region of interest. Although the device is evaluated based on the application of breast tumor margin assessment, it can also be scaled for use in many other clinical applications.

Using high-resolution imaging to deconstruct optical sources of contrast

S. A. Kennedy, J. Q. Brown, T. M. Bydlon, Duke Univ. (United States); L. Wilke, J. Geradts, Duke Univ. Medical Ctr. (United States)

The preferred treatment for early stage breast cancer is breast conserving surgery (BCS). In approximately, 40% of the nearly 200,000 BCS procedures performed each year, residual cancer is left behind during the first surgery due to a lack of suitable intra-operative tools. Optical spectroscopy has the potential to meet this clinical need. Our group has developed an intra-operative margin imaging device that is sensitive to different tissue types present in the breast, which we have applied to excised BCS specimens in over 100 patients. This device collects diffuse reflectance spectra, which are converted to tissue optical properties and parameter maps via an inverse Monte Carlo algorithm. The goal of the present work was to use a large-scale optimization algorithm to identify an optimum “cancer index” equation, which uses the extracted optical parameters from each individual pixel to separate malignant from normal pixels. This model can then be prospectively applied to all pixels on the margin images to identify areas on the margin that are likely to be cancer. A genetic algorithm (GA) implemented in MATLAB™, was used to optimize a form of the “cancer index” equation using the optically extracted values of the reduced scattering coefficient, [β]-carotene, and [Total Hemoglobin]. A cancer index was then computed which showed differences between positive and adipose pixels (p<5E-6), as well as all normal pixels (p=0.00058). Current work is aimed at determining the predictive accuracy of this cancer index in detecting cancer both at the site-level and margin-level.

Optical wire lumpectomy: frequency modulation measurements in breast tissue

A. L. Dayton, S. A. Prahl, Oregon Health & Science Univ. (United States)

In the U.S., nearly 300,000 lumpectomies are performed each year and 10-50% of patients had to undergo repeated surgery because negative margin status was not achieved. Non-palpable lesions are particularly challenging as the lesion cannot be identified during surgery. We have developed an optical wire that illuminates the lesion to be removed as well as the tissue surrounding the lesion. This creates a visual sphere of tissue to remove that is centered on the lesion. In addition, a hand held probe has been created that can measure the distance between the light source within the lesion and the probe placed on the surface of the tissue. The intensity of the light source was modulated, and the resulting phase lag measured at the probe with a computer controlled network analyzer. The phase lag was used to calculate the distance between the source and probe between 20 & 60mm at 5mm intervals; each measurement was repeated 3 times. Both 635 and 930nm laser diodes were used as individual light sources. The accuracy of the distance measurement of the optical wire system was tested in both infinite medium and bounded phantoms and found to be within 5-10% of the actual distance. The distance between source and detector in ex vivo breast tissue samples were measured to be within 20% of the actual values. The optical properties of the tissue samples were measured with a 5cm path length using an integrating sphere.

Confocal mosaicing microscopy of Basal-cell carcinomas ex vivo: progress in digital staining to simulate histology like appearance

J. M. Bini, Memorial Sloan-Kettering Cancer Ctr. (United States); V. Hazelwood, Stevens Institute of Technology (United States); J. Spain, K. S. Nehal, Memorial Sloan-Kettering Cancer Ctr. (United States); C. A. D’Marzio, Northeastern Univ. (United States); M. Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)
Confocal mosaicing microscopy enables rapid imaging of large areas of fresh tissue, without the processing that is necessary for conventional histology. Using acridine orange (1 milliMolar, 20 seconds) to stain nuclei, basal cell carcinomas were detected in fluorescence confocal mosaics of Mohs surgical excisions with sensitivity of 96.6% and specificity of 89.2%. A possible barrier, toward clinical acceptance, is that confocal mosaics are based on a single mode of contrast and appear in grayscale, whereas histology is based on two (hematoxylin for nuclei, eosin for cellular cytoplasm and dermis) and appears purple-and-pink. Toward overcoming this barrier, we report progress in digital staining: fluorescence confocal mosaics, which show only nuclei, are digitally stained purple and combined with reflectance confocal mosaics, which show only cellular cytoplasm and dermis, and digitally stained pink, to mimic the appearance of histology. Using multispectral analysis of H-stained and E-stained tissue and the CIE color matching functions, RGB components of each stain in tissue were determined. The resulting RGB components are then applied in a linear algorithm to transform the fluorescence and reflectance contrast of confocal microscopy to the absorbance contrast of pathology. Optimization experiments for staining with acridine orange showed reduced pooling artifacts and improved quality of digitally stained mosaics. Comparison of digitally stained confocal mosaics by our Mohs surgeon to the corresponding Mohs histology shows good correlation for normal versus tumor digit. Digitally stained confocal mosaicing microscopy may allow direct examination of freshly excised tissue and eventually serve as an adjunct for rapid pathology at-the-bedside.

7890-14, Session 3
An integrated time-resolved fluorescence and diffuse reflectance spectroscopy instrument for optical biopsy
Z. Nie, D. Cappon, J. E. Hayward, T. J. Farrell, M. S. Patterson, J. Provias, N. Murty, W. McMillan, Q. Fang, McMaster Univ. (Canada)

Times resolved fluorescence (TRF) spectroscopy and diffuse reflectance (DR) spectroscopy have been used in optical biopsy for clinical diagnostics as potential minimally-invasive, real-time alternatives to tissue biopsies. Fluorescence lifetime is independent of intensity variations and adds an additional source of contrast to steady-state fluorescence. Diffuse reflectance provides quantitative measurement of optical properties of tissue. Combining both DR and TRF spectroscopy in one optical biopsy instrument allows for the integration of diffuse reflectance, time-resolved, and steady-state spectra for tissue diagnosis. In addition, real-time correction of the fluorescence lifetime and spectrum can be achieved based on in situ optical property measurements. We designed and developed an integrated, clinically-compatible instrument capable of acquiring time-resolved fluorescence and diffuse reflectance signals simultaneously. The performance of the system including the evaluation of a dual modality, compact fiber-optic probe is discussed. Co-registrations and overlapping of the integration volume by both modalities using the dual-modality fiber probe is quantitatively calibrated. The overall performance of the integrated multi-modality instrument is evaluated using a fluorescence tissue phantom model.

7890-15, Session 3
Development of an intravascular diagnostic system integrating an IVUS-guided rotational fiber optics catheter for time-resolved fluorescence spectroscopy
H. Xie, J. Bec, Y. Sun, L. Marcu, Univ. of California, Davis (United States)

This communication describes an unique bi-modal intravascular technique that combines fast, time-resolved fluorescence spectroscopy (TRFS) to dynamically evaluate atherosclerotic plaque composition under pull-back motion, with intravascular ultrasound (IVUS) that allows for both visual reconstruction of plaque microanatomy and guidance of TRFS measurements. The system consists of two pathways (optical and ultrasonic) operated independently that allow for co-registration of data in the two channels. The optical pathway is based on a 200 to 400 nm side-viewing fiber optic and a fast (few microseconds) detection system for simultaneous TRFS data acquisition in four wavelength bands (390/40nm, 454/45nm, 542/45nm and 620/30nm) coupled to a fast-frame time-resolved fluorescence data acquisition system (20 GS/s sampling rate, 2.5s dead time). The ultrasonic pathway consists of a single element ultrasonic transducer (40 MHz) catheter (3 Fr) coupled to a standard IVUS platform (iLab, 30Hz frame rate, 256 Beam/frame). The fiber optic and the IVUS catheter are integrated in a compact (8 Fr) catheter sheath where in a pull-back sequence the ultrasonic channel can operate “ahead” of the optical channel so that IVUS images can be used to guide the TRFS data acquisition. A tissue-mimicking PVA- vessel phantom was used to validate the system ability for continuous measurement of TRFS data in radial and pull-back motion, to resolved molecular and morphological structures within the vessel wall, and to generate co-registered TRF-SIVUS data. Current results demonstrate the potential of this bi-modal system to operate intravascularly in conditions of blood flow and motion and to provide co-registered diagnostic information concerning plaque biochemical composition superimposed on plaque morphology and structure.

7890-16, Session 3
Fiber optic endomicroscopy for two-photon fluorescence imaging of human gastrointestinal mucosa
Y. Zhang, Y. Wu, J. Xi, E. J. Shin, M. I. Canto, X. Li, The Johns Hopkins Univ. (United States)

Two-photon fluorescence (TPF) endomicroscopy would be of significant clinical value for in vivo imaging of human gastrointestinal (GI) mucosa by helping in early detection of abnormalities at the cellular and subcellular level during routine endoscopy. A scanning fiber-optic TPF endomicroscopy system has been developed and demonstrated for imaging endoscopic mucosa resection (EMR) specimens. The system consists of a customized double-clad fiber (DCF) with the core for single-mode femtosecond pulse delivery and a large inner-cladding diameter of 200 μm for multimode TPF collection. A compact tubular PZT actuator was used to drive a fiber-optic cantilever to achieve high-speed, two-dimensional spiral beam scanning. A miniature compound aspheric lens with a high numerical aperture and reduced chromatic aberration allows for tight focus for the excitation light and efficient collection of the TPF signal. Preliminary ex vivo TPF imaging with the endomicroscopy system was performed on human esophageal EMR samples. The tissues were stained with Acriflavine, a clinically used dye for rapid staining of nuclei and cytoplasm enabling the visualization of cellular structure. Depth-resolved TPF images were also acquired to demonstrate the three-dimensional imaging capability of the system. The endomicroscopic images were compared with tissue histology. This pilot study indicated that the nonlinear endomicroscopy system has the ability to image GI mucosa at cellular and subcellular level has the potential for virtual detection of gastrointestinal abnormalities during a routine endoscopy procedure.

7890-17, Session 3
In-vivo multiphoton fluorescence microscopy of epithelial precancer
W. Zheng, D. Li, Y. Zeng, J. Y. Qu, Hong Kong Univ. of Science and Technology (Hong Kong, China)

Lots of human cancer arises from epithelium, the superficial layer covering the exterior of body or lining the internal body cavities.
Endogenous fluorophores such as aromatic amino acids, reduced nicotinamide adenine dinucleotide (NADH), flavoprotein (FAD), keratin, collagen, elastin can provide abundant information to reveal the changes in biochemistry, metabolism, and morphology of living tissues. Thus, autofluorescence spectroscopy and microscopy have been recognized as potential tools for discrimination of cancer from normal tissues. However, current fluorescence diagnostic studies mostly rely on spectrum analysis or morphological differentiation. It is challenged since the emission spectra of endogenous fluorophores are broad and usually overlapping and the fluorescence intensity could be affected by many factors. In addition, because the fluorescence of amino acids locates in UV wavelength band, few studies focus on these important endogenous fluorophores for early cancer diagnosis. In this study, we instrumented a multi-color excitation two-photon fluorescence microscopy system with the excitation source selected from a femtosecond Ti:sapphire laser and the ultrafast supercontinuum generation from a photonic crystal fiber to excite multiple endogenous fluorophores. The 7,12-dimethylbenz(a)anthracene-treated hamster cheek pouch were used as the animal carcinogenesis model. And the autofluorescence signals of tryptophan, NADH, collagen and elastin were recorded by a time- and spectral-resolution detection system. The results show that there are obviously differences in the morphology of three-dimensional autofluorescence resolved detection system. The results show that there are obviously differences in the morphology of three-dimensional autofluorescence resolved detection system. The results show that there are obviously differences in the morphology of three-dimensional autofluorescence resolved detection system.

NIR autofluorescence. By examining the anatomy and physiology of biological fluorophores in this region of the body which explain such peak found in parathyroid and thyroid tissues

Endocrine surgery traditionally involves meticulous dissection and resection of diseased glands based on visual recognition by the surgeon. Complications such as postoperative hypocalcemia and hypo-parathyroidism can occur due to accidental or incomplete removal of parathyroid glands. Initial fiber probe measurements have shown that the parathyroid exhibits markedly higher levels of near-infrared (NIR) autofluorescence in comparison to all other tissues in the neck with peak emission above 800 nm. However, there are no documented biological fluorophores in this region of the body which explain such NIR autofluorescence. By examining the anatomy and physiology of parathyroid and thyroid tissues the three primary candidates for the responsible fluorophore were determined to be Parathyroid Hormone, porphyrins and calcium-sensing receptor. The fluorescence of each compound was directly compared to excitation-emission measurements of frozen human thyroid and parathyroid tissues to assess the validity of each hypothesis. Additionally, protein and cell experiments were performed to correlate the NIR autofluorescence to expression of calcium-sensing receptor.

Analysis of near-infrared auto-fluorescence

Computer-aided diagnosis and classification of rheumatoid finger joints

L. D. Montejo, H. Kim, J. D. Montejo, C. D. Klose, Columbia Univ. (United States); U. J. Netz, Laser- und Medizin-Technologie GmbH, Berlin (Germany); S. Blaschke, G. A. Mueller, Georg-August-Univ. Göttingen (Germany); J. Reuthan, Laser- und Medizin-Technologie GmbH, Berlin (Germany); P. A. Zawaka, Georg-August-Univ. Göttingen (Germany); A. H. Hiescher, Columbia Univ. (United States)

In this work we report on the ability to diagnose Rheumatoid Arthritis (RA) from optical tomographic images of the peripheral inter-phalangeal (PIP) joints II-IV. The data presented was gathered through a clinical trial, resulting in 158 distinct fingers, including 60 healthy, 80 affected, and 18 unaffected. Imaging was performed with source-modulation frequencies of 0, 300, and 600 MHz with a frequency-domain optical tomographic imaging system. From each three-dimensional image data set a series of features was extracted that were used in various classification algorithms. The features included smallest and largest absorption and scattering coefficients as well as the respective ratios and the variance of these optical properties inside the finger. The statistical analysis and classification methods included classical methods such as ANOVA, MANOVA, receive-operator curves, linear and quadratic discriminate analysis. In addition we used machine learning type of algorithm, including support-vector machines, and SOM methods. Overall we found images obtained at 600MHz lead to better classification results than images obtained at 300 or 0 MHz. Furthermore, we found that combining multiple parameters also increases the classification accuracy. For example, the highest sensitivity Se = 0.91 and specificity Sp=0.86 were achieved by combining the variance, maximum and minimum of the scattering coefficient in the classification process. In this paper we discuss in detail the advantages and disadvantages of various classification approaches and how they can be used to make frequency-domain optical tomographic imaging a helpful tool in diagnosing RA.

Near-infrared fluorescence imaging of lymphatics in head and neck lymphedema

I. Tan, E. A. Maus, J. C. Rasmussen, M. V. Marshall, C. E. Fife, L. A. Smith, E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

[Background] Treatment of lymphatic disease is complicated and controversial, due in part to the limited understanding of the lymphatic circulatory system. Lymphedema (LE) could be a complication after surgical resection and radiation treatment in a number of cancer types, and is especially debilitating in regions where treatment options are limited. Although extremity LE could be effectively treated with manual lymphatic drainage (MLD) therapies or compression devices to direct proximal lymph transport, head and neck LE is more challenging, due to complicated geometry and complex lymphatic structure in head and neck region.

[Methods] Herein, we describe the compassionate use of an investigatory technique of near-infrared (NIR) fluorescence imaging to understand the lymphatic anatomy and function, and to help direct MLD in a patient with head and neck LE after surgery and radiation treatment for oral cancer. Immediately after 9 intradermal injections of 25 µg indocyanine green each around the face and neck region, near-infrared (NIR) fluorescence images were collected using a custom-built imaging system with diffused excitation light illumination. In addition, 3-dimensional (3D) surface profilometry was used to monitor response to therapy.

[Results] NIR fluorescence images of functional lymphatic vessels and abnormal structures were obtained. Precise geometries of facial structures were obtained using 3D profilometry, and detection of small changes in edema between therapy sessions was achieved.

[Conclusion] NIR fluorescence imaging provides a mapping of lymphatic architecture for direction of effective MLD therapy in the head and neck. 3D profilometry allows longitudinal assessment of edema to evaluate the efficacy of therapy.

Attenuation of motion artifact in near infrared spectroscopy signals using a wavelet based method

B. Molavi, B. Shadgan, G. D. Dumont, The Univ. of British Columbia (Canada)
Near Infrared Spectroscopy (NIRS) has become popular among researchers in studies involving tissue oxygenation monitoring. One limiting factor in utilization of NIRS is its sensitivity to subject's motion which results in non-physiological changes in NIRS signal commonly known as motion artifacts. Removal of these artifacts is an essential pre-processing step for proper analysis of the signal.

We propose a new wavelet based method for removing NIRS motion artifacts. Wavelet transform has good time and frequency localization. Abrupt changes in the signal will appear as a few large isolated coefficients in wavelet domain and therefore can be better identified and removed in wavelet domain. We assume a Normal distribution for wavelet coefficients corresponding to physiological signal of interest and treat the motion-related coefficients as outliers added to the signal. The mean and variance of the distribution are derived and the probability of observing each coefficient based on the normal distribution is calculated. If this probability is less than an arbitrary value, we decide the coefficient belongs to artifacts and remove it. We apply this thresholding scheme to a selected subset of decomposition levels and reconstruct the NIRS signal with new coefficients.

We applied our method to NIRS data collected from leg muscle in 3 patients undergoing leg fracture operation. Subjects had 21, 7 and 13 motion events with variable time durations, respectively. Results indicate an average of 18.32 db attenuation in artifact energy with an average of -14.62 dB of Normalized Mean Squared Error between the original and processed signal in artifact-free regions.

7890-22, Session 4

Skeletal muscle oxygenation assessment by near-infrared spectroscopy in intensive care medicine

C. Wang, National Chiao Tung Univ. (Taiwan); S. Liang, M. Chuang, China Medical Univ. Hospital (Taiwan); C. Chuang, National Taiwan Univ. (Taiwan); Y. Hsieh, C. Sun, National Chiao Tung Univ. (Taiwan)

In intensive care unit, sepsis and heart failure are the familiar disease of microcirculation. To investigate the disease-induced changes of local metabolic rate and local tissue perfusion adequacy, near-infrared spectroscopy (NIRS) was used for measuring the oxygenation signals on brachioradial muscle during a venous occlusion test (VOT). In this study, the oxygenation signals in ischemic and reperfusion phase via oxygenation response with time-variant pressure VOT (20-50 mmHg) in patients of sepsis, patients of heart failure show obvious different hemodynamics. The oxygenation signal plays an important role to assess the adequacy of oxygen delivery to and oxygen extraction from the microcirculation. Therefore, the measurement can provide a vital sign for clinical diagnosis by quantitative analysis of disease-induced changes from peripheral tissue oxygenation measurement.

7890-23, Session 5

Performance of line-scanning confocal microscopy in human skin: investigation of potential for clinical translation

B. A. Larson, G. Peterson, S. Abeytunge, M. Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Line-scanning, using 8-10 optical components, linear-array detectors and custom-FPGA electronics, may enable smaller, simpler and lower cost (< $15,000) confocal microscopes to accelerate translation to the clinic. The adaptability of commercially available low-cost array detectors for confocal microscopy and clinical cancer imaging is being investigated. With 830 nm illumination and 0.9 numerical aperture (NA), instrumental optical sectioning is 1.6-3.8 um and lateral resolution is better than 0.78 um as measured with a test target. These results showed good agreement with theory, and are comparable to that of point-scanning systems. LSFs through full thickness of human epidermis show two-fold degradation in sectioning performance. The LSFs are robust against variations in quarter-wave plate thickness and defocus of the line in the back focal plane of the objective. Analysis predicts the SNR to be 350, limited by shot noise after correcting for pattern noise. Imaging in vivo demonstrates nuclear and cellular detail down to the basal layer in human skin with a benchtop setup (830 nm, 0.9 NA) and also a compact clinical prototype (405 nm, 0.55 NA). Blood flow in oral mucosa is also imaged using the clinical prototype. However, speckle and background noise degrades contrast and resolution of the image. Fluorescence imaging shows improved contrast and resolution, compared to reflectance, due to absence of speckle. Future work will investigate the use of incoherent light sources and pupil engineering to explore the reduction of speckle and background in reflectance images and detectability of signal for fluorescence imaging with the new array detectors.

7890-24, Session 5

Deep-tissue optical imaging of decubitus ulcers

R. Moza, J. M. DiMaio, The Univ. of Texas Southwestern Medical Ctr. at Dallas (United States); J. Melendez, Spectral MD Inc. (United States)

Decubitus ulcers are a costly and widespread issue in healthcare today, that result from impaired blood flow in skin and underlying muscle and tissue. To address this need, a point of care multi-wavelength diagnostic imaging system has been developed to monitor hemodynamic processes via use of optical imaging of deep tissue. The resulting measurements demonstrate changes in light-tissue interaction to differentiate healthy and pathologic tissue without disturbing patients in a hospital setting. The identification of light source-detector illumination patterns uniquely map to spatial depths of tissue. The additional time dependent component, allows a novel four-dimensional analysis of tissue. The portable, noninvasive, and non-contact features provide quantitative in-situ measurements.

7890-25, Session 5

Characterization of burn injuries using terahertz time-domain spectroscopy

M. H. Arbab, T. C. Dickey, D. P. Winebrenner, A. Chen, P. D. Mourad, Univ. of Washington (United States)

Each year, over two million cases of burn injuries are reported for seeking medical attention in the United States. Grading of these burn wounds mainly rely on the physician’s experience and training. However, the accuracy rate of the clinical assessments alone, which are generally based on the wound appearance, is only about 64%-70% for partial thickness burns. This rate is even more disappointing (50%-85%) in differentiating a sub-group of such wounds that will not heal within three weeks without surgical procedures. In this paper, we present experimental results from terahertz characterization of burn wounds induced on a rat model. The male Sprague-Dawley rats used in this work were divided into two groups for both acute burn characterization as well as 72-hour survival studies. Throughout the study, the animals were anesthetized using isoflurane in accordance with the care protocol approved by the University of Washington Institutional Animal Care and Use Committee. The burns were induced by timed introduction of a 313 g metal brass heated to approximately 100°C in a water bath to the dorsal skin of the subject. Reflection measurements were obtained from the surface of both burn wounds as well as normal skin using ultra short pulsed terahertz radiation. Furthermore, spectroscopic signal processing techniques are described for interpretation of the acquired waveform. Differentiation of burn injuries from healthy, unburned skin is achieved with p-values as small as 0.0038.
Multispectral imaging system for imaging $O_2$Hb and HHb concentration changes in tissue for various clinical applications

J. H. Klaessens, R. de Roode, Univ. Medical Ctr. Utrecht (Netherlands); R. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); H. J. Noordmans, Univ. Medical Ctr. Utrecht (Netherlands)

Non-invasive, non-contact imaging techniques are useful to observe local variations in temperature, oxygenation and blood perfusion over large tissue areas.

Four imaging systems were developed and compared: Two systems consisting of white broadband light source and a CCD camera in combination with a Liquid Crystal Tunable Filter, one in the visual domain, 420-730 nm, and one in the infrared domain, 650-1100 nm. Thirdly, a CCD camera in combination with a software controlled multi-wavelength light source consisting of a panel with 600 LEDs divided in 16 spectral groups in the range from 370 to 880 nm. Specified spectral distributions can be generated instantly at high repetition rate (>1000 Hz). And, fourthly a standard IR thermal camera (FLIR ThermoCam SC640) for comparison.

From the acquired images at the selected wavelengths chromophores concentration images of oxy and deoxy hemoglobin can be calculated applying different algorithms.

These imaging techniques were applied and compared for various clinical applications: Tumor demarcation, early inflammation, effectiveness of peripheral nerve block anesthesia, and localization of epileptic seizure. The relative changes in oxygenation and temperature could be clearly observed in good correlation with the physiological condition. The algorithms and data collection/processing can be optimized to enable a real-time diagnostic technique.

Early increase in blood supply associated with premalignant colonic lesions detected by fiber optic polarization-gated spectroscopy

A. J. Gomes, S. Ruderman, J. D. Rogers, V. Backman, Northwestern Univ. (United States)

Endoscopic examination has proven effective in both detecting and preventing colorectal cancer; however, only about a quarter of eligible patients undergo colonoscopic screening. We have developed an endoscopically compatible fiber-optic probe and clinical data collection system that utilizes polarization-gating to depth-selectively measure microvascular blood content. The penetration depth of the light collected by the probe is a crucial design parameter and we report on methods to control and characterize the penetration depth of polarization-gated spectroscopy. Using a probe with a penetration depth suitable for the colonic mucosa, we have taken measurements from human patients in vivo during colonoscopy procedures. Results show an increase in blood supply localized to the mucosal layer in patients harboring neoplastic lesions. Furthermore, this increase is present both near and distant to the site of the lesion. Detection of microvascular blood content via polarization-gated spectroscopy could provide a low cost and patient friendly means of identifying high risk patients most in need of a colonoscopy.

Imaging tumor specific peptide-targeting using spectral-domain optical coherence tomography

P. Yu, L. Ma, Univ. of Missouri-Columbia (United States)

In vivo imaging of targeting molecular probes, or molecular imaging, is an emerging field for early detection of cancer. Optical imaging is particular suited for molecular imaging, as optical probes are sensitive and can be specifically conjugated to small molecules, antibodies and proteins. In this work, we report recent studies on tumor specific peptide-targeting
Multimodality optical imaging of ovarian cancer in a post-menopausal mouse model

J. M. Watson, P. F. Rice, D. L. Bently, S. L. Marion, The Univ. of Arizona (United States); M. A. Brewer, Univ. of Connecticut Health Ctr. (United States); P. B. Hoyer, J. K. Barton, The Univ. of Arizona (United States)

In the US over 21,500 new cases of ovarian cancer arise each year (American Cancer Society). Our goal is to use multi-modality optical imaging, including optical coherence tomography (OCT) and multi-photon microscopy (MPM), to detect cancer development on the sub cellular scale in a mouse model. Using multiple modalities will allow us to collect more information than either technique alone. By determining the microscopic changes that precede ovarian cancer we may be able to develop a minimally invasive screening test for high risk patients. A mouse ovarian cancer model has been developed by treating mice with 4-Vinylcyclohexene Diepoxide (VCD) to induce ovarian failure and 7,12-Dimethylbenz[a]anthracene (DMBA) to induce ovarian cancer. The drug treatment has successfully led to tumor development in mice. By mounting a 3D OCT system on the MPM stage, we have obtained co-registered en face images of twenty mouse ovaries ex vivo. Images have been analyzed and compared to histology. Preliminary analysis indicates that OCT can visualize ovarian microstructure, multi-photon excited fluorescence microscopy highlights cells with high metabolic rates, second harmonic generation microscopy shows detailed collagen structure, and that all parameters differ in control and treated ovaries. We have an approved protocol for sterile imaging in vivo and have successfully completed two survival surgeries. During the next year we will be completing a long term survival study using post-menopausal mice that have been treated with DMBA to induce cancer and imaged in vivo at time points before and after treatment.

Optical coherence tomography in agriculture applications

C. Lee, S. Lee, S. Han, H. Jung, J. Kim, Kyungpook National Univ. (Korea, Republic of); C. Na, D. Youn, C. Choi, Dongshin Univ. (Korea, Republic of)

Optical coherence tomography is a powerful, non-invasive diagnostic imaging technology that provides micro resolution, cross-sectional images. Advanced in OCT technology have made it possible to apply OCT in wide variety of application such like medical applications: ophthalmology, dermatology, gastroenterology, cardiology, and oncology. Since its first demonstrates, study trends have been focused on the development of 3D real-time OCT, high resolution OCT, and endoscopic OCT. Recently, these significant technological advances opened the new application field such as micro size electro components screening, crack detection and plants applications. However, in the case of plant application, the study scopes were limited to reveal the morphological features. In this paper, we demonstrated the possibility of the diagnosis of plant disease: apple disease 'Marssonina blotch', abnormal seeds screening: melon seed, cucumber seed, tobacco leaves infected by virus. By comparing the normal with abnormal samples, we could find distinctive changes in substance boundary. These results will contribute the expansion of the application in OCT.

Versatile display mounted handheld OCT probe for primary care diagnostics

W. Jung, Univ. of Illinois at Urbana-Champaign (United States); J. Kim, M. Jeon, Kyungpook National Univ. (Korea, Republic of); E. J. Chaney, Univ. of Illinois at Urbana-Champaign (United States); K. Sayegh, S. I. Sayegh, EYE Ctr. (United States); C. N. Stewart, Blue Highway, LLC (United States); S. A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

We have developed a new type of handheld OCT probe which is able to not only image various tissues, such as the eye, ear, and skin, but also provide the physician with a convenient imaging tool to be used in the primary care clinical environment where both space and time are limited. The handheld OCT probe utilizes a pair of galvanometer scanners and interchangeable lens mounts for imaging different tissue sites. The lens mounts were designed to conform to the outer shape of the ear and eye to provide ready comfortable access to tissue in short periods of time. In our probe, a miniature CCD-based color video camera and 3.5 inch display were integrated. During acquisition of OCT images, real-time OCT and video images are shown both at the control computer monitor and a flexible handheld displays.
and on the small display on the probe. Physicians can also operate the system and save OCT images using button controls mounted on the probe handle. In order to demonstrate the use and performance of this new handheld OCT probe design, in vivo human retina, cornea, skin, and tympanic membrane were imaged, which clearly identified the normal tissue morphology. This new instrument and approach will advance the diagnostic capabilities of primary care medicine and point-of-care, enabling screening and earlier detection of disease, as well as provide quantitative image-based data for more efficient referral to subspecialty care.

7890-36, Session 7

Simultaneous confocal fluorescence microscopy and optical coherence tomography for in-vivo drug distribution and tissue integrity assessment

M. T. Rinehart, J. T. LaCroix, M. H. Henderson, D. F. Katz, A. P. Wax, Duke Univ. (United States)

Effectiveness of microbialidal gels, topical products developed to prevent infection by sexually transmitted diseases including HIV/AIDS, is governed by extent of gel coverage, pharmacokinetics of active pharmaceutical ingredients (APIs), and integrity of vaginal epithelium. While biopsies provide localized information about drug delivery and tissue structure, in vivo measurements are preferable in providing objective data on API and gel coating distribution as well as tissue integrity.

Our system combines confocal microscopy with optical coherence tomography (OCT) to simultaneously measure local concentrations and diffusion coefficients of APIs during transport from microbialidal gels into tissue, while assessing tissue integrity. The confocal module acquires 2-D images of fluorescent APIs multiple times per second allowing analysis of lateral diffusion kinetics. The custom Fourier domain OCT module has a maximum a-scan rate of 54 kHz and provides depth-resolved tissue integrity information co-registered with confocal fluorescence measurements. Each imaging modality uses separate scanning systems, allowing independent control of scan rates and beam shaping, prior to combination in a mutual imaging objective. The confocal illumination uses the full objective numerical aperture and achieves an axial slice thickness of 5µm while the OCT imaging is designed to provide 10µm lateral resolution and a 400µm depth of field. The combined system is validated by imaging phantoms and ex vivo epithelial tissue coated with experimental microbialidal gels containing the APIs UC-781 and Dapivirine. Time-resolved API concentration measured as the throughput at a given resolution. Traditionally resolution may be increased by placing a slit at a conjugate focal point but the throughput decreases as a result. Our group has come up with a robust device called a virtual slit that fundamentally improves the performance of a spectrometer by use of a virtual slit.

Unfortunately, such OCT needle probes have a very small field of view, limited to approximately 1-2mm from the needle, making it extremely difficult to locate the tissue to be imaged.

Ultrasound guidance of biopsy needles and of fine needle aspiration is commonly used in many clinical settings. We describe a proof-of-principle experimental setup where the ultrasound probe is used to visualize tissue structures, and provide real-time guidance for OCT needle placement.

During needle guidance, ultrasound images were acquired along the long axis of the needle, visualizing both the needle and the target tissue. Once the OCT needle probe was correctly placed, the ultrasound probe was re-oriented to image the short axis of the needle, allowing simultaneous visualization of structures in both the OCT and ultrasound images. We demonstrate the technique on several tissue phantoms.

7890-39, Session 7

Fundamental performance improvement in Raman and SD-OCT systems by use of a virtual slit

J. T. Meade, B. B. Behr, Univ. of Waterloo (Canada); P. B. Christensen, Tornado Medical Systems (Canada); A. T. Cenko, A. R. Hajian, Univ. of Waterloo (Canada); J. Hendrikse, F. D. Sweeney, Tornado Medical Systems (Canada)

The fundamental performance of a dispersive based spectrometer is measured as the throughput at a given resolution. Traditionally resolution may be increased by placing a slit at a conjugate focal point but the throughput decreases as a result. Our group has come up with a robust device called a virtual slit that fundamentally improves the performance of a spectrometer by being able to increase the spectral resolution without decreasing the throughput. This device fundamentally impacts speed sensitive biomedical technologies that are limited by the performance of their spectrometer subsections. Raman microscopes require very high resolution and have very low throughput so integration times must be long to obtain a statistically significant signal. A virtual slit placed into a Raman microscope increases the throughput while maintaining
the required resolution so integration times may be decreased. Faster operating Raman microscopes may be used for better quality control in pharmaceutical drug development. Spectral domain optical coherence tomography (SD-OCT) systems are limited in their penetration depth and image quality by their spectral resolution. A virtual slit placed in an SD-OCT system fundamentally increases the maximum penetration depth, image quality, and dynamic range of the instrument. The virtual slit operates on a collimated beam of light by redistributing the étendue between the dispersion and cross-dispersion axes to change the focused spot profile to increase the resolution. The operation and performance characteristics of the virtual slit will be presented.

7890-40, Session 7
Spectroscopic swept-source optical coherence tomography for hemoglobin saturation level detection
J. D. Cho, H. S. Lee, M. Jeong, C. Kim, Pusan National Univ. (Korea, Republic of); H. Song, B. K. Kim, Electronics and Telecommunications Research Institute (Korea, Republic of)
Spectroscopic optical coherence tomography (OCT) is a recently developed technical extension of OCT for functional images. As an isosbestic point of hemoglobin lies on 800 nm wavelength, wavelength dependency of optical attenuation can be used to detect the different saturation level between oxy- and deoxy-hemoglobin. Conventional spectroscopic OCT’s have used a line scan camera with broadband light source centered at 800 nm, however, they suffered several limitations, such as limited pixel number of camera, non-Gaussian shape of light spectra, low sensitivity and high cost. In this work, we present a spectroscopic swept-source based on an 800 nm optical semiconductor amplifier gain for hemoglobin saturation level detection and functional imaging. Bandwidth of wavelength swept laser is measured around 40 nm centered at 800nm and instantaneous linewidth is also measured around 0.1 nm. By using of this novel swept-source, we can achieve a higher sensitivity image due to balanced detector and simpler design of all-fiber spectroscopic OCT system. For a simple design of optical system, we employed all-fiber ring cavity swept source and modified Michelson interferometer.

7890-41, Session 8
Diagnosing lung cancer using coherent anti-Stokes Raman scattering microscopy
L. Gao, Rice Univ. (United States) and Methodist Hospital Research Institute (United States); F. Li, J. Xing, Methodist Hospital Research Institute (United States); M. J. Thrall, The Methodist Hospital (United States); Y. Yang, Z. Wang, P. Luo, Methodist Hospital Research Institute (United States); K. K. Wong, Methodist Hospital Research Institute (United States) and The Methodist Hospital (United States); H. Zhao, Methodist Hospital Research Institute (United States); S. T. C. Wong, Methodist Hospital Research Institute (United States) and The Methodist Hospital (United States) and Rice Univ. (United States)
Lung carcinoma is the most prevalent type of cancer in the world, and it is responsible for more deaths than other types of cancer. Early detection of lung cancer has attracted major research interest because it dramatically increases the survival rate. However, less than 1% of patients with early-stage lung cancer can be diagnosed because of the asymptomatic nature of the lesion and limitations of current detection techniques. To increase the diagnostic efficacy, we investigated the feasibility of using coherent anti-Stokes Raman scattering (CARS) microscopy as a label-free strategy to image lung lesions. Different mouse lung cancer models were developed by injecting human lung cancer cell lines, including adenocarcinoma, squamous cell carcinoma and small cell carcinoma, into lungs of the nude mice. CARS images were acquired from normal lung tissues and different subtypes of cancer lesions in vivo using intrinsic contrasts from symmetric CH2 bonds. These images showed good correlation with the hematoxylin and eosin (H&E) stained sections from the same tissue samples with regard to cell size, density and cell-cell distance. These features are routinely used by pathologist to diagnose lung lesions. Next, classification software was developed to quantitatively extract above features and utilize them to discriminate normal from cancer lesions as well as different subtypes of cancers from each other. Our results showed that the CARS technique is capable of providing a visualizable platform to differentiate different kinds of lung cancers using pathologically prevalent features and thus has the potential to be applied in vivo for minimally-invasive differential diagnostic applications.

7890-42, Session 8
Combined Raman spectroscopy: in-vivo confocal microscopy for the detection of skin cancers
M. A. Mackanos, Vanderbilt Univ. (United States); C. L. Arrasmith, Montana State Univ. (United States); C. A. Patil, C. Paras, I. J. Pance, Vanderbilt Univ. (United States); D. L. Dickensheets, Montana State Univ. (United States); A. Mahadevan-Jansen, Vanderbilt Univ. (United States)
Skin cancer is the most common cancer in the United States, with a continuously rising rate of incidences however, it can be highly curable if detected at an early stage. The best clinical practices require physicians to screen large areas of skin, identify suspicious lesions, perform biopsies, and wait for the pathology for diagnosis. However, identification of lesions can be subjective, biopsy is invasive, and pathological analysis is time consuming and costly. The potential of novel optical techniques such as Raman spectroscopy and in vivo confocal microscopy to perform rapid, non-invasive “optical biopsy” has been widely touted; however, these methods suffer limitations. The biochemical sensitivity of Raman spectroscopy facilitates classification of lesions with high accuracy; however it is unable to relate lesion microstructure. Confocal microscopy can image tissue microstructure but lacks molecular specificity. The two methods can be combined in a single instrument to provide for in vivo analysis without the need for a biopsy. We will demonstrate the ability of a Raman spectroscopy - in vivo confocal microscope system to perform image guided acquisition of Raman spectra and biochemical identification of features in confocal images. The instrument utilizes independent system backbones with integrated sampling optics. We report the design, characterization, and performance of a clinical system for performing Raman spectroscopy - in vivo confocal microscopy of the skin, and the results of an ongoing pilot study demonstrating the systems feasibility for diagnosing cancerous lesions. Images and spectra acquired from cancerous and non-cancerous lesions using the clinical instrument will be presented.

7890-43, Session 8
In-vivo diagnosis and detection of precancer and cancer in the stomach using multimodal image-guided Raman endoscopy
Z. Huang, National Univ. of Singapore (Singapore)
We report on the first implementation of multimodal image-guided Raman endoscopy technique developed for improving in vivo detection of gastric premalignant and malignancies at endoscopy. A total of 126 gastric patients have been recruited for this study. High-quality in vivo Raman spectra can be acquired from normal and abnormal gastric tissue within 0.5 second under wide-field endoscopic imaging guidance. Significant Raman spectral differences are observed among precancer, cancer and normal gastric tissue. PCA-LDA modeling on in
vivo Raman spectra acquired yields diagnostic sensitivities of 94.4% and 96.2%; and specificities of 99.3% and 98.5%, respectively, for distinction of precancer and cancer from normal gastric tissue. This study illustrates that image-guided Raman endoscopy associated with PCA-LDA algorithms has potential for improving the noninvasive, in vivo diagnosis and detection of gastric precancer and cancer during clinical gastroscopic inspections.

7890-44, Session 8
Optical biopsy of pre-malignant or degenerative lesions: the role of the inflammatory process

H. da Silva Martinho, Univ. Federal do ABC (Brazil)

Recent technological advances in fiber optics, light sources, detectors, and molecular biology have stimulated unprecedented development of optical methods for monitoring pathological changes in tissues. These methods, collectively termed “optical biopsy,” are nondestructive in situ and real-time assays. Optical biopsy techniques as fluorescence spectroscopy, polarized light scattering spectroscopy, optical coherence tomography, confocal reflectance microscopy, and Raman spectroscopy had been extensively used to characterize several pathological tissues. In special, Raman spectroscopy technique had been able to prove several biochemical alterations due to pathology development as change in the DNA, glycogen, phospholipid, non-collagenous proteins. All studies claimed that the optical biopsy methods were able to discriminate normal and malignant tissues. However, few studies had been devoted to the discrimination of very common subtle or early pathological states as inflammatory process, which are always present on, e.g., cancer lesion border. In this work we will present a systematic comparison of optical biopsy data on several kinds of lesions were inflammatory infiltrates play the role (cervical cancer, oral cancer, tendonitis lesions). It will be discussed the essential conditions for the optimization of discrimination among normal and altered states based on statistical analysis (Principal Components Analysis, Logistic Regression, Receiving-Operating Curve). Perspectives concerning the use of high-wavenumber spectral region for this kind of discrimination will also be presented.

7890-45, Session 8
Using Raman spectroscopy to study the onset of labor

E. Vargis, N. Webb, B. C. Paria, J. Reese, K. Bennett, Vanderbilt Univ. (United States); A. Al-Hendy, Meharry Medical College (United States); A. Mahadevan-Jansen, Vanderbilt Univ. (United States)

Preterm labor is the second leading cause of neonatal mortality and can lead to a number of complications for the mother and her child. Having a tool to predict preterm labor could lead to an increased amount of births that come to term. While there are few ways to predict preterm labor with fetal fibronectin screening and cervical length measurements, over half of all preterm births are not diagnosed and do not fall into any high-risk category. This study seeks to predict the onset of labor by using Raman spectroscopy to detect changes in the cervix during pregnancy. Raman spectra were acquired from the cervix of non-gravid and gravid mice and humans, all of whom delivered at full term. We believe significant changes will occur in the Raman spectra obtained during the course of pregnancy. Preliminary results show that there are differences based on cycling status alone, in both mice and humans. Furthermore, there are significant changes that occur during pregnancy. This study will determine if Raman spectroscopy can be used to predict when labor will occur, most likely due to the effect of cervical softening and changes in hormonal levels on the spectra. Any algorithm that can be developed to predict when a woman will enter labor will greatly benefit the outcome for pregnant women and their children.

7890-46, Session 8
Clinical Raman-spectroscopy system for accurate non-invasive glucose monitoring

C. Kong, I. Barman, N. C. Dingari, J. W. Kang, R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

While Raman spectroscopy has been studied as a possible non-invasive optical technique for measuring glucose, it is yet to be seen in the everyday clinic due to difficulties associated with tissue turbidity, lag between blood and interstitial glucose levels, and inherently weak intensity of Raman scattered light. In this study, a portable Raman spectroscopy unit has been designed to investigate the feasibility of using Raman spectroscopy as a non-invasive glucose monitoring technique. The instrument is used to collect Raman spectra from healthy human volunteers undergoing oral glucose tolerance tests and the glucose levels measured from Raman spectra are compared against conventional blood glucose measurements. The main components include an 830 nm diode laser, a thermoelectrically cooled deep depletion CCD, a spectrograph and a light collection unit. For enhanced light collection, a non-imaging optical element converts the wide angular range of the scattered photons into a limited range of angles. The light collection is achieved in back-scattered mode using a flexible optical fiber probe coupled with a compound parabolic concentrator, as well as in transmission mode with a compound hyperbolic concentrator. The glucose detection performances of these two methods are compared. In addition, the instrument includes a broadband light source to collect diffuse reflectance spectra for turbidity correction, and strategies are employed to overcome the effects of tissue autofluorescence and the lag between the blood and interstitial fluid glucose. The information obtained from this study provides valuable insight for implementing Raman spectroscopy as a practical clinical technique for non-invasive glucose monitoring.

7890-47, Session 8
The correlation kernel and support vector machines for the classification of Raman spectra

A. Kyriakides, Univ. of Cyprus (Cyprus); E. Kastanos, Univ. of Nicosia (Cyprus); K. Hadjigeorgiou, C. Pitris, Univ. of Cyprus (Cyprus)

The classification of Raman spectra can be very useful in a wide range of diagnostic applications including bacterial identification. Raman spectra of bacteria can be used to discriminate different species and strains and even provide antibiotic sensitivity. Before classification of the Raman spectra, some form of pre-processing is commonly applied. This pre-processing greatly affects the accuracy of the results and introduces user bias and over-fitting effects. In this work, we propose a novel approach of using Support Vector Machines with a correlation kernel. This kernel defines a similarity metric which captures the appropriate similarities between Raman spectra from the same type of sample. Our experiments show that this kernel is a better choice than the linear SVM or Discriminant Analysis especially on noisy, low resolution, data. A model with cross-validation accuracy of 88% was created, using a training set, which was then used to classify a test set with 87% accuracy (compared to 53% with DA and 60% with linear SVM.) It is important to note that we have used two separate data sets for training and testing. The test set was completely separate from the training set in that it was obtained at a later time period. Most other work quotes only the leave-one-out cross-validation results of the training set creating models which fail when applied to unknown test sets. The correlation kernel is “self-normalizing,” generalizes effectively to unknown data, and produces superior classification performance with minimal pre-processing, even on highly-noisy data obtained using inexpensive equipment.
7890-48, Session 8

Design of handheld clinical Raman spectroscopic systems based on feature selection and nonlinear calibration

N. C. Dingari, I. Barman, C. Kong, J. W. Kang, R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

Raman spectroscopy offers a powerful tool for non-invasive and real-time diagnostics of biological samples. However, its translation to the clinical setting has been encumbered by the lack of robustness of spectroscopic calibration models and the inability to match the spatial footprint constraints. Specifically, linear multivariate calibration models employing full spectrum analysis are often misled by spurious correlations. To develop enhanced robustness in the calibration models and to reduce over-fitting, we propose the application of kernel-based non-linear support vector regression (SVR). Further, we observe that the whole spectrum analysis may not yield optimal predictions due to the presence of uninformative regions in the spectrum. We perform wavelength interval selection based on a moving window approach and minimization of cross-validation error in the calibration set using SVR.

Using glucose detection in tissue phantoms as a representative example, we show that even a three-fold reduction in the number of wavelengths analyzed using SVR lead to calibration models of comparable prediction accuracy as linear full spectrum analysis. With clinical datasets obtained from human subject studies, we also demonstrate the prospective applicability of the selected wavelength subsets from one subject to another, while maintaining essentially constant prediction errors. This key result enables the design of hand-held clinical systems that is programmed for tunable excitation and detection of application-specific selected subsets of the full spectrum. The proposed systems significantly reduce the spatial footprint and we anticipate that they will play a vital role in disease diagnosis as well as in pharmaceutical applications and forensic analysis.
processes such as adhesion, endocytosis, and apoptosis are in progress
this technique to the identification of characteristic features of cellular
Two perpendicularly oriented dye molecules into two separate images
rotating linearly polarized excitation field. Spectral separation of the
in the membrane of liposomes and cells are probed under a constantly
the membrane and BODIPY-PC-labeled lipids aligned perpendicularly
controlled polarization angle of the excitation field. With this system,
fluorescence images is obtained with a camera synchronized with the
combined with a quarter-wave plate, the system
retarder is employed to achieve retardance tunability in the polarized
and its calibration and validation methods to monitor the orientation of
the "real" ones. Results show that the calibration method with standard
phantom gets the best agreement with the results from Monte Carlo
simulation.

Two solid tubular phantoms with known optical properties are adopted to
evaluate the proposed calibration methods. Endoscopic measurements
on the phantoms were carried on to obtain the amplitude attenuation and
phase delay while Monte Carlo simulation was employed to calculate
realistic results. These results show that the calibration method with standard
phantom gets the best agreement with the results from Monte Carlo
simulation.

Calibration methods of frequency domain measurement system with near-infrared
diffused light
H. Zhao, Y. Fan, X. Zhou, J. Liang, T. Wang, F. Gao, Tianjin Univ.
(China)
Optical diagnostics has the potential to provide real-time diagnosis of
tissue noninvasively, and many optical diagnostic techniques are
receiving extensive attention and being developed. Frequency domain (FD)
near-infrared diffuse spectroscopy (NIRS) is one of the three
common techniques in NIRS field. Generally, a FD system modulates the
light intensity in radio frequency and measures the amplitude attenuation and
phase delay of the diffused light using heterodyne detection.
This article deals with the method for eliminating or calibrating the
intrinsic parameters of the measurement system, which include the
intrinsic amplitude attenuation and intrinsic phase delay. Several
 calibration methods are proposed, namely, calibration with standard
phantom, calibration based on multiple source-detector separations
(SDS), and calibration with the combination of standard phantom and
multiple SDS. To eliminate the uncertain contact condition of the optical
fibers and the tissue, a method is also proposed to eliminate the optode
coupling coefficient, which is the main source of the inaccuracy in FD
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simulation.

Real-time polarization microscopy for probing local distribution of biomolecules
Hwang, National Institute of Standards and Technology (United States)
We present real-time full-field fluorescence polarization microscopy
and its calibration and validation methods to monitor the orientation of
absorption dipoles of fluorescent molecules. A liquid crystal variable
retarder is employed to achieve retardance tunability in the polarized
excitation light. Combining with a quarter-wave plate, the system
provides linear polarization rotation of the excitation field which is
coupled to a fluorescence microscope. A series of full-field polarimetric
fluorescence images is obtained with a camera synchronized with the
controlled polarization angle of the excitation field. With this system,
the dynamic orientations of the dipoles of fluorescent lipid analogs such as
catonic tetramethylindocarbocyanine dye-labeled lipids aligned perpendicularly
in the membrane of liposomes and cells are probed under a constantly
rotating linearly polarized excitation field. Spectral separation of the
two perpendicularly oriented dye molecules into two separate images
provides both an internal control and the ability to quantitatively correlate
the membrane structure and fluctuations, within an optical section,
with the incident polarization direction in real-time. Applications of
this technique to the identification of characteristic features of cellular
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this technique to the identification of characteristic features of cellular
processes such as adhesion, endocytosis, and apoptosis are in progress

Improving patient and user safety during endoscopic investigation of pancreatic and
biliary ducts
J. E. Chandler, C. D. Melville, C. M. Lee, M. D. Saunders, E. J.
Seibel, Univ. of Washington (United States)
Endoscopic investigation of the main pancreatic duct and biliary ducts
is called endoscopic retrograde cholangiopancreatography (ERCP),
and carries a risk of pancreatitis for the patient. During ERCP, a metal
guidewire is inserted into the pancreatobiliary duct from a side-viewing
large endoscope within the duodenum. To verify correct placement
of the ERCP guidewire, an injection of radiopaque dye is required for
fluoroscopic imaging, which exposes the patient and clinical team
to x-ray radiation. A safer and more effective means to access the
pancreatobiliary system can use direct optical imaging, although the
endoscope diameter and stiffness will be significantly larger than a
guidewire’s. To quantify this invasiveness before human testing, a
synthetic force-sensing pancreas was fabricated and attached to an
ERCP training model. The invasiveness of a new, 1.7-mm diameter,
stereable scanning fiber endoscope (SFE) was compared to the standard
ERCP guidewire of 0.89-mm (0.035") diameter that is not stereable.
Although twice as large and significantly stiffer than the ERCP guidewire,
the SFE generated significantly less average force during insertion at all
4 sensor locations (P<0.05) within the main pancreatic duct. Therefore,
the addition of steering and forward visualization at the tip of the
endoscope reduced the invasiveness of the in vitro ERCP procedure.
Since fluoroscopy is not required, risks associated with dye injection and
x-ray exposure can be eliminated when using direct optical visualization.
Finally, the SFE provides wide-field high resolution imaging for image-
guided interventions, laser-based fluorescence biomarker imaging, and spot spectral analysis for future optical biopsy.

7891-04, Session 1

Reduction of noise floor for molecular, fluorescence-enhanced optical imaging
B. Zhu, J. C. Rasmussen, Y. Lu, E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

While near infrared fluorescence (NIRF) optical imaging has been demonstrated and proposed for molecular imaging in preclinical and clinical studies, its success depends upon sensitivity for detection of pico- to femto- molar quantities of imaging agents deep within tissues. Yet despite the plethora of studies, there has been virtually no attention given to evaluation of the “noise floor” which limits sensitivity. In this presentation, we show that imaging sensitivity is limited by the strong backscattered excitation light and its leakage through interference filters that have been extensively employed in NIRF imaging studies. Herein, we describe the method for optimizing filter combination and collimation optics which is adapted to our NIRF clinical optical imagers to reduce backscattered excitation light leakage and collect NIR fluorescence signals from deeper tissues with greater performance. First, spectral contributions were assessed due to the excitation light backscattered from the tissue and from non-normal-incidence of the excitation light on the optical filters used. Then a collimation optical system was designed to account for non-normal incidence of light through interference filters and correct for efficient excitation light rejection and optimal fluorescence collection. Finally, the optimized filtering scheme was validated on a phantom before being demonstrated in an actual clinical study. Performance of fluorescence enhanced molecular imaging approaches depend upon validated spectral discrimination of collected signals and comparison of devices require a consistent and standardized metric of sensitivity.

7891-05, Session 1

Analysis of reliability of multiple Raman spectroscopy systems in vivo for clinical implementation
I. J. Pence, C. A. Patil, E. Vargas, A. Walsh, H. Krishnamoorthy, J. M. Cayce, C. Paras, A. Makowski, Vanderbilt Univ. (United States); M. D. Keller, Vanderbilt Univ. (United States) and Lockeed Martin Aculight (United States); X. Bi, M. A. Mackanos, E. D. Jansen, Vanderbilt Univ. (United States); D. L. Ellis, Vanderbilt Univ. Medical Ctr. (United States); A. Mahadevan-Jansen, Vanderbilt Univ. (United States)

During the course of any large study for which multiple data collection systems are required it is necessary to understand the response of each system to ensure device reliability. Because the relatively weak Raman signal can be several orders of magnitude weaker than the inherent fluorescence generated by biological tissue it is necessary to fully understand the effects of data collection on different systems for subsequent comparison. During a large study of benign skin lesions, spectra of both lesion and normal skin tissue were collected in vivo from human patients using four different Raman Spectroscopy systems. Here we present our findings of the reliability of cross-system data collection and the process by which we assessed the systems in order to understand the data collected. With multiple systems it is necessary to account for the differences between the systems, including CCD cameras, spectographs and fiber-optic probes. By using a single standard to calibrate system response, we aim to relate the systems and obtain a basis for comparison. Also the alteration of fluorescence subtraction and white light correction for individial systems has been investigated. The goal of this study is to determine how data collected with different machines can be compared in a meaningful way that does not reduce the quality of the detection technology.

7891-06, Session 2

Industry perspective: Regulatory issues

No abstract available

7891-07, Session 3

Design and validation of a robust, user-friendly modulated imaging device for clinical research
D. J. Cuccia, Modulated Imaging, Inc. (United States)

Quantitative characterization of tissue structure and function is one of the most challenging problems in Medical Imaging. We are advancing the Modulated Imaging (MI) technique, toward its application as a clinical research device to provide objective parameters for in-vivo tissue status determination. MI is a spatial frequency domain imaging (SFDI) method, which employs spatially-patterned illumination to non-invasively obtain subsurface images of biological tissues. This non-contact approach enables rapid quantitative determination of the absorption and scattering optical properties of tissues over a wide field-of-view. When combined with multi-spectral imaging, the optical properties at several wavelengths can be used to quantitatively determine the in-vivo concentrations of chromophores that are relevant to tissue health, namely, oxy- and deoxy-hemoglobin. We present the design, fabrication, and testing of a robust, user friendly MI instrument appropriate for deployment at clinical sites. This instrument is compatible with a plurality of light sources, provides a wide field of view (~180x135mm), and is sufficiently compact and robust for human subject measurements. It possesses sufficient spatio-temporal resolution to study both fast (<1s) and localized (<1mm) events at depths of several millimeters in thick tissues. The device performance was characterized in a laboratory setting in terms of linearity, dynamic range, reproducibility, and drift. A turnkey software interface was developed facilitating clinical deployment for real-world testing and evaluation. Ultimately, we envision that this platform will enable quantitative insight into disease progression and therapeutic response in areas such as wound healing, dermatology, skin cancer and reconstructive surgery.

7891-08, Session 3

The device for registration of biological cellular object by light Fraunhofer diffraction
O. I. Bilyy, V. B. Getman, R. Y. Yaremky, Y. P. Ferensovich, O. M. Vasyliv, Ivan Franko National Univ. of L'viv (Ukraine); I. Y. Kotsyumbas, H. I. Kotsyumbas, SSRC Institute of Veterinary Preparations (Ukraine)

The device for registration of biological cellular object after changes by them spatial distributing of intensity of light in their pictures of light Fraunhofer diffraction is described. The novelty of work consists in the use of intercommunication between distributing of intensity in the scattering pattern of light Fraunhofer diffraction on biological cellular objects, and by their type and size. This regularity is given allows to distinguish the different types of blood cells and different types of microorganisms after the known dependences of the spatial distributing of intensities in the picture of theirs diffraction. Principle of operation the device consists in registration and treatment after the chosen algorithm picture of light Fraunhofer diffraction, which crosses at crossing of test of liquid, which contains biological cells, of focused laser beam. The laser beam is designed on a diaphragm, placed before a photodetector. Amplitude of signal on the output of photodetector is multiple memorized.
Calibration schemes of a field-compatible optical spectroscopic system to quantify neovascular changes in the dysplastic cervix

V. T. Chang, Duke Univ. (United States); D. Merisier, Family Health Ministries (Haiti); B. Yu, D. K. Walmer, N. Ramanujam, Duke Univ. (United States)

A significant challenge in detecting cervical pre-cancer in low-resource settings is the lack of effective screening techniques and trained personnel to detect the disease before it is advanced. Light-based technologies have the potential to provide an effective, low-cost, and portable solution for cervical pre-cancer screening in these communities. The goal of this study is to construct and field-test an optical spectroscopic system in Haiti to aid in the diagnosis of high-grade cervical intraepithelial neoplasia (CIN), or severe dysplastic lesion that is most likely to progress into cervical cancer. We have developed and characterized a portable USB-powered optical spectroscopic system to quantify total hemoglobin content, HB saturation, and reduced scattering. The system consists of a high-power LED, a bifurcated fiber optic assembly, and 2 spectrometers for sample and calibration spectra acquisitions. The system was subsequently tested in Leogane, Haiti, where diffuse reflectance spectra from 33 colposcopically normal sites and 16 colposcopically abnormal sites in 21 patients were acquired. The effect on optical property extraction using two different reflectance standards, i.e., a Spectragon® puck and a built-in self-calibration channel was elucidated. Our results suggest that a self-calibration channel led to a more accurate extraction of scattering contrast through simultaneous correction of intensity drifts in the system. Extracted scattering was also significantly associated with applied contact probe pressure in reflectance from colposcopically normal sites. Hence, future contact spectroscopy or imaging systems should incorporate a self-calibration channel and acquire spectra at a consistent tissue-probe contact pressure to reliably extract scattering contrast.

The use of Monte Carlo simulations to determine the spatial distribution of sensitivity of Raman fiber probes

I. Gerstonde, Laser- und Medizin-Technologie GmbH, Berlin (Germany); C. Reble, Technische Univ. Berlin (Germany) and Laser- und Medizin-Technologie GmbH, Berlin (Germany); G. Illing, Laser- und Medizin-Technologie GmbH, Berlin (Germany)

Raman spectroscopy is a powerful analytic tool to discriminate tissue compositions. For the interpretation of the measurement results information about the sampling volume is required, especially in inhomogeneous tissue. Since biological tissues are optically turbid, the sampling volume is determined by both the tissue optical properties and the measurement geometry.

With our Monte-Carlo method we determine a weight function, which defines the spatial distribution of sensitivity of the Raman measurement. This is then used to calculate the influence of absorption and reduced scattering coefficients on the sampling volume of different fiber configurations. Furthermore, the influence of the measurement geometry characterized by different fiber diameters and the overlap or spatial offset of excitation and detection fiber is compared.

In order to correct Raman measurements for the influence of the optical properties, combinations of Raman and elastic scattering spectroscopy have been suggested [1]. However, a simple ratio of Raman and elastic reflectance, even for identical measurement geometry, can not completely correct for the turbidity-induced variations of the Raman signal [2]. We therefore investigate differences in the influences of optical properties on the sampling volume of Raman and elastic scattering spectroscopy.

If the excitation light is focused on a small spot of the sample, heating becomes critical especially for biological samples. We show a simple approach to extend our Monte-Carlo calculation to obtain the temperature distribution for continuous (cw) illumination. No mesh or triangulation is necessary.


Therefore a design that can amplify the AC component and have a sampling rate and an anti-aliasing filter appropriate to the signal bandwidth would be beneficial. An additional advantage of custom made sensors is that on-chip processing of blood flow allows the data bottleneck that exists between the photo-detector array and processing electronics to be overcome, as the processed data can be read out from the image sensor to a PC or display at a low data rate.

A fully integrated 64x64 pixel array for imaging blood flow is presented. On-chip analog signal processing is used to amplify the AC component, normalize the AC signal by the DC light intensity and provide anti-aliasing. On-chip digital signal processing is used to implement the filters required to calculate blood flow.

The imaging array has been incorporated into a device that has been used in a clinical setting. Results from the clinical study are presented demonstrating changes in blood flow in occlusion and release tests and imaging of inflammatory responses.

7891-13, Session 4

A blood perfusion mapping by utilizing a remote opto-physiological imaging system

Y. Sun, J. Zheng, J. A. Chambers, S. Hu, Loughborough Univ. (United Kingdom)

A study of blood perfusion mapping was performed using a remote opto-physiological imaging (OPI) system coupling a sensitive CMOS camera and a dual-wavelength customized RCLED ringlight. The present setup is suitable for remote assessment of blood perfusion in tissue over a wide range of anatomical locations. This study intends to evaluate the reliability and stability of the OPI system through the measurement of cardiovascular properties, e.g., pulse and respiration rate. To this end, OPI and contact photoplethysmography (CPPG) were recorded simultaneously from 24 subjects before and after 5-min of cycling exercise. The time-frequency-representation (TFR) method was used to visualize the time-dependent behavior of the signal frequency. Compared to conventional contact pulse waveform measurements, the physiological parameters derived from the images captured by the OPI system exhibit comparable functional characteristics in both the time and frequency domains. More importantly, a 3-D representation of blood perfusion mapping in human tissue can be interpreted by our opto-physiological modeling. The present research could lead to a new insight in the clinical assessment and diagnosis of circulatory pathology in various tissue segments.

Keywords: Opto-physiological imaging (OPI), photoplethysmography (PPG), resonant cavity light emitting diode (RCLED), blood perfusion mapping

7891-14, Session 4

Broadband UV-Vis optical property measurement in layered turbid media

Q. Wang, U.S. Food and Drug Administration (United States); D. Le, J. C. Ramella-Roman, The Catholic Univ. of America (United States); J. Pfefer, U.S. Food and Drug Administration (United States)

Quantitative data on the fundamental optical properties (OPs) of biological tissue, including absorption ($\mu_a$) and reduced scattering ($\mu_s'$) coefficients are important for elucidating light propagation during optical spectroscopy, facilitating diagnostic device design and optimization, and may enable rapid detection of early neoplasia. However, systems for in situ broadband measurement of mucosal tissue OPs in the UV-Vis have not been realized. In this study, we evaluate a fiberoptic-based reflectance system coupled with a neural network for multi-layered tissue. The experimental system incorporated a broadband light source, a fiberoptic probe and a CCD camera. The calibration method involved a set of standard nigrosin-microsphere phantoms as well as a more permanent spectrally phantom for quality assurance testing and recalibration. With this method, we are able to obtain highly accurate measurements in the wavelength range of 350-630 nm. The system was evaluated using two-layer hydrogel phantoms with hemoglobin and polystyrene microspheres. The effect of fiberoptic probe design, tissue layer thickness and fitting approaches based on known absorption and scattering distributions are discussed.

7891-15, Session 4

Pseudo-random single-photon counting system: a high-speed implementation and its applications

Q. Zhang, N. Chen, National Univ. of Singapore (Singapore)

As a new time-resolved method which combines the spread spectrum time-resolved method with single photon counting, pseudo-random single photon counting (PRSPC) has been proved to have the potential for high speed data acquisition due to high count rate achievable. A continuous wave laser modulated by a pseudo-random bit sequence is used to illuminate the sample, while single photon counting is used to build up the optical signal in response to the excitation. Periodic cross-correlation is performed to retrieve the temporal profile. Besides the high count rate, PRSPC also offers low system cost and portability which are not with the conventional time-correlated single photon counting (TCSPC). In this paper, we report a high speed PRSPC system that can be used for real time acquisition of the temporal spread function (TPSF) of diffuse photons. We also present preliminary experimental work in which Laplace transformed TPSF of diffuse photons is used to predict relative change in human blood glucose level.

7891-16, Session 5

Multiphoton imaging for deep-tissue penetration and clinical endoscopy

C. Xu, Cornell Univ. (United States)

The main advantages of multiphoton microscopy (MPM) lie in two areas of applications: (1) imaging deep into scattering tissues, and (2) imaging intrinsic fluorescence and harmonic generation, particularly for in vivo investigations. The intrinsic excitation localization and the longer wavelength used enable MPM to image deep into scattering biological specimens. Nonlinear excitation allows the use of near IR wavelength to excite fluorophores that normally absorb in the UV or deep UV region, enabling imaging of intrinsic fluorescence without the limitation of UV photodamage. Nonlinear harmonic generation also provides a unique contrast mechanism for MPM. Multiphoton imaging may potentially become a useful tool for clinical diagnosis. In this paper, we present our efforts in improving the penetration depth of MPM and the development of a multiphoton endoscope for imaging intrinsic tissue fluorescence and harmonic generation in vivo, with a main focus on instrument design and optimization

7891-17, Session 5

Ultra-thin 350-micrometer diameter high-resolution fiber optic confocal probe

D. Lorensen, R. S. Pillai, R. A. McLaughlin, D. D. Sampson, The Univ. of Western Australia (Australia)

Endoscopic confocal microscopy allows high-resolution in vivo imaging. However, it is restricted to imaging the superficial layers of hollow organs. Further miniaturization of the probe to hypodermic needle dimensions is required to enable interstitial in-vivo imaging of deep tissue, including
many tumors. The miniaturization of the high-numerical-aperture microscope objectives used in confocal microscopy poses significant challenges in terms of optical design and fabrication, resulting in a trade-off between size reduction and optical performance. We demonstrate a 350-µm diameter probe using graded-index (GRIN) optics with a lateral resolution of 1 µm and an axial resolution of 11 µm. To the best of our knowledge this is the highest resolution reported for a confocal probe of this size. The custom-designed objective has a length of 27.2 mm and consists of a 0.28-NA GRIN lens bonded to a 1.75-pitch GRIN relay rod, allowing it to be inserted into a 22-gauge needle. The GRIN relay rod avoids the necessity of a highly complex distal scanning mechanism as scanning can be performed at the back end of the needle using low-cost conventional optics and scanning actuators. These could potentially be integrated into a handheld probe unit connected to a fiber-based excitation and detection system. We discuss the optical design of the GRIN objective using ray-tracing software (ZEMAX), and present the experimental characterization of the fabricated probe in a prototype scanning setup. The measured lateral and axial resolutions correspond closely to the design values, and we demonstrate the imaging performance using fluorescent test samples.

7891-18, Session 5

Lensfree telemedicine microscopy for global health challenges
O. Mudanyali, D. K. Tseng, S. O. Isikman, C. Oztoprak, I. Sencan, W. Bishara, O. Yaglidere, A. Ozcan, Univ. of California, Los Angeles (United States)

The current renaissance that we have been experiencing in optical microscopy is truly fascinating. However, together with this progress, the complexity, size and the cost of optical imaging also increased. Unfortunately, microscopy in resource-poor environments has needs significantly different from those of advanced settings, and such imaging devices should be cost-effective, compact, and lightweight, also permitting integration with a telemedicine network.

To provide a tool for this need, here we demonstrate a new telemedicine microscope that is based on lensfree digital in-line holography. This on-chip imaging modality does not require any lenses, lasers or other bulky optical/mechanical components. Instead, it utilizes a simple LED and a compact opto-electronic sensor-array to record lensfree in-line holograms of the objects. These lensfree holograms are formed by the interference of the scattered light from each object with the background illumination, and they permit rapid digital reconstruction of the microscopic images of the specimen with sub-cellular resolution (~1.5µm at ~600nm) over ~24mm2 imaging field-of-view, i.e., more than an order-of-magnitude larger than the field-of-view of a typical 10X objective-lens. This wide-field telemedicine microscope is implemented on a light-weight (~46 grams) stand-alone unit as well as on a commercially-available cellular phone that is modified with a simple hardware weighing ~38 grams. The performance of this lensfree on-chip microscope is validated by imaging various sized micro-particles, red- and white-blood cells, platelets, sperms, as well as waterborne parasites; and we believe that it may provide a cost-effective telemedicine tool to combat various global-health challenges such as HIV, malaria and tuberculosis.

7891-19, Session 5

Design and implementation of an optical contact sensor to automate in-vivo data acquisition upon mucosa contact with a fiber optic light scattering probe
S. Ruderman, Northwestern Univ. (United States); S. Mueller, American BioOptics (United States); J. D. Rogers, V. Backman, Northwestern Univ. (United States)

Contact fiber-optic probes are useful in a wide variety of applications for noninvasive and real-time analysis of tissue properties. The nature of interactions between these fiber-optic probes and the tissue surface presents a challenging problem with respect to the variability of in vivo measurements, such as effects due to variations in the length of contact time between the probe tip and tissue surface. In order to minimize variability due to temporal effects, in vivo measurements could be automated in a consistent, reliable manner. In addition to mitigating temporal changes, it has the potential to speed up data acquisition.

Utilizing the same polarization-gated spectroscopic probes previously designed by our group, we developed an optical method to automatically trigger data acquisition upon contact between the probe tip and tissue. Analysis of previous in vivo data was used to determine a short wavelength range with expected reflected intensity values for establishing thresholds to indicate contact. Through continuous monitoring of the reflected intensity in this spectral range, proximity to tissue can be detected. To improve data quality, as well as allow compatibility with different endoscopic techniques, several additional configurable controls and thresholds were implemented. Initial in vivo studies are presented that assess reliability and timing of contact detection, reduction in measurement variability, and probe-user techniques.

7891-20, Session 5

Optimal illumination for the direct visualization of oral cavity
Y. Chen, C. Yeh, National Chung Cheng Univ. (Taiwan); C. Chiang, National Taiwan Univ. (Taiwan); F. Cheng, Chung Hua Univ. (Taiwan); H. Wang, National Chung Cheng Univ. (Taiwan)

Oral diseases are significant worldwide health problems, and the way to diagnose these diseases more early has been concerned by researchers. Detection of the traditional biopsy procedure is invasive, and it will not only destroy the tissue structure, may also lead to other side effects, such as taking high cost and time to complete the pathological diagnosis. We demonstrate a multispectral-based approach with image processing, which can simulate presents of the same image under different light sources. Also we investigate the visual effect caused by wavelength and color temperature of light sources to identify the most suitable illumination for detection of oral diseases. The main purpose of this study is to enhance the color difference between lesion and normal tissue, so that doctors can diagnose symptoms directly by naked eye and make early treatment in time.

7891-22, Session 6

Ultra-high axial resolution high-speed FD-OCT using broadband astigmatism-corrected spectrometer
K. Lee, S. K. Mahalik, J. P. Rolland-Thompson, Univ. of Rochester (United States)

Optical coherence tomography (OCT) is a non-invasive optical imaging technique that permits micron-scale three-dimensional visualization of biological tissue and materials. In contrast to conventional microscopy, the axial resolution in OCT imaging is determined by the coherence length of the light source. The width of the coherence length, the twice of the axial resolution in OCT imaging is determined by the coherence length of the light source. The width of the coherence length, the twice of the axial resolution in OCT imaging is determined by the coherence length of the light source. The width of the coherence length, the twice of the axial resolution in OCT imaging is determined by the coherence length of the light source. The width of the coherence length, the twice of the axial resolution in OCT imaging is determined by the coherence length of the light source.

Recently sub-micron axial resolution OCTs were developed using extremely broadband source (i.e. supercontinuum) generated by photonic crystal fiber pumped with a femtosecond laser. However, the ultrahigh resolution OCTs using the supercontinuum sources until now are based on time-domain acquisition that requires reference mirror scanning, thus much slower than frequency-domain (FD) acquisition. The main challenge to FD-OCT using supercontinuum sources is the development of the broadband custom spectrometer supporting high speed commercial line CMOS or CCD cameras. We have designed a Czerny-Turner type spectrometer using an off-the-shelf cylindrical lens to provide a broadband astigmatism-corrected spectrometer. This design...
enables to collect most power with the high speed line cameras where the width of the detector area is limited to maximize signal to noise ratio. We achieved sub-micron axial resolution in skin tissue with an acquisition speed of 70 A-scans using the implemented FD-OCT, which consists of the supercontinuum source (i.e. 360nm bandwidth centered at 800nm) and the custom broadband astigmatism-corrected spectrometer (i.e. 0.1 nm spectral resolution over 360nm) that will be detailed. The performance of the ultrahigh resolution and high speed FD-OCT is demonstrated with various biological in vivo specimens.

7891-23, Session 6

Doppler imaging with dual-detection full-range frequency domain optical coherence tomography

P. Meemon, K. Lee, J. P. Rolland-Thompson, Univ. of Rochester (United States)

Most of full-range techniques for Frequency Domain Optical Coherence Tomography (FD-OCT) reported to date utilize the phase relation between consecutive axial lines to reconstruct a complex interference signal, and hence may exhibit degradation in either mirror image suppression performance or detectable velocity dynamic range or both when monitoring a moving sample such as flow activity. Recently, we have reported a method of simultaneous detection of the quadrature components of a complex spectral interference so-called Dual-Detection Frequency Domain OCT (DD-FD-OCT) [Lee et al., Opt. Letter 35, 1058-1060, 2010]. The technique enables full range imaging without any loss of acquisition speed and is intrinsically insensitive to the movement of the sample. In this paper, the implementation of DD-FD-OCT for a phase-resolved Doppler imaging is presented. Because the full-range signal is achieved without manipulation of the phase relation between consecutive axial lines, the phase information of the full-range signal is almost identical to that acquired by the conventional FD-OCT. Therefore, the full-range DD-FD-OCT is fully applicable to phase-resolved Doppler imaging without either degradation in the full-range performance or reduction in the velocity dynamic range as normally encountered in other full-range techniques. In addition, phase-resolved DD-FD-OCT can utilize the maximum SNR provided by the full-range capability of DD-FD-OCT to improve Doppler sensitivity. The velocity sensitivity of Doppler DD-FD-OCT and the relation between the measured Doppler phase shift and the set flow velocity of a flow phantom were quantified. Finally, we demonstrate Doppler imaging using DD-FD-OCT in a biological sample.

7891-24, Session 6

Compact polarization diverse receiver for biomedical imaging applications

D. Neill, L. Stewart, Finisar Australia (Australia); H. Li, Finisar Shanghai, Inc. (China); T. Killin, Finisar Australia (Australia); F. Chen, Finisar Shanghai, Inc. (China); S. Frisken, G. Baxter, S. Poole, Finisar Australia (Australia)

Advances in biomedical optical technology have often leveraged advances and economies of scale created in the telecommunication space and in particular fiber optic componentry. Recent developments in high speed optical transmission are focusing on coherent detection of phase encoded data and many of the challenges are analogous to those faced in high speed optical imaging based on Swept Frequency Optical Coherence Tomography (SW-OCT). Here we report a novel compact Dual Polarization Quadrature Receiver that has been developed for Telecom applications and present applications including an optical engine for OCT and coherent spectroscopy.

The receiver is based on the polarization mixing of a signal and local source. It is implemented as a compact 2x4 photodiode array, with a 250 micron spacing between the detectors, and balanced receivers. The scheme involves 3 polarization splitting elements and a quarter waveplate and manages both polarizations simultaneously. Integration into an OCT system is achieved by mixing a signal reflected and separated (with a circulator) from the sample under test and a path length matched tap source (1%).

A prototype has been constructed and characterized, with the response of the quadrature phase being experimentally determined for each polarization. Advantages of the reported device, including insensitivity to mechanical perturbations and applications to birefringence and real time Doppler shift measurements will also be presented.

7891-25, Session 6

Polarimetric scattering signature imaging of highly photon: scattering bio-medium

S. H. Wu, P. Chen, National Yang-Ming Univ. (Taiwan); D. Yang, Taipei Veterans General Hospital (Taiwan); H. Wang, A. E. T. Chiou, S. F. Nee, T. Nee, National Yang-Ming Univ. (Taiwan)

Tissue is an optically anisotropic and highly photon-scattering medium. It has long been treated as an optically diffusive medium in bio-medical applications. In our recent transmission Stokes imaging experiment of the rat liver samples, the 5 fundamental Mueller matrix elements were measured and analyzed. The set of simultaneous images of four independent optical properties: anisotropy, scattering, depolarization and retardation phase of the test sample has provided a new biosensoring technology to inspect the structures of tissue samples and is useful for critical disease discrimination and medical diagnostics applications. This system is extended to measure reflectance/backscattering images. The measured scattering imaging data of (i) Liposyn II intravenous emulsion solution samples of different concentrations, and (ii) bio-tissues of different thicknesses will be reported. The correlation with our theory and existing PDW theory will be analyzed and reported.

7891-34, Session 6

Optical coherence microscopy using Bessel beams

K. Lee, Univ. of Rochester (United States); S. Vo, Univ. of Rochester (France); J. P. Rolland, Univ. of Rochester (United States)

No abstract available

7891-26, Session 7

Correction of axial optical aberrations in hyperspectral imaging systems

Z. Spiclin, F. Pernuš, B. Likar, Univ. of Ljubljana (Slovenia)

In hyperspectral imaging systems that have a broad spectral range, the acquired spectral images can be totally or partially out-of-focus in certain parts of the spectral range. This phenomenon is mainly due to axial optical aberrations originating from variations of the refractive index of lens elements with respect to the wavelength of incident light. To correct axial optical aberrations and recover in-focus images, the image formation process has to be inverted by deconvolution of the out-of-focus images with the point-spread function of the optical system. For the correction of total or partial out-of-focus spectral images the width of the point-spread function should vary with respect to image coordinates and corresponding wavelength of the spectral image. We propose a method that simultaneously corrects the axial optical aberrations of multiple spectral images: width parameters of the point-spread functions are tuned so as to increase the density of samples in the joint intensity space of multiple spectral images. By intuition, the deconvolution of the spectral images with appropriately sized point-spread functions tends
to sharpen the spectral images and thus tighten the samples in the joint intensity space. Experiments were conducted on microscopy samples using a hyperspectral imaging system based on acousto optic tunable filter in the visible spectral range (0.4 - 0.9 µm). By running the proposed method, the image quality of raw spectral images was substantially improved. Image quality improvements were quantified by the Fisher information measure and demonstrate the potential of the proposed method for correction of axial optical aberrations.

7891-27, Session 7

Illumination system design for hyperspectral imaging

J. Katrašnik, F. Pernuš, B. Likar, Univ. of Ljubljana (Slovenia)

Near-infrared hyperspectral imaging is becoming a popular tool in the biomedical field, especially for detection and analysis of different types of cancers, analysis of skin burns and bruises, imaging of blood vessels and for many other applications. As in all imaging systems, proper illumination is crucial to attain optimal image quality that is needed for best performance of image analysis algorithms. In hyperspectral imaging based on filters (AOTF, LCTF and filter wheel) the acquired spectral signature has to be representative in all parts of the imaged object. Therefore, the whole object must be equally well illuminated - without shadows and specular reflections. As there are no restrictions imposed on the material and geometry of the object, the desired object illumination can be achieved with two distinct designs: 1) completely diffuse illumination and 2) orthogonal polarized axial illumination. In order to minimize shadows and specular reflections in diffuse illumination the light illuminating the object must be completely spectrally and spatially homogeneous. We present and test the two special illumination system designs, with optimized geometry, that try to achieve optimal homogeneity of the above mentioned properties, and high efficiency. The illumination homogeneity properties were measured with an AOTF based hyperspectral imaging system utilizing a standard reference diffuse reflectance target and a specially designed calibration target for estimating the spatial and angular illumination homogeneity. The two illumination designs, namely the diffuse reflectance illumination and axial orthogonal polarization illumination, were compared on images of different biological samples.

7891-28, Session 7

Hyperspectral imaging characterization for a perfused oximetry phantom

R. C. Chang, M. L. Clarke, D. Samarov, J. Hwang, M. Litorja, D. W. Allen, National Institute of Standards and Technology (United States)

In hyperspectral imaging microscopy, a continuous spectrum for each imaged cell is possible by implementing a spectrally tunable source consisting of a spectrally dispersed white light source spatially fitted onto a digital micro-mirror array whereby each mirror in the array can be selectively and rapidly switched on or off, thereby allowing selective wavelengths to be generated. This technique can be applied to characterize local blood perfusion or tissue oxygenation in vivo by creating maps of hemoglobin saturation in blood vessels. As stable engineered biopolymers become more reproducible in vitro, the potential of implementing biomimetic constructs as optical phantoms and mainstream tools in molecular imaging studies becomes more viable. Herein, we fabricate alginate hydrogel microbeads through external gelation with a direct cell writing deposition process. The biopolymer microbeads are integrated onto a biological equivalent of an integrated circuit, or microfluidic device. The deployment of a microfluidic platform with an in vivo simulating tissue construct with facile incorporation of biological constituents is amenable to a variety of optical measurements. In this paper, hemoglobin serves as the representative oxygen-binding model protein for oximetry studies. The hemoglobin is encapsulated within the biopolymer microbead and subsequently perfused with a mixture of gases to serve as an in vitro optical phantom testbed for quantitative absorption measurements of varying hemoglobin saturations by coupling with a tunable spectral source to enhance feature contrast.

7891-29, Session 7

Low-light hyperspectral imager for characterization of biological samples based on an sCMOS image sensor

J. E. Hernandez-Palacios, Norsk Elektro Optikk AS (Norway); L. L. Randeberg, I. J. Haug, Norwegian Univ. of Science and Technology (Norway); I. Baarstad, T. Løke, Norsk Elektro Optikk AS (Norway); T. Skau, Norwegian Defense Research Establishment (Norway)

Hyperspectral imaging is an effective technique to identify and characterize biological samples according to spectral signatures. In environments in which the number of available photons per spectral band is scarce, the overall signal-to-noise ratio is decreased for a given exposure time. Consequently, the quality of the spectral information is reduced. This is a major restriction for accurate imaging and further characterization of biological samples. A hyperspectral imaging system capable of imaging biological samples in low light conditions has been developed using an electron-multiplying CCD (EMCCD) sensor. The EMCCD provides enhanced sensitivity to light and has a performance close to the fundamental photon noise limit. The system has been designed to lower the risk of damaging photosensitive samples, delay the bleaching of fluorophores and detect weak fluorescence signals. The device works in the VNIR spectral region (400 nm - 900 nm) with a spectral sampling of 4 nm. Images with high spatial resolution are obtained using a close up lens yielding a scene pixel size of 25 µm and a 25 mm FOV. This allows the analysis of relatively large samples while maintaining microscopic resolution. The imaging system also features a multi-filter module for acquiring polarimetric, fluorescence and direct illumination signals from samples of varying dynamic range in one exposure sequence. The system has been tested using skin samples and skin mimicking phantoms in polarimetric and UV light-induced fluorescence measurements. Preliminary results indicate that the developed hyperspectral imaging system outperforms the comparable imaging systems and offers a tool for optical analysis in biomedical applications.
Characterization of hyperspectral imaging and analysis via microarray printing of dyes

M. L. Clarke, M. Litorja, D. W. Allen, J. Hwang, National Institute of Standards and Technology (United States)

The application of hyperspectral imaging requires rigorous characterization of the spatial and spectral imaging domains of the system. We present a microarray printing methodology for the testing of absorption or reflectance microscopy measurements. This controlled system can serve as a platform for inter-system calibration and provides a common framework for the development of post-processing algorithms. Calibration of the illumination at the objective plane using a transfer standard spectroradiometer allows comparison of light levels regardless of the illumination used, different apertures, and different microscopes. The method uses standard commercial optomechanical components. Printed dyes enable multiplexed testing of the spectral capability of a hyperspectral instrument. The spectral signatures of individual or blended dyes can be analyzed and applied to the testing of spectral image processing tools. Customized programming of the microarrayer allows for arbitrary patterning of dye samples onto the substrate, allowing for the testing of image processing algorithms involving the spatial distribution of spectral features.
7892-01, Session 1
Quantitative, wide-field characterization of tissue optical properties and chromophores with spatial frequency domain imaging (SFDI)
D. J. Cuccia, Beckman Laser Institute and Medical Clinic (United States)

Evaluation of the accuracy of brain optical properties estimation at different ages using the frequency domain multidistance method
M. Dehaes, P. E. Grant, D. Sliva, Children's Hospital Boston (United States); N. Roche-Labarbe, Massachusetts General Hospital (United States); R. Pienaar, Children's Hospital Boston (United States); D. A. Boas, M. A. Franceschini, J. J. Selb, Massachusetts General Hospital (United States)

Breast coil for multiplanar MRI/optical spectroscopy in vivo
M. A. Mastanduno, S. Jiang, B. W. Pogue, K. D. Paulsen, Dartmouth College (United States)

Snapshot spectrally and spatially resolved measurement of turbid media
N. A. Hagen, Rice Univ. (United States); A. Mazhar, Univ. of California, Irvine (United States); N. Bedard, T. S. Tkaczyk, Rice Univ. (United States); B. J. Tromberg, Univ. of California, Irvine (United States)

We present a rapid, noncontact imaging technique which can obtain the spectral- and spatially-resolved scattering and absorption coefficients of a turbid medium. The measurement involves combining a spatially modulated illumination pattern with a snapshot imaging spectrometer for measurement. After capture of an (x,y,lambda) datacube, a simple image demodulation scheme is applied in post-processing to obtain the spatial maps of diffus reflectance, absorption coefficient, and reduced scattering coefficient. The resulting system is used to show the dynamic changes (in 1s intervals) in oxy- to deoxyhemoglobin concentrations due to venous occlusion and reactive hyperemia.
Hyperspectral imaging and modeling of bruises

B. Stam, M. J. van Gemert, T. G. van Leeuwen, M. C. Aalders, Jr., Academisch Medisch Ctr. (Netherlands)

Age determination of bruises as aid for diagnosing child abuse is currently based on difficult and subjective methods, such as comparing the bruises color with standardized color schemes. Such a method heavily relies on the judgment of the physician to determine the age of the bruise. We are developing a method that is objective and accurate using reflectance spectroscopy. This technique has a great advantage over the previous mentioned method, as it is non-invasive and quantitative. Combining spatiotemporal spectral information with algorithms based on physiological processes may lead to a technique that can objectively determine a bruises age.

We developed a numerical 3D model to simulate the spatial kinetics of hemoglobin and bilirubin during the formation and healing of bruises. The different spatio-temporal dynamics of hemoglobin and bilirubin allows age determination of bruises. We did measurements on bruises with two systems: one fiber based and the second a hyper spectral imaging system. For the data analysis, we use a modified Lambert-Beer with the healthy skin as reference. The known chromophores (oxygenized hemoglobin, deoxygenized hemoglobin, bilirubin, water) were fitted to the measured spectra.

Results show that chromophores present in a bruise can be identified individually and the spatio-temporal dynamics can be assessed. Due to a difference in diffusivity between these two chromophores, differences in the area the chromophores take in occur. Combining spectral information of chromophore concentrations with the spatial information, the actual location of the chromophores, allows precise simulation of the kinetics in these real bruises and age determination.

Applications of multimodal microscopy in pathology and thick tissue imaging

S. Yazdanfar, GE Global Research (United States)

No abstract available

Integrated scanning laser ophthalmoscopy and optical coherence tomography for quantitative multimodal imaging of retinal degeneration and autofluorescence

A. Issaei, L. Szczygiel, N. Hossein-Javaheri, M. Young, Simon Fraser Univ. (Canada); L. L. Molday, R. S. Molday, The Univ. of British Columbia (Canada); M. V. Sarunic, Simon Fraser Univ. (Canada)

Non-invasive retinal imaging has the potential to reduce the number of animals required to study diseases causing blindness, and investigate new therapies. Optical Coherence Tomography (OCT) is emerging as the preferred modality for in vivo depth-resolved structural imaging of the retina. Scanning Laser Ophthalmoscopy (SLO) is another common imaging modality used to obtain high resolution en face images of the retina, and can be integrated with fluorescent detection for the detection of molecular contrast signals. Integration of both structural and functional imaging modalities makes ISLO and OCT highly complementary.

We have previously reported on a customized multimodal FDOCT prototype combined with fluorescent SLO (ISLO) and its functionality was successfully demonstrated. In this report, we have investigated the integrated ISLO-OCT multimodal system customized for imaging a rodent model of Stargardt’s Macular Dystrophy which is characterized by retinal degeneration and accumulation of toxic autofluorescent lipofuscin deposits. High-resolution cross sectional OCT images are important to monitor the progression of retinal degeneration associated with Stargardt’s. Detection of autofluorescence associated with the lipofuscin deposits with SLO is necessary since OCT images cannot resolve the small structures.

New results have been demonstrated on the rodent model of Stargardt’s disease (ABCA4 knock out). Multimodal images were acquired from both central and knock out eyes. Fundus autofluorescence from lipofuscin deposits was visualized with the ISLO and quantified against the control mice. The results demonstrate a system which could assist researchers investigate new therapies for Stargardt’s using ABCA4 knock out mice by monitoring retinal degeneration and fundus autofluorescence simultaneously.

Fiber-based combined optical coherence and multiphoton microscopy

G. Liu, Z. Chen, Beckman Laser Institute and Medical Clinic (United States)

This manuscript demonstrates a multimodal imaging system which combined multiphoton microscopy (MPM) imaging modality with Fourier domain (FD) optical coherence microscopy (OCM) imaging modality. The system used a single fiber-based femtosecond laser as the light source for both MPM and OCM modality. The femtosecond fiber laser has a central wavelength of 1.03um , a pulse width of 120fs and bandwidth of 29nm. The systems used fiber based devices for both MPM and OCM imaging. The MPM and OCM shared the same excitation light path. The excitation light was delivered with the core of a dual-clad fiber. The MPM and OCM signal was collected by different parts of the dual-clad fiber. The MPM signal was collected by the clad of the dual-clad fiber and the OCM signal was collected by the core of the dual-clad fiber. The FD OCT used a home-built InGaAs detector array spectrometer with a maximum A-line speed of 7.7 KHz. The multiphoton signal collection efficiency was analyzed and several imaging modality including second harmonic generation imaging, two-photon excitation fluorescence and optical coherent microscopy imaging were demonstrated.

Combined OCT and CARS using a single ultra-short-pulse Ti:sapphire laser

C. Hoffmann, Leibniz Univ. Hannover (Germany); B. Hofer, A. Unterhuber, B. Považay, Medizinische Univ. Wien (Austria) and Cardiff Univ. (United Kingdom); U. Morgner, Leibniz Univ. Hannover (Germany) and Laser Zentrum Hannover e.V. (Germany); W. Drexler, Medizinische Univ. Wien (Austria) and Cardiff Univ. (United Kingdom)

Optical coherence tomography (OCT) is a non-invasive in vivo biomedical imaging modality capable of three-dimensional visualization of tissue morphology. Coherent Anti-Stokes Raman Scattering (CARS) is a nonlinear spectroscopic technique which provides molecular information due to a four wave mixing process.

In order to extend the performance of OCT towards detecting the molecular fingerprint of biological samples a combined OCT/CARS instrument has been developed that only employs a single ultrashort pulse Ti:Sapphire laser emitting up to 240nm optical bandwidth (at full-width-at-half-maximum) equivalent to sub 5-fs pulses. This laser enables three-dimensional ultrahigh axial resolution (1-2 µm) OCT imaging and at the same time in combination with a spectral shaper a variable CARS setup when interfaced to a microscope.

During first measurements the same area of a sample was imaged
twice, applying OCT and CARS consecutively. OCT was used to perform three-dimensional morphological screening. Due to CARS additional chemical information could be gained for two dimensions. The spectrum was modified computer controlled to match the requirements for the generation of a CARS signal whereas for OCT the unmodified spectrum was applied. Fluids such as dimethylsulfoxide (DMSO) and PBS were compared in a cuvette with two cells to demonstrate the functionality of the multimodal setup. As a biological sample a 100 µm thick cross section through a human optic nerve surrounded by solina was investigated. A characteristic Raman resonance of collagen type I was probed. The challenges and limitations of this hybrid imaging modality using only a single ultrafast light source will be discussed.

Towards a robust hybrid imaging modality

The challenges and limitations of this hybrid imaging modality using only a single ultrafast light source will be discussed.

7892-11, Session 2

The origin of refractive index variation in a living cell: integration of quantitative phase and confocal Raman microscopy

J. W. Kang, N. Lue, I. Barman, N. C. Dingari, C. Kong, R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

Live cell imaging without staining is a big challenge. Most widely used techniques is phase contrast method. Sub-cellular components inside of a cell make optical path length changes and this phase variation can be transformed into intensity variation. Recently, quantitative phase microscopy (QPM) based on interferometry technique started providing quantitative morphological structure of a living cell in 2D or even in 3D. QPM is fast and full-field technique but it has fundamental questions: “What is the origin of refractive index variation in a cell?” Due to this unanswered question, the interpretation of QPM image is not trivial without proper background knowledge.

Confocal Raman microscopy could provide answer to this question. Raman scattering has been widely used as an analytical tool in many fields. Small amounts of inelastically scattered light from the sample include “finger print” vibrational information about the sample which can be used for both qualitative and quantitative analysis. Confocal Raman can obtain accurate chemical information with high spatial resolution. It can distinguish DNA, protein, lipid, mitochondria and so on from their characteristic Raman spectra. Despite its great promise, confocal Raman microscopy has not been widely used for biological research, in comparison to fluorescence imaging, due to its intrinsically weak signals.

We combined confocal Raman and quantitative phase microscopy. Both imaging modalities use intrinsic contrast mechanisms. Combined system obtains both fast/morphological and slow/chemical information. Using this hybrid system, we are investigating the origin of refractive index variation in a living cell.

7892-12, Session 3

‘Spectral a priori’ to ‘spatial a posteriori’ in continuous-wave image reconstruction in near-infrared optical tomography

G. Xu, D. Piao, Oklahoma State Univ. (United States); H. Dehghani, The Univ. of Birmingham (United Kingdom)

It has been demonstrated that the “spectral prior” may be more suitable than the “spatial prior” in frequency-domain optical tomography, for characterization of tissue constituents such as total hemoglobin concentration, oxygen saturation, water and scattering parameters. This work examines the robustness of “spectral prior” in continuous-wave based image reconstruction for separately recovering the spatial maps, which are referred to as “spatial a posteriori”, of chromophores and the scattering parameters including scattering power and scattering amplitude. The study begins with analytic understanding for recovering the chromophore concentrations and scattering properties of a homogeneous medium using a single source-detector pair. Simulation studies are conducted to compare the reconstruction accuracy obtained among three measurement configurations, namely direct-current (DC), DC-excluded frequency-domain and DC-included frequency-domain. The findings include: 1) in agreement with the uniqueness of multi-spectral NIR tomography, the analytical solution shows that each of the unknown parameters can be independently reconstructed by DC measurements, since the spectral prior can substantially reduce the coupling between the absorption and scattering parameters; 2) the level of artifacts in scattering-power is intrinsically the highest; 3) with spectral prior the DC-based reconstruction produces least background artifacts, and; 4) within spectral ranges where the extinction coefficients of the chromophores are highly correlated, increasing the number of wavelengths does not necessarily improve the reconstruction accuracy; 5) when spectral-prior is available, including DC in frequency-domain reconstruction generally improves reconstruction outcome more than neglecting DC. This demonstrates the advantage of using multi-modal data-types in DOT to provide more stability in parameter recovery.

7892-13, Session 3

An efficient time-resolved adjoint Monte Carlo method for fluorescence molecular tomography

J. Chen, V. Venugopal, X. Intes, Rensselaer Polytechnic Institute (United States)

MC methods are computationally expensive, especially in the time domain. We report on a new method that combines a forward and an adjoint MC approach to calculate efficiently time-resolved Jacobians for fluorescence lifetime multiplexed tomography. The technique is tested with in silico studies on anatomically accurate synthetic murine models. We compare the computing time and reconstruction performances between a previously developed perturbation MC method and the proposed method. This new approach to perform time resolved molecular tomography is particularly attractive when combined with GPU computing and allow for efficient time resolved MC computations without a massive computing environment.

7892-14, Session 3

Fully parallel adaptive finite element simulation using the simplified spherical harmonics approximations for frequency domain fluorescence molecular imaging

Y. Lu, B. Zhu, The Univ. of Texas Health Science Ctr. at Houston (United States); H. Shen, Virginia Polytechnic Institute and State Univ. (United States); J. C. Rasmussen, The Univ. of Texas Health Science Ctr. at Houston (United States); G. Wang, Virginia Polytechnic Institute and State Univ. (United States); E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

Fluorescence molecular imaging/tomography may play an important role in preclinical research and clinical diagnostics owing to the potential for high photon count rates and rapid imaging. While optical tomography entails the solution of an ill-posed problem, time- and frequency-domain fluorescence imaging can acquire more measurement information, improving the reconstruction quality of fluorescence molecular tomography. Although diffusion approximations (DA) theorems have been extensively applied in optical tomography, high-order photon migration models must be further investigated for quantitative imaging. In this paper, a frequency-domain fully parallel adaptive finite element solver is developed with the simplified spherical harmonics (SPH) approximations.
To fully evaluate the performance of the SPN approximations, a fast tetrahedron-based Monte Carlo simulator suitable for complex heterogeneous geometries is developed using the convolution strategy to realize the simulation of the fluorescence excitation and emission. Due to the use of triangular and tetrahedral elements, arbitrary shaped illumination and fluorescent inclusions can be set, enabling the simulation of the complicated cases. When compared to DA, the SPN approximation has poor performance in void-like and near-source domains. However, validation results show that the SPN approximation can effectively correct the modeling errors of diffusion equation especially when the tissues have high absorption characteristics and/or when high modulation frequencies are used. Furthermore, performance with the digital mouse phantom shows that significant precision and speed improvements are obtained from the parallel adaptive mesh evolution strategy. This solver provides a useful platform for fluorophore reconstruction in fluorescence molecular tomography using high-order approximations to the radiative transfer equation.

Three-dimensional time-reversal optical tomography

B. Wu, W. Cai, M. Alrubaiiee, The City College of New York (United States); M. Xu, Fairfield Univ. (United States); S. K. Gayen, The City College of New York (United States)

Time reversal optical tomography (TROT) approach is used to detect and locate absorptive targets embedded in a highly scattering turbid medium to assess its potential in breast cancer detection. TROT experimental arrangement uses multi-source probing and multi-detector signal acquisition and Multiple-Signal-Classification (MUSIC) algorithm for target location retrieval. Light transport from multiple sources through the targets to the detectors is represented by a response matrix constructed using experimental data. A TR matrix is formed by multiplying the response matrix by its transpose. The eigenvectors with leading non-zero eigenvalues of the TR matrix correspond to embedded objects.

The approach was used to: (a) obtain the location and spatial resolution of an absorptive target as a function of its axial position between the source and detector planes; and (b) study variation in spatial resolution of two targets at the same axial position but different lateral positions. The target(s) were glass sphere(s) of diameter ~9 mm filled with ink (absorber) embedded in Intralipid-20% suspension in water with an absorption coefficient $\mu_a$ ~ 0.003 mm$^{-1}$ and a transport mean free path $l_t$ ~ 1 mm at 790 nm, which emulate the average values of those parameters for human breast tissue. The spatial resolution and accuracy of target location depended on axial position, and target contrast relative to the background. Both the targets could be resolved and located even when they were only 4-mm apart. The TROT approach is fast, accurate, and has the potential to be useful in breast cancer detection and localization.

Preclinical multimodal optical and radionuclide imaging

S. R. Cherry, Univ. of California, Davis (United States)

We are interested in working at the intersection of optical and radionuclide imaging, and exploring the synergistic use of these modalities for in vivo molecular imaging applications. Two examples will be explored. In the first, a fluorescence optical tomography (FOT) system based on a conical mirror design and compatible with existing preclinical positron emission tomography (PET) systems is used for hybrid imaging. In the second example, we demonstrate a system based on the detection of Cerenkov radiation, to use optical techniques to noninvasively image a range of radionuclides inside small animals.

In vivo reconstruction of NIR FRET using wide-field time resolved optical tomography

V. Venugopal, J. Chen, Rensselaer Polytechnic Institute (United States); M. Barroso, Albany Medical College (United States); X. Intes, Rensselaer Polytechnic Institute (United States)

We investigate the feasibility of 3-D localization of Forster resonance energy transfer (FRET) between two NIR fluorophores (Alexa Fluor 700 and Alexa Fluor 750) in small animal models based on lifetime contrast. A time-resolved optical tomography system based on a wide-field excitation is used to acquire spatially and temporally dense datasets using a time-gated camera. Anatomical information obtained using a small animal MRI in a non-concurrent setting is used to compute the Monte Carlo based forward model. The MC model reconstructs both the time delay and FRET efficiency of the donor fluorophore and the FRET complex using the time-gate data type. This technique is validated by experimental studies in euthanized murine models.
The cancer imaging community recognizes the importance of augmenting high-resolution anatomical images with quantitative functional images for accurate tumor characterization. Within the limited range of abilities for functional imaging that are currently available, tumor oxygenation is recognized as a potential marker for assessing individual chemotherapy responses. Diffused optical tomography (DOT) using near IR light has been used to obtain accurate functional images based on the spectral differences between healthy and hypoxic tissue. Although contrast (1:4) and spatial resolution (2-4 mm) of DOT images are poor compared to high-resolution magnetic resonance images (MRI), the optical information can be used to augment concurrently images with crucial functional information.

We have developed a new system designed for imaging luminescent agents that respond to tissue oxygenation to improve the contrast and spatial resolution of functional optical images. High-resolution spatial and anatomical information from simultaneously obtained MRI images is used to improve the accuracy of the reconstructed optical images. The time domain lifetime imaging module has parallel acquisition across a cooled 16-element avalanche photodiode array for high resolution and high throughput imaging. The low-cost, compact lifetime imager is a cooled 16-element avalanche photodiode array for high resolution and 2-4 mm spatial resolution (2-4 mm) of DOT images are poor compared to high-resolution magnetic resonance images (MRI). Using this APD module in a dual-modality imaging system, the optical information can be used to augment concurrently images with crucial functional information.

In this report we will evaluate three alternative strategies, which allow to recover optical parameters accurately with the ill-posedness of its inverse problem. A hybrid frequency domain lifetime imaging module has parallel acquisition across a cooled 16-element avalanche photodiode array for high resolution and high throughput imaging. The low-cost, compact lifetime imager is a cooled 16-element avalanche photodiode array for high resolution and 2-4 mm spatial resolution (2-4 mm) of DOT images are poor compared to high-resolution magnetic resonance images (MRI). Using this APD module in a dual-modality imaging system, the optical information can be used to augment concurrently images with crucial functional information.

Despite of its high sensitivity, DOT has low spatial resolution due to the ill-posedness of its inverse problem. A hybrid frequency domain MRI-DOT system has been previously developed by our group and demonstrated to be able to recover optical parameters accurately with phantom studies. In this setting, both MR and multi-wavelength DOT data are acquired simultaneously. The MR images structural a priori information is utilized for constraining and guiding DOT chromophore reconstruction.

Even though multi-wavelength DOT has been evaluated with phantom and human studies, there have been very few small animal studies in the literature. In this study, in vivo small animal studies have been undertaken and chromophore reconstruction results for three tumor types, viable, edematous and necrotic, are shown and compared. The ultimate aim of the study is to evaluate the performance of the hybrid MRI-DOT system in vivo.
attaining deep tissue Raman imaging. The first one is a modified time-gated imaging. By utilizing a near-IR (>1000 nm) excitation, much deeper penetration depth is possible. However, the detection of long-wavelength photon is rather problematic. We use an optical parametric amplifier pumped in the visible, which uses the collected Raman signal as an idler wave, to increase the number of detected photons above the dark noise level of a typical CCD and to convert the long-wavelength radiation into light, which can be conveniently detected using Si-based CCD technology.

The second approach utilizes near-IR excitation with coherent anti-Stokes detection. We show that CARS microspectroscopy is capable of providing deep tissue information in highly turbid media [1], while providing an enhanced sectioning through optical focusing and nonlinear optical interaction.

Finally, we evaluate a near-IR excitation scheme with non-optical detection. We utilized stimulated Raman photoacoustic imaging and demonstrate that under proper conditions in can be superior to all the above mentioned methods for deep tissue chemically-specific bone imaging [2].

References:

7892-23, Session 4
PpIX fluorescence contrast detects the presence of diffuse gliomas more accurately than magnetic resonance imaging

K. S. Samkoe, H. H. Yang, Dartmouth College (United States); S. L. Gibbs-Strauss, Beth Israel Deaconess Medical Ctr. (United States); J. A. O’Hara, P. J. Hoopes, R. A. Kauppinen, Dartmouth Medical School (United States); B. W. Pogue, Dartmouth College (United States)

Protoporphyrin IX (PpIX) guided surgical resection of gliomas is the standard of practice in Germany and is currently in clinical trials in the USA. In diffuse gliomas, or at tumor margins, there is often PpIX fluorescence originating from tissue that may not be visible by standard morphology- or contrast-based magnetic resonance imaging (MRI). In order to determine whether these diffuse regions of PpIX fluorescence can be evaluated as cancerous lesions, we undertook a study to compare image contrast arising from PpIX fluorescence and a variety of standard MRI sequences. U251 cells stably transplanted with green fluorescent protein (U251-GFP) were implanted into the brain of nude mice. The mice were imaged in a 7T small animal MRI 14-20 days post-implantation. Following MRI imaging, the mice were injected with 100mg/kgaminolevulinic acid (PpIX metabolic precursor), sacrificed two-hours later and ex-vivo brain sections imaged for GFP and PpIX fluorescence. Regions of interest (ROIs) were determined using both standard H&E and GFP fluorescence, and the contrast in each ROI (where contrast = [intensity in ROI][intensity in contralateral region]) was compared. Contrast from PpIX fluorescence and the T1W contrast enhanced and T2 map MRI groups were statistically significant from the control group. However, in both the H&E and GFP ROIs, PpIX fluorescence most accurately determined the presence of diffusely growing U251-GFP (AUC = 1 (H&E-ROIs) and 0.95 (GFP-ROIs)). This implies that, if validated in humans, surgeons could trust the presence of PpIX fluorescence even if the pre-surgical MRI does not indicate tumor in that region.

7892-24, Poster Session
Different optical spectral characteristics in a necrotic transmissible venereal tumor and a cystic lesion in the same canine prostate observed by triple-band transrectal optical tomodiography under transrectal ultrasound guidance

J. Zhen, G. R. Holyoak, J. W. Ritchey, K. E. Bartels, K. Rock, C. L. Ownby, Oklahoma State Univ. (United States); G. Slobodov, The Univ. of Oklahoma Health Sciences Ctr. (United States); C. F. Bunting, D. Piao, Oklahoma State Univ. (United States)

Different optical spectral characteristics were observed in a necrotic transmissible venereal tumor (TVT) and a cystic lesion in the same canine prostate by triple-wavelength transrectal optical tomodiography under trans-rectal ultrasound (TRUS) guidance. The NIR imaging at 705nm, 785nm and 808nm was able to reliably quantify both the total hemoglobin concentration (HbT) and oxygen saturation (StO2) in the prostate. The TVT tumor in the canine prostate as a model of prostate cancer was induced in a 7-year old, 27 kg dog. A 2 mL suspension of 2.5×10^6 cells/mL of homogenized TVT cells recovered from an in vivo subcutaneously propagated TVT tumor in an NOD/SCID mouse were injected in the cranial aspect of the right lobe of the canine prostate. The left lobe of the prostate had a cystic lesion present before TVT inoculation. After the TVT homogenerate injection, the prostate was monitored weekly over a 9-week period, using trans-rectal NIR and TRUS in grey-scale and Doppler. A TVT mass at the right lobe developed into a necrotic nodule during the later stages of this study, as the mass presented with substantially increased [HbT] in the periphery, with an area of reduced StO2 less than the area of the mass itself. Conversely, the cystic lesion presented with slightly increased [HbT] with oxygen-reduction in the periphery of the lesion. There was no blood flow change evident on Doppler US. The slightly increased [HbT] in the periphery of the cystic lesion was found in histopathology as related to intra-lesional hemorrhage.

7892-25, Poster Session
An investigation on fluorescent-optical dual-mode tomography using time-resolved data

L. Zhang, W. Zhang, F. Gao, J. Li, H. Zhao, Tianjin Univ. (China)

Diffuse optical tomography (DOT) is a noninvasive method to image the optical properties of tissue and has great potential applications in some medical imaging, such as optical mammography and functional brain imaging. At present, the main application barriers of DOT are low spatial resolution, quantitativeness and non-specific. Fluorescence diffuse optical tomography (FDOT) with the aid of specific fluorescent probes promises to open new pathways for the characterization of biological processes in living animals. As a result of using exogenous fluorescent agents, the specificity and the contrast of DOT can be greatly enhanced. The continuous wave, frequency-domain and time-domain (TD) systems are three main modes in FDOT and DOT. Compared with the two former, time domain system has the advantages of simultaneous recovery of fluorescent yield and lifetime or absorption and reduced scattering coefficients, and the analysis of multiple components in a direct way. In addition, due to the image information limitation of different imaging principle, the use of image fusion technology to improve image quality is a trend for achieving an accurate diagnosis.

We present a scheme for fluorescence-optical dual-mode tomography to reconstruct the fluorescence parameters (yield and lifetime) and optical parameters (absorption and reduced coefficients) based on time-resolved data. This method utilizes the advantage of greatly improving target contrast by injecting fluorescence contrast agent into tissue. In this paper, the fluorescence parameters were reconstructed at first, then the fluorescence images were used to guide and constrain the diffusion
optical tomography reconstruction, and the binary image segmentation strategy was applied to improve the image quality in DOT. To validate the proposed method, the numerical simulation was performed. The results showed the feasibility of this method, and the spatial resolution, quantification and computational efficiency in DOT were enhanced evidently. Finally the image fusion was realized accurately.

7892-26, Poster Session

Macroscopic optical imaging to measure fluid flow in microfluidic phantoms

R. J. Hennessy, California Polytechnic State Univ., San Luis Obispo (United States); C. Koo, P. Ton, A. Han, R. Righetti, K. C. D. Maitland, Texas A&M Univ. (United States)

Ultrasound poroelastography can quantify structural and mechanical properties of tissue such as stiffness, compressibility, and fluid flow rate. This novel ultrasound technique is being explored to detect tissue changes associated with lymphatic disease. We have constructed a macroscopic optical imaging system to validate fluid flow measurements and to provide high resolution imaging of microfluidic phantoms.

The optical imaging system is composed of a white light source, excitation and emission filters, and a camera with zoom lens. The field of view can be adjusted from 10 mm x 7.5 mm to 90 mm x 70 mm. The microfluidic device is made of PDMS and has 9 channels, each 40µm deep with widths ranging from 30 µm to 200 µm. A syringe pump is used to propel water containing 15 µm fluorescent microspheres through the microchannels, with flow rates ranging from 0.5 µl/min to 10 µl/min. Video is captured at a rate of 25 frames/s. The velocity of the fluid in the microchannels is calculated using an algorithm that tracks the movement of the fluorescent microspheres. The imaging system is capable of measuring velocities ranging from 0.2 mm/s to 20 mm/s. The range of flow velocities of interest in lymph vessels is within 1 mm/s to 10 mm/s; therefore, our imaging system is sufficient to validate ultrasonic fluid flow measurements in phantoms modeling lymphatic flow.

7892-27, Poster Session

The finite element method for three-order (P3) approximation of radiative transfer equation

W. Ma, F. Gao, L. Wu, H. Zhao, Tianjin Univ. (China)

In this article, the flux at point r in direction takes the form of an expansion in spherical harmonics, and we derive the two-dimensional spherical harmonics equations to three-order for anisotropic scattering. We also solved this equations using Galerkin finite element method and compared the solutions with the first-order diffusion equation and Monte Carlo simulation. Two benchmark problems (inhomogeneous model and homogeneous model) are tested, and we found that the developed three-order model with high absorb coefficient is able to significantly improve the diffusion solution in circle geometry, and the radiance distribution close to light source is more accurate. It is significant for accurate modeling of light propagation in small tissue geometries in small animal imaging.

7892-28, Poster Session

Integrating optical system designed for multimodal analysis of pearls and its mother oyster to distinguish and appraise cultured pearls

M. J. Ju, S. J. Lee, E. J. Min, Y. Kim, D. H. Kim, Gwangju Institute of Science and Technology (Korea, Republic of); H. Y. Kim, Korea Pearl Lab. (Korea, Republic of); D. S. Lee, B. Lee, Gwangju Institute of Science and Technology (Korea, Republic of)

Integrating optical system combining optical coherence tomography (OCT) and fluorescence spectroscopy (FS) has been designed and utilized to distinguish mother oyster used in pearl culture, and to differentiate and evaluate pearls, nondestructively. By fabricating a wavelength-division multiplexing (WDM) and a double clad fiber (DCF) coupler, a FS system was combined with a fiber based swept source OCT (SS-OCT) system. Utilizing a common-path configuration, the integrating system could be implemented in a simple and effective way with highly minimized polarization and group velocity dispersion (GVD) mismatch problems. Different two types of measurements previously performed by two independent apparatus were made as using the proposed system: the internal structure measurement and the fluorescence spectrum measurement. From the internal structure measurement of a pearl, we could measure the thickness of the nacre layer, observe the fine sub-structure of the nacre, and inspect the nucleus through the nacre. And with the fluorescence spectrum measurement, we could match the pearls and their mother oysters, which made it possible to distinguish among Pinctada fascata, Pinctada maxima, Pteria penguin, and Pinctada margaritifera. In addition, identification of freshwater pearls became feasible with the analysis of the fluorescence spectrum. With this multifunctional modality, we believe it would be possible multimodal analyzing for distinguishing and estimating cultured pearls.

7892-29, Poster Session

Use of graphic processing unit in spectral domain optical coherence tomography to increase real-time display speed

U. Jung, H. Jeong, C. Lee, N. Cho, S. Han, J. Kim, Kyungpook National Univ. (Korea, Republic of)

Optical coherence tomography (OCT) is widely used in the medical diagnostic fields. A real time displaying feature becomes an important parameter to extend the applications in dynamic environment. As the higher data throughput needed, the real time displaying requires better computation power. So, we realized a Spectral domain OCT (SD-OCT) system that utilizes both a central processing unit (CPU) and graphics processing unit (GPU). The CPU take a role to acquire data and to display images. A GPU performs signal processing including background removing algorithm, k-linearization, Fast Fourier Transform (FFT), and the log operation. Also the data acquisition set a real time priority, and during the data acquisition of next frame, the signal processing and the image display are to complete for it to reduce lag time between data acquisition. As a result, we developed a SD-OCT system display images at the rate 60 frames/sec (2048 FFT size x 512 A-scans). The real time displaying speed is increased up to 4 times faster compared to the method using a single processor.
Skin penetration studies are crucial for the development of dermal drug carrier systems. The skin is a very efficient barrier to protect the human body against xenobiotics as well as pharmaceutical drugs. To overcome the skin barrier and thus to improve dermal therapy new drug carrier systems are developed and have to be tested. We use two approaches to obtain information about the transport of model drugs within the skin. First, fluorescence-labeled drugs are used to optically assess images by using widefield and laser-scanning microscopy techniques. Second, a 7-tesla MRI Scanner is used for developing a method that is not influenced by the optical properties of the skin. The NMR results might be used to compare the results of optically assessed skin samples.

We use two sets of pig skin, untreated and water-saturated, to determine the feasibility of the NMR-technique. The results of the untreated control samples show good morphological information. The water-saturated samples have enhanced image signal in epidermis und dermis layers. The advantages of the NMR-technique compared to optical assessment are the unlimited imaging depth and the possibility to differentiate and analyze the fat and water components of the skin separately. The main drawbacks are its lower resolution of about 100 microns and that there has to be an internal standard to quantify the measurements. Topically applied contrast agents encapsulated in drug carrier systems are used as model agent for skin penetration studies by NMR imaging.

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Intravascular Near-Infrared Fluorescence (NIRF) imaging is a promising imaging modality to image vessel biology and high-risk plaques in vivo. We have developed a NIRF fiber optic catheter and present the ability to image atherosclerotic plaques in vivo, using appropriate NIR fluorescent probes. Our catheter consists of a 100/140 µm core/clad diameter housed in polyethylene tubing, emitting NIR laser light at a 90 degree angle compared to the fiber’s axis. The system utilizes a rotational and a translational motor for true 2D imaging and operates in conjunction with a coaxial Intravascular Ultrasound (IVUS) device. IVUS data yield 3D images of the internal structure of a vessel and are used in our system for anatomical guidance. Using the IVUS images we are building an accurate hybrid fluorescence-IVUS data inversion scheme that takes into account photon propagation in the vessel. This hybrid imaging approach can then correct for the non-linear dependence of light intensity on the distance of the fluorescence region from the fiber tip, leading to quantitative imaging. The experimental and algorithmic developments will be presented and the effectiveness of the algorithm showcased with experimental results in both saline and blood-like preparations. The combined structural and molecular information obtained from these two imaging modalities are positioned to enable the diagnosis of biologically high-risk atherosclerotic plaques in the coronary arteries that are responsible for heart attacks.

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We have developed a NIRF fiber optic catheter and present the ability to analyze the fat and water components of the skin separately. The main drawbacks are its lower resolution of about 100 microns and that there has to be an internal standard to quantify the measurements. Topically applied contrast agents encapsulated in drug carrier systems are used as model agent for skin penetration studies by NMR imaging.
system.
We have successfully monitored the enhancement kinetics of both MRI and optical contrast agents simultaneously in vivo. Currently, we are evaluating the enhancement kinetics of both Gd-DTPA and ICG for different stages during tumor growth.

7892-38, Poster Session

In-vivo small animal optical imaging enhanced with indocyanine green and saline

N. Liu, Y. Lin, M. Hsing, O. Nalcioglu, G. Gulsen, Univ. of California, Irvine (United States)

Extrinsic contrast agents such as indocyanine green (ICG) have been used to enhance near-infrared optical imaging, and studied in recent years for both small animal and human breast tumors. ICG has large absorption peak near 800nm. When ICG is injected into blood stream, it increases tumor/tissue contrast and along with pharmacokinetic information improves tumor detection in vivo. Saline solution (sterile solution of sodium chloride in water), on the contrary, has very low absorption and scattering in the near-infrared wavelength range. When injected into a vessel, however, it dilutes the blood and decreases both its absorption and scattering properties. Here, we present a comparison of in vivo mice optical images enhanced with ICG and saline. The multimodality imaging system used here combines frequency-domain optical techniques with magnetic resonance imaging (MRI) for small animal studies. Intensity-modulated light emitted by a laser diode at 785 nm is delivered to the mice by eight 62.5 µm fibers. Eight collection fibers, 1 mm in diameter, were arranged alternatively with illumination fibers. All sixteen fibers were radially arranged to obtain transverse mice images revealing the tumor. The temporal resolution of the DOT system was 16 seconds. The dynamic optical data acquisition lasted for 50 minutes. MRI images were also recorded simultaneously and used for finite element mesh generation for optical image reconstruction. The reconstructed tomograms show the enhancement kinetics at tumor location in both cases. The pharmacokinetics of ICG and saline at the same tumor location show comparable levels of relative but opposite enhancement change.

7892-39, Poster Session

Comparison of monte carlo and diffusion in rat leg imaging for raman and fluorescence signals

J. H. Demers, B. W. Pogue, F. Leblond, Dartmouth College (United States); M. D. Morris, Univ. of Michigan (United States)

Recovery of Raman or Fluorescence signatures from within thin tissues benefits from model-based estimation of where the signal came from, especially if the signal passes through layers in which the absorption or scattering signatures distort the signal. Estimation of the signal strength requires appropriate normalization or model-based recovery, but the key to achieving good results is a good model of light transport. While diffusion models are routinely used for optical tomography of tissue, there’s some thought that more precise radiation transport modeling is required for accurate estimation. However, diffusion is often used for small animal imaging, because it’s a practical approach, which doesn’t require knowledge of the scatter phase function at each point in the tissue. Using more accurate radiation transport models implies that this phase function can be known to reasonable accuracy, even though this seems unlikely. The question asked in this study is, under what situations can diffusion theory reasonably model light transport in small volumes such as a rodent leg, and if it’s possible to use radiation transport modeling with estimations of the scatter phase function distribution. This study uses simple and complex leg geometries extracted from animal CT scans, to study the diffusion approximation and Monte Carlo simulations that can readily incorporate the accurate exterior and interior boundaries. The results show that under certain conditions there can be substantial similarity, but differences remain. The precise conditions under which diffusion and Monte Carlo disagree are outlined for the case of Raman and fluorescence tomography of the rat.

7892-40, Poster Session

Development of a hybrid MRI and fluorescence tomography system for small animal imaging

M. T. Ghijsen, Y. Lin, O. Nalcioglu, G. Gulsen, Univ. of California, Irvine (United States)

Fluorescence diffuse optical tomography (FT) is a molecular imaging technique that can create images of spatially resolved fluorophore concentrations and fluorescence lifetimes. One problem faced by FT is that the recovered fluorophore parameters greatly depend on the size and depth of the inclusion due to the ill-posedness of the FT inverse problem. Structural a priori information from imaging modalities with high spatial resolution is demonstrated to significantly improve the accuracy of the FT reconstruction. We have constructed a small animal imaging FT/ MRI system in this study. Near-infrared light was delivered and collected by optical fibers that connect the FT/MRI system to the interface in the MRI bore. We investigated the feasibility of a photo-multiplier tube (PMT) based detection system that acquires time-resolved data in the frequency domain. Phantom studies were used to evaluate the performance of the combined system. We show reconstructions of the concentration and lifetime maps with and without the structural a priori information obtained from MRI. ICG and DTTCI, two fluorophores with similar excitation and emission spectra but different lifetimes, were used in this evaluation. Specifically, we showed that the PMT-based frequency domain hybrid system can recover fluorescence intensity and lifetime accurately for two 5mm objects with different lifetimes in a 38 mm phantom, when MRI structural a priori information is used.
A hybrid approach combining microCT and fluorescence tomography: imaging workflow and system of coordinate registration

R. Holt, F. El-Ghussein, Dartmouth College (United States); K. M. Tichauer, Lawson Health Research Institute (Canada); F. Leblond, B. W. Pogue, Dartmouth College (United States)

A fluorescence imaging system was designed to interface with an x-ray microCT device. The in vivo microCT is capable of high spatial resolution, but is limited in its ability to image soft-tissue contrast. The fluorescence imaging system relies on Fluorescence Tomography (FT) to provide functional and molecular information. In order to properly orient the subject in both instruments, an imaging bed has been designed that can be translated between the imaging modalities. We focus on refining the workflow for the co-registration of the two systems, beginning with creating a finite elements mesh of the interrogated medium.

The meshing process begins with taking projection images in the microCT. These images are reconstructed, and exported as a stack of DICOMs. These are then used to create a binary mask of the object, which is used to generate a mesh using the NIRFAST software package.

A procedure is presented whereby a cylindrical phantom is used in order to derive a transformation matrix allowing seamless co-registration of the coordinate systems of the two instruments. The position of the phantom is initially adjusted manually to be located in the geometrical center of the FT instrument. It is then moved to the microCT and imaged. By determining the location of the geometrical center of the phantom in both instruments we derive an (x,y,z) translation matrix which can be used for all subsequent scans performed with the instruments. The evaluation of the co-registration procedure is presented using fluorescence tomography images of a synthetic mouse phantom, as well as actual mice where fluorescent tubes have been implanted.
Conference 7893: Endoscopic Microscopy VI

7893-01, Session 1

Standards in endoscopic microscopy
G. J. Tearney, Wellman Ctr. for Photomedicine (United States)
No abstract available

7893-02, Session 1

Endoscopic detection of murine colonic dysplasia using a novel fluorescently labeled peptide
S. J. Miller, B. Joshi, A. Gaустad, E. R. Fearon, T. D. Wang, Univ. of Michigan (United States)
Colorectal cancer is the 2nd leading cause of cancer-related deaths in the US. Current screening endoscopy does not detect all pre-malignant (dysplastic) colorectal lesions and thus, the development of more sensitive, targeted techniques to improve detection sensitivity is needed. The present work uses phase display to identify a novel affinity peptide that binds to pre-malignant mucosa in a CPC:Apc mouse model of colorectal dysplasia. Several rounds of in vivo T7 library biopanning revealed a candidate peptide that repeatedly bound to dysplastic mucosa. A wide-field, small animal endoscope capable of fluorescence excitation (450-475nm) was used to identify murine polyps via white light (175W Nova Xenon source delivered via 3 mm diameter fluid light cable) and to collect fluorescence images (510 nm barrier filter) of selective peptide binding. Candidate and control peptide sequences conjugated to 6'-FITC with an 6-amino hexanoic acid (Aha) linker were independently delivered at 100 µM to the distal colon of CPC:Apc mice via the instrument port of the endoscope. Quantitative analysis of peptide adsorption to distal colonic adenomas revealed the fluorescent-labeled peptide (Target/Background: 2.17 +/- 0.61) binds ~2-fold greater to the colonic adenomas when compared to the control peptide (Target/Background: 1.14 +/- 0.15), p<0.01. Our results demonstrate that preferential binding of affinity peptides towards dysplastic colonic mucosa can be identified via in vivo phage panning. More importantly, this work is first to image fluorescence-labeled peptide binding in vivo that is specific towards colonic dysplasia, demonstrating targeted detection with optical imaging.

7893-03, Session 1

Monitoring the esophageal response to radio frequency ablation with optical frequency domain imaging
Recent studies suggest that endoscopic radiofrequency ablation (RFA) may be used to effectively treat Barrett’s esophagus. RFA treatment may however be associated with an increased risk of subsquamous intestinal metaplasia that may not be effectively assessed with current standard biopsy protocols. Optical frequency domain imaging (OFDI) provides cross-sectional images of tissue microstructure with a penetration depth in tissue of approximately 2 mm, which may enable the assessment of tissue at a greater depth than that achieved with standard forceps biopsy. The goal of this pilot study is to use OFDI as a tool to assess the esophageal tissue response to RFA therapy in Barrett’s patients. 13 patients with a prior diagnosis of Barrett’s esophagus who were scheduled to receive RFA treatment were enrolled in this study. Volumetric OFDI imaging was performed using a 1.67 mm diameter imaging catheter immediately prior to treatment, post treatment, and at their regularly scheduled 3 and 6 month follow-up visits. During each imaging session, 4-quadrant spiral cross-sectional OFDI images were obtained over the entire length of the Barrett’s segment. The OFDI images were subsequently assessed at each time interval to evaluate the extent of Barrett’s epithelium, and to calculate the percentage of subsquamous buried glands. The OFDI images spanned the entire longitudinal extent of the involved Barrett’s segment in >93% of pullbacks. Following RFA treatment, endoscopy with biopsy suggested that most patients achieved full eradication of the Barrett’s epithelium, however the OFDI images revealed an increase in the size and number (148% increase; p=0.014) of buried glands post treatment. Comprehensive OFDI imaging of the esophagus may be a useful tool for assessing adequate treatment of Barrett’s epithelium and may provide a more thorough means of assessing subsquamous Barrett’s epithelium. This may be achieved by both reducing the sampling error associated with biopsy acquisition, and by enabling the assessment of tissue at a greater depth than that typically achieved with forceps biopsy.

7893-04, Session 1

Endoscopic 3D-OCT for in-vivo assessment of endoscopic treatments of Barrett’s esophagus and esophageal cancer
C. Zhou, T. Tsai, H. Lee, Massachusetts Institute of Technology (United States); D. C. Adler, J. M. Schmitt, LightLab Imaging Inc. (United States); Q. Huang, VA Boston Healthcare System (United States); J. G. Fujimoto, Massachusetts Institute of Technology (United States); H. Mashimo, VA Boston Healthcare System (United States)
Patients with Barrett’s esophagus (BE) have a greatly increased cancer risk than the general population. If detected and treated early, a high percentage of regression and significant improvements in 5-year survival rates can be expected. Radiofrequency ablation (RFA) allows broad and superficial ablation for BE, and was demonstrated to achieve a high rate of complete dysplasia eradication in patients with high-grade and low-grade dysplasia. Cryospray ablation (CSA) is used for endoscopic management of esophageal cancers in cases when esophagectomy is not indicated. Endoscopic three-dimensional OCT (3D-OCT) uses a thin fiberoptic imaging catheter placed down the working channel of a conventional endoscope to image a large field (8mm x 20mm x 1.6mm) within 20s with less than 10µm axial resolution and ~60k A-lines per second. Patients were recruited and imaged with 3D-OCT before and after (up to 24 months) endoscopic treatments (RFA or CSA) of BE or esophageal cancer. Characteristic features of normal esophagus, BE, and esophageal cancers (adenocarcinoma, papillary carcinoma and squamous cell carcinoma) were identified from 3D-OCT. In patients after RFA treatment of BE, a small number of isolated glands were found buried beneath the neo-squamous epithelium (NSE). NSE is a marker of successful ablative therapy, while buried glands may have malignant potential and are difficult to detect using conventional video endoscopy and random biopsy. Ablation (RFA and CSA) induced tissue morphological changes were also successfully observed and compared with 3D-OCT. These results suggest 3D-OCT as an important tool for on-site assessment and follow-up evaluation of endoscopic therapies.
High-resolution in-vivo targeted imaging of colorectal dysplasia with a LED-based confocal microendoscope

S. F. Elahi, S. J. Miller, T. D. Wang, Univ. of Michigan (United States)

Transformed epithelial cells that develop into colon cancer express molecular targets that can be used for diagnosis and therapy. These targets can be studied using mouse models that spontaneously develop colorectal dysplasia. New instruments are needed with sub-cellular resolution, fast frame speeds, and sufficiently small package dimensions to directly image molecular targets expressed in the epithelium of living mice. We demonstrate targeted fluorescence-labeled peptide imaging of adenomas in mice using a LED-based, flexible, fibered microscope that passes through the instrument channel of a small animal endoscope. We coupled a LED, centered at 470 nm, to a fiber-optic bundle with an aspheric lens. We selected a peptide via phage display that preferentially binds to adenomas in a transgenic mouse model of colorectal dysplasia, and synthesized FITC-labeled selective and control peptides. Independently, the peptides were applied topically to the mucosal surface containing adenomatous polyps. Polyps were located using wide-field small animal fluorescence endoscopy, and the microendoscope was placed directly in contact with adenomatous and normal mucosa. En face images were collected at 10 Hz and fluorescence intensities from adenomas and normal appearing surrounding mucosa were compared. The instrument delivers 0.7 mW illumination and achieves lateral resolution of 4 µm. Selective and control peptides preferentially bound to adenomas with a target-to-background ratio of 3.39 ± 0.75 and 1.01 ± 0.54, respectively. Selective peptide showed binding to crypts. This instrument may improve our ability to perform early detection, risk stratification, and therapeutic monitoring of cancer in the digestive tract.

Miniature 3D confocal microendoscope used to in-vivo identification of Eosinophils trigger symptoms

Z. Liu, J. Domke, Z. Qiu, N. Safdarian, K. Oldham, E. Wang, T. D. Wang, Univ. of Michigan (United States)

Eosinophil trigger symptoms in allergic rhinitis. New diagnostic methods for identifying nasal eosinophils are needed. Flavin adenine dinucleotide (FAD) in eosinophil granules produce intense autofluorescence. A novel fiber-based miniature confocal microendoscope has been developed to image the eosinophils in vivo. 471nm laser is used for excitation. A single mode fiber delivers the laser light into a 8 mm diameter instrument, a size compatible with imaging human nares. After collimation, the laser beam is directed to a MEMs scanning mirror via a smaller fixed mirror. A miniature aspheric relay lenses system is inserted in between the MEMs scanning mirror and the high NA (0.6) GRIN lens. The relay lenses system expands the beam diameter and images the MEMs mirror to the back aperture of the objective lens, so the light will overfill the objective lens during scanning to provide sufficient numerical aperture at the tissue surface. To perform z-axis (into tissue) scanning, a novel piezoelectric microactuator has been designed. The distal lens in the relay lens system is translated by the piezoelectric microactuator. Based on the simulation and experiment result, the field of view is larger than 270µm(x) x 260µm(y) x 240µm (z) and the lateral and axial resolution is ~1 µm and ~5 µm, respectively, and achieves sub-cellular resolution. The image of fluorescent beads and live eosinophils cell are demonstrated.
Pulses with 45 fs duration were obtained at the output of a standard 2 meter long unimodal fiber. Using this ultrashort pulse as an excitation source, a spectroscopic study of the endogenous signal (TPEF + SHG) generated from human pulmonary fixed tissue samples of various thicknesses was performed. Forward (sample thickness 20 micrometers) generated from human pulmonary fixed tissue samples of various source, a spectroscopic study of the endogenous signal (TPEF+SHG)

7893-10, Session 2

Epifluorescence light collection for multiphoton microscopic endoscopy
C. M. Brown, D. R. Rivera, C. Xu, W. W. Webb, Cornell Univ. (United States)

Multiphoton microscopic endoscopy is a promising medical in vivo diagnostic imaging technique because it captures intrinsic fluorescence and second harmonic generation signals to reveal real-time anatomical and histological information about disease states in tissue. However, maximizing light collection from multiphoton endoscopes remains a challenge: weak nonlinear emissions from endogenous structures, miniature optics, large imaging depths, and light scattering in tissue all hamper light collection. In this experiment we use a light collection scheme that utilizes two configurations of dual-clad optical fiber (DCF) to quantify both scattered and unscattered collection. We investigated excitation and collection of multiphoton emission light from a tissue phantom composed of a 10 µm Fluorescein solution mixed with microspheres; the tissue phantom approximates the scattering properties of human bladder tissue. Light from a Ti:Sapphire laser is dispersion compensated and focused through an illumination DCF and lens system into the tissue phantom at depths of 0, 100 and 200 µm. Emitted fluorescence returns from the tissue phantom through the lens system via both the illumination DCF and the collection DCF, whose lateral position varies from 75 um to 1450 um spacing from the illumination DCF. Results showed that the ratio of light detected from the illumination DCF compared to the collection DCF area decreased from 13:1 to 0.76:1 on increasing the imaging depth from 0 to 200 µm. From these results, a light collection scheme employing an illumination DCF for collection of unscattered light as well as an optical fiber array for collection of scattered emission light is proposed.

7893-11, Session 3

Autoclaveable miniaturized video endoscopes with simplified flip-chip assembly
E. Beckert, F. Wippermann, S. Walther, T. Burkhardt, Fraunhofer-Institut für Angewandte Optik und Feinmechanik (Germany); B. Messerschmidt, Grintech GmbH (Germany); T. Bartnitzek, VIA electronic GmbH (Germany); T. Vahrenkamp, ficonTEC Service GmbH (Germany); R. Eberhardt, Fraunhofer-Institut für Angewandte Optik und Feinmechanik (Germany); A. Tünnermann, Friedrich-Schiller-Univ. Jena (Germany)

Video endoscopes with an imager located at the distal end possess an opto-mechanical layout simplified for assembly since an optical relay system is obsolete. Based on a CMOS imager with 650x650 pixels of 2.8um pitch straight view systems with 75° and 110° field of view at f/4.3 mm have been developed and built. Including a protective stainless steel shell these systems provide an outer diameter of 3 mm and a length of approx. 8 mm and can be integrated into working channels of standard endoscopes. The optics contain of injection molded polymer lenses in combination with a GRIN and a dispersed lens. Using this optical system a simple flip chip assembly that uses concentric outer diameter alignment along with high precise optic and mounting adhesive dispensing was developed that resulted in an optical axis alignment better 10 µm and a final MTF of 150 lp/mm at 30% contrast. The 75° FOV system was sealed using a solderjetted front window, providing 10-9 mbar/s leakage rates even after several autoclave cycles. The technologies combined to assemble the miniaturized endoscopic video cameras allow for the flexible manufacturing of up to medium quantities and successfully showed that optoelectronics assembly can be carried out using passive alignment techniques.

7893-12, Session 3

Vertical cross-sectional imaging by handheld dual-axes confocal microscope
Z. Qiu, Z. Liu, C. Rhee, K. Oldham, K. Kurabayashi, T. D. Wang, Univ. of Michigan (United States)

In vivo high resolution imaging of the epithelium, the thin layer of mucosa where many important cancers originate, can be performed with microendoscopes. Vertical cross-sections show the relationship among tissue micro-structures as they vary with depth, and are the preferred view of pathologists. We aim to develop a miniature dual axes confocal microscope that has sufficient dynamic to perform vertical cross-sectional imaging to a depth of 400 microns in a scattering medium. We designed and fabricated a single axis in-plane MEMS mirror with dimensions of 3 mm (X) by 1 mm (Y) with “dumbbell” geometry, and integrated this mirror with a bulk PZT z-axis actuator that has travel of 400 microns. We packaged this novel scanning and actuation mechanism into a 10 mm diameter handheld instrument using a 785 nm semiconductor laser, collimators, a parabolic mirror, and a solid immersion lens. We acquired vertical cross-sectional images at 2 Hz (fps) with a field-of-view (FOV) of 800 µm × 400 µm, sub-cellular resolution (<5 µm axial, <5 µm lateral), and deep tissue penetration (~400 µm). The near infrared LICOR dye labeled 10 µm polystyrene beads embedded with gelatin were imaged using scattering phantom. Furthermore, harvested tissue organs from CPC-APC mouse model of colonic dysplasia were also imaged with LICOR dye. We demonstrate a novel scanning and actuation mechanism for a handheld dual-axes confocal microscope that performs vertical cross-sectional imaging with a depth of 400 microns and validate performance on a new 3D phantom with near infrared LICOR dye labeled beads.

7893-13, Session 3

3D tumor imaging by self interference fluorescence endoscopy
M. de Groot, J. F. de Boer, Vrije Univ. Amsterdam (Netherlands)

We will demonstrate a novel 3D fluorescence imaging technique ideally suited to be incorporated in small (less than 2 mm diameter) endoscopes. The technique is based on the principle of self-interference: by presenting the fluorescent photons with two alternative optical paths they are forced to interfere with themselves. Alternating constructive and destructive interference modulates the detected fluorescence spectrum. The phase of this spectral modulation uniquely identifies the depth location of the fluorescent source without the need for a mechanical depth scanning mechanism. We demonstrate that sub 10 µm depth sensitivity can be obtained over a depth of field of 1 mm. To simulate the effect of wavefront distortion by scattering in tissue, we imaged a multilayer sample through 1 mm 1% intralipid solution and found that the sensitivity was still 20 µm.

Early cancer diagnosis can be greatly improved by employing fluorescent labels that selectively target tumors. However, effective endoscopic imaging tools are needed to optimally exploit the potential of these markers. Depth resolved imaging of fluorescence will aid in the determination of the extent of invasion of a tumor in the underlying tissue in real time during intervention. The potential integration with OCT will
provide both depth resolved tumor location and information about the surrounding tissue architecture. This could improve the effectiveness of therapy and response monitoring.

**7893-14, Session 3**

**Design of illumination system in ring-field capsule endoscope**

W. Jeng, O. Mang, National Chiao Tung Univ. (Taiwan); Y. Chen, National Central Univ. (Taiwan); Y. Wu, National Chiao Tung Univ. (Taiwan)

This paper is researching about the illumination system in ring filed capsule endoscope. It is difficult to obtain the uniform illumination on the observed object because the light intensity of LED will be changed along with its angular displacement and same as luminous intensity distribution curve. As the location of light source (i.e., LED) is approaching imaging lenses which light source incident into the cone mirror and then the reflected light will be reflected through the lenses to enter CMOS sensor directly. On the other, the strong reflection rays from the transparent window will incident into the cone mirror and then the reflected light will be reflected by cone mirror indirectly and through the lenses to enter CMOS sensor. The result of above reasons is bound to make over-blooming on the image plane. Regarding for this problem, we use the optical design software which is Advanced Systems Analysis Program (ASAP) to build a photometric model for the optimal design of LED illumination system in ring field capsule endoscope. setting the several key parameters that are LED quantities, location of LED, geometric shape of LED, tilt angle of surface of LED, distance between LED and cone mirror etc. That is why to decrease the stray light and increase the whole illumination uniformity of endoscope. In this paper, the optimal design of illumination uniformity in the endoscope is from origin 0.128 up to optimum 0.603 and it would advance the image quality of endoscope greatly.

**7893-15, Session 3**

**Fused oblique incidence reflectometry and confocal fluorescence spectroscopy**

M. D. Risi, College of Optical Sciences, The Univ. of Arizona (United States); A. R. Rouse, A. F. Gmitro, The Univ. of Arizona (United States)

Confocal microendoscopy provides real-time high resolution cellular level images via a minimally invasive procedure, but relies on endogenous fluorophores, has a relatively limited penetration depth (100 μm), and produces a high rate of detailed information to the user. A new catheter based multi-modal imaging system has been developed that combines confocal imaging, fluorescence spectroscopy, and oblique incidence reflectometry (OIR); a non-invasive method capable of rapidly extracting tissue absorption and reduced scattering spectra at depths of 1-2 mm. The system builds on previous development of a custom slit-scan multi-spectral confocal microendoscope and results in a device that can rapidly switch between diffuse spectroscopy and confocal fluorescence modes. The new probe consists of a fiber bundle and dye delivery channel for traditional confocal fluorescence imaging combined with two offset OIR source fibers whose white-light illumination is reflected at +/-45 degrees. Diffusely scattered light from each of the source fibers is collected via the fiber bundle with a frame of data representing spectra collected at a range of distances from the OIR source points. Computer controlled shutters allow the rapid switching of OIR illumination fibers. The improved imaging depth and tissue characterization speed allows for rapid identification of suspicious regions prior to collecting optical biopsy images in confocal mode, which greatly improves the diagnostic potential of the device.

**7893-16, Session 4**

**Imaging of flowing red blood cells using spectrally encoded confocal microscopy**

L. Golan, L. Minali, D. Yelin, Technion-Israel Institute of Technology (Israel)

Spectrally encoded confocal microscopy (SECM) has been shown promising for imaging through single fiber endoscopic probes, with a large number of resolvable points. In SECM, lateral imaging is obtained by encoding one transverse axis with wavelength, using a lens-grating arrangement at the distal end of the probe, while the second axis is mechanically scanned at relatively slow rates. The backscattered light is then collected by the imaging optics and detected by a high-speed spectrometer.

In this work, we report a novel approach which allows endoscopic imaging of flowing cells using SECM with no mechanical scanning. The lateral motion of the cells flowing perpendicularly to the spectrally encoded line eliminates the need for scanning, allowing the acquisition of full two dimensional images of the cells. The approach is experimentally demonstrated by repeatedly acquiring a spectrally encoded line within a 0.25 mm² cross-section chamber containing a 1 mm/s steady flow of red blood cells. The sub-cellular resolution images provide valuable information on the cells including their size, shape, orientation in space, and flow velocity profiles, with no staining or fluorescent labeling required. An SECM probe for minimally invasive imaging of blood flow below the tissue surface could be made free of moving parts, and be mechanically connected to the light source and detection system by a single optical fiber. The technique could be useful for monitoring various hematological disorders by imaging the flow in visceral blood vessels.

**7893-17, Session 4**

**Spectrally encoded confocal microscopy for identifying eosinophilic esophagitis**


Eosinophilic esophagitis (EoE) is an inflammatory condition of the esophagus that is diagnosed by endoscopic biopsies and the histologic assessment of esophageal eosinophilia. Since the histopathologic diagnosis is the only objective way currently available, repeated upper endoscopic biopsies are involved in monitoring of EoE. Spectrally-encoded confocal microscopy (SECM) is a form of reflectance confocal microscopy that enables visualization of sub-cellular features of upper GI tissues through large area. In this study, we investigate the capability of SECM to identify and count esophageal eosinophils for diagnosing EoE. A total of 43 biopsy samples from 35 pediatric patients undergoing routine esophagogastroduodenoscopy (EGD) for EoE were imaged by SECM immediately after their removal and then sent for histologic processing. 17 biopsy samples were diagnosed as EoE by histopathologic assessment. A large number of eosinophils were detected and identified in corresponding SECM images, confirmed by the histology with cellular level matches. There was a good correlation for maximum eosinophil counts between SECM and histopathology (r=0.82, p<0.0001). When an eosinophil count cutoff threshold of 15 eos/HFP was used, SECM had a sensitivity of 74-100% (95% confidence interval) and a specificity of 70-100% (95% confidence interval) for diagnosing EoE. The results suggest that future development of this technique could be a potential diagnostic and/or monitoring tool for EoE.
Spectrally encoded confocal microscopy for intra-operative margin assessment

D. Kang, E. F. Brachtel, B. L. Smith, B. E. Bouma, G. J. Tearney, Massachusetts General Hospital (United States)

Lumpectomy followed by radiation therapy is a standard surgical treatment for patients with early stage breast cancer. However, surgically-removed tissues show positive margins upon postoperative histologic analysis in as many as 50% of the lumpectomy patients. In such cases, patients are required to undergo additional surgeries, resulting in increased patient morbidity and procedure costs. Spectrally encoded confocal microscopy (SECM) is a high-speed confocal microscopy technology that can achieve 10 to 100 times faster image acquisition speed than conventional confocal microscopy systems. By utilizing the high image acquisition speed, SECM can be utilized to image entire surgical specimens and provide accurate margin status during surgery. The capability of assessing margin status intraoperatively with SECM can enable surgeons to acquire tumor-free margins during the initial surgery, reducing the needs for additional surgeries or re-excisions. Here, we report results from a preliminary study of imaging large areas of surgically-removed breast tissues with an SECM benchtop system. Large area SECM images (4 mm by 2 mm to 5 mm by 4 mm) of 18 breast tissues were shown to visualize architectural and cellular features similar to those used in histologic analysis: SECM images of normal breast tissues clearly differentiated adipose cells from stroma; low-grade invasive ductal carcinoma showed small glands invading stroma in a disorganized fashion; and high-grade invasive carcinoma exhibited poorly differentiated tumor cells with little stroma. These preliminary results showed that SECM has the capability to visualize key histomorphologic features relevant to breast cancer diagnosis.

Non-confocal detection in spectrally encoded endoscopy

D. Kang, M. S. Shishkov, B. E. Bouma, G. J. Tearney, Massachusetts General Hospital (United States)

Spectrally encoded endoscopy (SEE) is an optical imaging technology that can be integrated into a sub-millimeter miniature endoscope. At the tip of an SEE endoscopic probe, a grating is utilized to encode one transverse coordinate of the specimen in the wavelength. Previously, a 350-µm-diameter SEE probe has been demonstrated for laparoscopic animal imaging through a fine gauge needle. However, the image quality was limited in previous studies due to speckle noises and relatively weak back reflection from the SEE probe itself caused by the coherent and single-path illumination/detection method. In this paper, we present a new method of conducting SEE that generates speckle-free images with a high signal-to-background ratio. In the new method, broadband light from a supercontinuum source was coupled into a small core (diameter = 3.7 µm) of an illumination fiber, focused by a GRIN lens (diameter = 500 µm), and produced a small focused spot, the size of which determined the lateral resolution. A multi-mode fiber (core diameter = 93 µm) was assembled on the side of the GRIN lens with its distal tip meeting the distal tip of the GRIN lens and used for detection. The new method was evaluated in a benchtop setup comprising a 1800-lp/mm grating and a galvo scanner. Images of a human finger were obtained in vivo at the image acquisition rate of 10 frames/sec, and unprocessed images showed clear evidence of the detailed structure of fingerprints without any noticeable speckle noises.

Wavelength-swept laser-based spectrally encoded fluorescence imaging

M. Strupler, N. Goulamhoussen, E. De Montigny, C. Boudoux, Ecole Polytechnique de Montréal (Canada)

Spectral encoding enables high pixel density, video rate imaging through sub-millimeter diameter endoscopes. However, the technique typically relies on reflectance signal and is therefore restricted to structural information. We present herein a spectrally encoded imaging method which rapidly acquires fluorescence and reflectance images based on a wavelength-swept laser centered at 1310 nm (80 nm bandwidth). Based on a transmission diffraction grating, we acquired up to 9920 lines of 1024 pixels per second of a textile sample stained with near infrared quantum dots (EvidentTech PbS Quantum Dots). This imaging rate compares favorably with a previously reported scheme which was based on a broadband source and wavelength dependent amplitude modulation which was much more sensitive to vibrations and misalignments. Near infrared quantum dots are however not commonly used in biology. To overcome this limitation, we developed a new wavelength swept laser centered at 780 nm (30 nm bandwidth). Based on a transmission diffraction grating, we acquired up to 9920 lines of 1024 pixels per second of a textile sample stained with near infrared quantum dots (EvidentTech PbS Quantum Dots). An instantaneous linewidth of 0.03 nm was achieved, resulting in 1070 resolvable points. This laser allows efficient excitation of indocyanine green, an FDA approved dye staining plasma proteins. Combined with a dual-clad fiber coupler, this approach paves the way for large area endoscopic imaging providing structural and functional information with high pixel density and acquisition rates.
Reduced speckle, large-area comprehensive imaging by spectrally encoded confocal microscopy

P. Pal, D. Kang, B. E. Bouma, G. J. Tearney, Massachusetts General Hospital (United States)

Spectrally encoded confocal microscopy (SECM) is a reflectance microscopy technique, which employs confocal selection of light reflected from the sample and encodes one dimension of spatial information in the optical spectrum. Broadband light is dispersed using a diffraction grating and is subsequently focused onto the sample by an appropriate objective lens. Light scattered from different spatial locations is collected by an optical fiber core (or cladding), which acts as a confocal aperture and provides optical sectioning by rejecting out-of-focus light. Mechanical scanning is employed for obtaining the dimension transverse to the spectrally encoded dimension. The detected light is spectrally decoded by a high-speed camera-based spectrometer. Ensuring that the sample to be imaged stays within the focal plane of the objective requires an additional mechanism for optimizing the lens position to accommodate for tissue surface irregularities. In this paper, we present our prototype for an SECM endoscopic probe designed to perform rapid, helical, large-area scans of luminal organs by focusing the objective and we demonstrate a significant reduction of speckle-related artifacts in the images by employing a double-clad fiber as the illumination/detection conduit. By tilting the objective lens, which also enables multiple planes within a depth of 50 µm to be interrogated simultaneously, we derive the feedback signal for the adaptive focus algorithm. The position of the objective lens is optimized in real-time by a miniature transducer-based actuator. With our prototype, we successfully imaged a phantom tissue sample and reduced the speckle contrast by a factor of 5.

In-vivo imaging of human lung microvasculature using Doppler optical coherence tomography

A. Lee, P. M. Lane, The BC Cancer Agency Research Ctr. (Canada); B. Standish, Ryerson Univ. (Canada); C. E. MacAulay, A. McWilliams, The BC Cancer Agency Research Ctr. (Canada); V. X. D. Yang, Ryerson Univ. (Canada); S. Lam, The BC Cancer Agency Research Ctr. (Canada)

Architectural changes in and remodeling of the bronchial and pulmonary vasculature are important pathways in diseases such as asthma, chronic obstructive pulmonary disease (COPD), and lung cancer. However, there is a lack of methods that can find and examine small bronchial vasculature in vivo. Structural lung airway imaging using Optical coherence tomography (OCT) has previously been shown to be of great utility in examining bronchial lesions during lung cancer screening under the guidance of autofluorescence bronchoscopy. Here we present a new OCT instrument that is capable of detecting lung vasculature in addition to providing morphological information.

This new OCT instrument uses Doppler and velocity variance algorithms to detect blood vessels and estimate flow velocities within them. Our imaging system consists of a swept-source laser centered at 1310nm, and a side-looking, circumferentially-scanning fiber optic probe. The fiber optic probe was passed down the instrument channel of a standard bronchoscope to image airways from segmental bronchi down to the respiratory bronchioles. Blood vessels greater than 40µm were readily detected in a selection of patients with normal lung function and COPD while simultaneously collecting the structural OCT images.

Dynamic three-dimensional imaging of lung parenchyma by OCT in mice

S. Meissner, Technische Univ. Dresden (Germany); A. Tabuchi, St. Michael's Hospital (Canada); M. Mertens, Charité Universitätsmedizin Berlin (Germany); W. M. Kübler, St. Michael's Hospital (Canada); E. Koch, Technische Univ. Dresden (Germany)

The development of protective ventilation strategies to improve mechanical ventilation of patients is of great importance. However, approaches to improve artificial ventilation are hampered by our lack of understanding of the effects on the alveoli. One approach to circumvent this limitation is the development of numerical models of the lung. Thereby alveolar dynamics can be simulated and different protective ventilation strategies can be characterized concerning the mechanical stress exerted on the alveolar structures. The basis for such models is detailed knowledge of the dynamic alveolar behavior in an in vivo situation.

In this feasibility study, we present a method for dynamic 3-D imaging of healthy and injured subpleural lung tissue in the ventilated mouse. We use triggered swept source optical coherence tomography (OCT) with an A-scan frequency of 20 kHz to image murine subpleural alveoli during the inspiratory phase. The data acquisition is gated to the ventilation pressure to take single B-scans in each respiration cycle for different pressure levels. The acquired B-scans are combined off-line into one volume scan for each pressure level. The air fraction in healthy lungs and injured lungs is measured using 2-D OCT en-face images. Upon lung inspiration from 2 to 12 cmH2O ventilation pressure, the air fraction increases in healthy lungs by up to 11% and in injured lungs by 8%. This expansion correlates well with results of previous studies, reporting increased alveolar area with increased ventilation pressures. We demonstrate that OCT is a useful tool to investigate alveolar dynamics in spatial dimensions.

Optimization of the focal parameters for three-dimensional optical frequency domain imaging of air-filled alveoli

C. I. Unglert, E. Namati, B. E. Bouma, G. J. Tearney, Massachusetts General Hospital (United States)

Visualizing the interdependence of alveolar networks in vivo has been challenging due to the high resolution, imaging speed and imaging depth required in order to investigate these anatomical structures. Optical Frequency Domain Imaging (OFDI) is a promising new imaging modality for this task, with axial resolutions in the order of 10µm and imaging speeds in excess of 100fps. In this study, we investigate variations in focal parameters, namely numerical aperture (NA) and multi-depth focusing, for OFDI of air filled alveoli. We hypothesized we could identify an optimal set of parameters maximizing imaging depth while maintaining sufficient lateral resolution.

We evaluated four different NAs (0.02, 0.03, 0.08, and 0.1) and the potential of multi-depth focusing to further increase the usable imaging depth. Two rabbit lungs were instillation fixed and dried using a modified Heitzman fixation technique at 20 cm H2O pressure, and then cut in 2 mm thick slices. One rabbit lung was freshly excised and inflated to 20 cm H2O pressure for imaging. Three-dimensional data sets from the upper right lobe were obtained with OFDI for each of the focal parameter sets. Images were analyzed to determine the best compromise between imaging depth and resolution for the visualization of alveolar networks. The imaging depths at which alveolar septal walls were clearly identified varied from 80 to 160 µm depending on the NA. Acquisition of images obtained at different focal locations substantially increased imaging depths for the 0.08 and 0.1 NA lenses.
We conclude that the choice of optimal focal parameters is crucial for investigating alveoli. Recommendations for focusing parameters and methods depend on the specific research question and study design.

7893-26, Session 5
Improved imaging of alveolar structures by index matching
S. Meissner, L. Knels, E. Koch, Technische Univ. Dresden (Germany)

One fundamental requirement for developing numerical models of mechanical ventilation is the quantification of alveolar geometries of complete acinus as close to an in vivo situation as possible. However, in vivo imaging of 3-D alveolar geometries has proved to be difficult due to the limitations in resolution and penetration depth presented by established imaging modalities. OCT imaging of air-filled lung tissue is hampered by the penetration of light to a depth of less than 200 µm. In deeper areas of the lung, the different refraction indices lead to total reflection at the interface between lung tissue and air, resulting in pseudostructures in the OCT datasets, which were observed in previous studies.

To eliminate the diffraction index interfaces between alveolar pockets and walls, we fill the lungs, after perfusion fixation with a mixture of glutaraldehyde and paraformaldehyde, with ethanol by perfusing with gradually increasing concentrations. This bottom-up filling process leaves no remaining air bubbles in the alveolar structures, thus drastically improving the resolution and penetration depth of 3-D FDOCT imaging. We observe an approximately 18% increase in alveolar area after ethanol filling, likely due in large part to elimination of the air/tissue interfaces. 3-D OCT datasets acquired from ethanol-filled lungs allow segmentation of the ethanol-filled structures, which were formerly air-filled, and 3-D reconstruction of larger areas of subpleural alveolar structures. Our innovative process of filling the lungs with ethanol post perfusion fixation thus enables more accurate quantification of alveolar geometries, a critical component of modeling lung function.

7893-27, Session 5
Smoke inhalation induced airway changes quantified using 3D endoscopic optical coherence tomography
D. Yoon, Beckman Laser Institute and Medical Clinic (United States); A. E. Heidari, OCT Medical Imaging, Inc. (United States); S. Lee, Beckman Laser Institute and Medical Clinic (United States); T. S. Ramalingam, OCT Medical Imaging, Inc. (United States); J. Lee, S. B. Mahon, D. S. Mukai, Z. Chen, M. Brenner, Beckman Laser Institute and Medical Clinic (United States)

Smoke inhalation injury is frequently accompanied by cyanide (CN) poisoning that may result in substantial morbidity and mortality. We utilized a prototype rapid acquisition swept source laser 3-D-optical coherence tomography (OCT) system we constructed to investigate morphological airway changes following smoke and CN exposure in rabbits.

New Zealand White male rabbits were intubated and mechanically ventilated prior to exposure to smoke from unbleached burned cotton administration. Sodium Cyanide solution was administered via continuous IV infusion with a 10mg total dose administered over 60 minutes. A flexible fiber optic OCT probe was inserted through the endotracheal tube to image the trachea. Images of airway mucosa and submucosa were obtained at baseline and at specified intervals post exposure. The thicknesses of the epithelial, mucosal, and submucosal layers of the rabbit airway were measured using 3-D OCT system from reconstructed longitudinal images. Less than 15 minutes after smoke inhalation, OCT was able to detect increases in the thickness of the tracheal walls of the rabbit. OCT is capable of quantitatively detecting regional changes in airway swelling following inhalation injury. 3-D OCT is capable of quantifying progressive regional changes in airway injury following smoke inhalation in this animal model. 3D-OCT may provide a method for more accurate determination of extent of airway injury, and potentially assessing response to therapeutic interventions.

7893-28, Session 5
Quantitative measurement of airway wall thickness by OCT
P. M. Lane, A. Lee, A. McWilliams, The BC Cancer Agency Research Ctr. (Canada); H. O. Coxson, Vancouver General Hospital (Canada); C. E. MacAulay, S. Lam, The BC Cancer Agency Research Ctr. (Canada)

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease that causes shortness of breath due to remodeling and narrowing of the small airways (bronchiolitis) and destruction of alveoli (emphysema). The disease is the 4th leading cause of death in the United States and affects more than 600 million people worldwide. Airway thickness is correlated with lung function and the progression of COPD. However, in vivo measurement of airway thickness as a biomarker for assessing COPD severity has been challenging. The best CT scanners don’t have sufficient spatial resolution to accurately quantify the dimensions of the small (<2mm diameter) airways. Currently the only way to accurately quantify the dimensions of these airways is by histopathology following lung resection, transplantation, or post-mortem examination. Endobronchial optical coherence tomography (OCT) has the required spatial resolution and depth penetration required to quantify the dimensions of the small airways in vivo. In a previously-published pilot study of 44 patients we showed that OCT can be used to measure airway dimensions and that OCT may be more sensitive at detecting small airway wall changes in individuals with COPD. In this talk we will present high-resolution OCT images of the small airways from study participants with normal lung function and COPD. We will also show preliminary results from a study designed to validate the thickness of the small airway walls as measured by OCT using histopathology as the gold standard.

7893-29, Session 6
In-situ 3D imaging of alveoli using an OCT needle probe
B. C. Quirk, R. A. McLaughlin, A. Curatolo, R. W. Kirk, P. B. Noble, D. D. Sampson, The Univ. of Western Australia (Australia)

Artificial ventilation is an important therapy in respiratory failure but can induce lung injury. Selection of optimal ventilation strategies could be improved by a better understanding of the regional lung structure and the dynamic response of alveoli to different pressures. However, this is currently limited by a lack of suitable in situ imaging techniques. We have developed a miniaturized optical coherence tomography (OCT) probe, encased within a 23-gauge hypodermic needle, that is capable of imaging alveoli in situ. The focusing optics consists of no-core and GRIN fiber spliced to a length of single-mode fiber. The beam was deflected at 90 degrees by a highly polished copper mirror positioned within the needle, and passed through a small window etched into the needle wall. The probe was mounted on a stepper motor rotating at 2Hz, and a translation stage enabling insertion and retraction. The probe was attached to a frequency-domain OCT system with source central wavelength of 840nm and bandwidth of 50nm. The use of this OCT needle probe allowed the acquisition of images several centimeters below the surface of the lungs. Fresh sheep lungs were imaged in two
settings: under artificial inflation with air, and after perfusion with saline. Multiple 3D OCT data sets were acquired with the OCT needle probe. Results demonstrated the ability of the system to image individual alveoli and respiratory bronchioles. Perfusion with saline was found to give improved OCT penetration depth.

7893-30, Session 6

Using optical coherence tomography to investigate the compliance of the airways in vivo

C. Robertson, S. Lee, Y. Ahn, S. B. Mahon, Z. Chen, M. Brenner, S. C. George, Univ. of California, Irvine (United States)

The airways of asthmatic patients may change compliance during disease progression. This change reflects broadly the effects of airway tissue remodeling that occurs in a chronic, repetitive, inflammatory disease. Altered airway compliance may affect the resistance to air flow through the lung, thus exacerbating symptoms observed in asthma. It is not currently possible to measure the compliance of airway tissue in vivo, as both extremely high spatial (sub-millimeter) and temporal (sub-second) resolution are needed to track the sub-millimeter deformations caused by normal breathing.

Optical coherence tomography (OCT) allows for non-destructive imaging of tissue with the resolution in space and time necessary for strain tracking during normal tidal breathing. We have developed algorithms to control for probe motion, determine strain in a polar geometry, and to compare strains to ventilator pressures. Thus, the algorithms allow estimation of airway tissue elastic properties in vivo. These algorithms were initially used to analyze the compliance of elastic phantoms with known mechanical properties demonstrating reasonable accuracy: modulus according to standard tensile mechanical testing: 1.3±0.3MPa, compared to OCT imaging 1.6±0.1MPa. These methods were then used to analyze the compliance of the trachea of an anesthetized rabbit, and its changes during and following wound healing from an epithelial-scrape injury.

This work represents the first estimate of tissue elastic properties from imaging with an endoscopic OCT probe in vivo. The extremely high spatial and temporal resolution of OCT imaging allows for elastographic imaging of small tissues, and has applications to the study of airway fibrosis.

7893-31, Session 6

Four-dimensional peripheral lung dynamics assessed through catheter-based optical frequency domain imaging

E. Namati, C. I. Unglert, Wellman Ctr. for Photomedicine (United States); B. E. Bouma, G. J. Tearney, Massachusetts General Hospital (United States)

Investigating the structure and function of pulmonary alveoli is crucial for understanding the normal and diseased lung. In particular, understanding the dynamic four-dimensional relationship to neighboring alveoli, alveolar ducts and small airways during respiration would be a major advance. However, the intact lung is an inherently difficult organ to image and the peripheral lung has many compounding challenges not limited to its highly scattering micro architecture, large motion artifacts and difficult access through the bronchial tree.

In this study, we investigate the dynamic relationship between alveoli and small airways in an isolated swine lung model using a catheter based high-speed optical frequency domain imaging (OFDI) system. Imaging components include a rapidly swept wavelength 1300nm source providing an A-line scan rate of 62,500Hz, a polarization diverse dual balanced receiver and a sample arm consisting of a 0.8mm fiber optic catheter connected via a high speed rotary pullback system. Whole lungs were excised from normal swine, placed inside a custom-imaging chamber, and ventilated under pressure control. The fiber optic catheter was inserted through the trachea and into the peripheral lung where high-speed (100fps) helical pullbacks were performed at multiple inflation and deflation airway pressures from 5-25cmH2O in 5cmH2O increments. Mean alveolar and small airway dimensions were extracted from OFDI datasets at each airway generation for the length of the pullback (100mm).

For the first time to our knowledge, catheter based OFDI of dynamic alveolar and small airway measurements have been simultaneously made via the bronchial tree during air filled ventilation, providing unique insight into this organ.

7893-32, Session 6

Multigeneration optical frequency domain imaging of the pulmonary airways in patients

M. J. Suter, Massachusetts General Hospital (United States); D. R. Riker, Lahey Clinic (United States); M. Mino-Kenudson, K. A. Gallagher, M. S. Shishkov, J. R. Thiesse-Namati, B. E. Bouma, Massachusetts General Hospital (United States); J. F. Beamus, Lahey Clinic (United States); G. J. Tearney, Massachusetts General Hospital (United States)

Lung cancer accounts for 28% of all cancer deaths in the United States, more than colorectal, breast and prostate cancer combined. Survival is stage dependent, however despite recent efforts to increase early detection and diagnosis, the current 5-year survival rate remains under 15%. Early detection is especially problematic in squamous cell and neuroendocrine cancers that typically arise in the bronchial mucosa, as they are radiographically occult, are heterogeneously distributed, and are therefore not readily detected by standard x-ray based screening or biopsy assessment techniques. The goal of this study was to demonstrate the potential of optical frequency domain imaging (OFDI) for studying airway pathology, and to demonstrate the potential of OFDI for conducting multi-generation volumetric microscopy in vivo. OFDI images of the bronchial mucosa were obtained with a custom in-house developed 2.4 Fr catheter and imaging system that acquired images at a rate of between 25-100 frames per second. Patients undergoing bronchoscopy procedures for the suspicion of central airway cancers were enrolled in the pilot clinical study. The OFDI images revealed features including the epithelium, lamina propria, submucosa, cartilage, alveolar attachments, blood vessels and glands. Volumetric OFDI images were acquired in multi-generation segments up to 5.9 cm in length. Histopathology and OFDI interpretation was in agreement in 86% of cases. This study represents the first demonstration of multi-generation volumetric microscopy of the human airways. The tremendous volume of tissue assessed with OFDI, which is considerably larger than that possible with standard forceps biopsy approaches, suggest that OFDI may be a useful screening and surveillance tool for the detection and assessment of airway cancers and other airway pathology, and for the guidance of interventional procedures.
Visualizing respiratory ciliary motion and mechanosensitivity of ciliated cells using spectral-domain optical coherence tomography

L. Liu, Massachusetts General Hospital (United States); M. Mazur, S. Parker, The Univ. of Alabama at Birmingham (United States); B. E. Bouma, Massachusetts General Hospital (United States); S. M. Rowe, The Univ. of Alabama at Birmingham (United States); G. J. Tearney, Massachusetts General Hospital (United States)

Our understanding of mechanism which regulates the cilia beating has been limited by our inability to capture ciliary motion and interactions between cilia and mucus in intact epithelia and in three dimensions. Current imaging modalities to study the ciliary motion are either destructive (SEM) or lack of anatomical information (video microscopy). We hypothesize that visualizing the cilia motion and mucus transport in intact respiratory epithelia in three dimensions could yield new findings in a variety of important topics such as ciliary beat pattern and function, cell mechanosensitivity and cell-cell communication. We show that: (1) SD-OCT can visualize ciliary motion in individual active epithelial area; (2) SD-OCT measurements are sensitive to ciliary activity and increase as a response to the mechanical stimulus brought about by heavy mucus load.

Resting phase, recovery phase and effective phase in a ciliary beat cycle were clearly recognized. Coupled with knowledge from SEM images in previous studies, ciliary beat pattern in three dimensions was also identified. Cilia were found to respond to heavy mucus load by increasing the beat frequency by up to 50% and recruiting more cilia to propel mucus.

Our results demonstrate SD-OCT can be used to monitor ciliary motion and study mechanosensitivity of the cell in an intact mucociliary system. The future development of a catheter could allow three or four-dimensional imaging (three spatial dimensions and time) in vivo and provide new avenues for improving our understanding of activities of the motile cilia and mechanisms by which they are regulated.

Ex-vivo ultra-high-resolution optical coherence tomography imaging of fine lung structure by use of a high-power Gaussian-like supercontinuum at 0.8-um wavelength

N. Nishizawa, S. Ishida, Nagoya Univ. (Japan); T. Ohta, K. Itoh, Osaka Univ. (Japan); M. Kitatsuji, H. Oshshima, HOYA Corp. (Japan); M. Matsushima, T. Kawabe, Nagoya Univ. (Japan)

Optical coherence tomography (OCT) is an emerging technology for non-invasive cross-sectional imaging of biological tissue and material with um resolution. Recently, non-invasive high resolution cross-sectional imaging is desired for investigation of diseases in lung in the field of pulmonary medicine. So far, a few works have been reported about OCT imaging of lung. Since the lung consists of alveoli separated by thin wall, ultrahigh resolution (UHR) OCT is supposed to be effective for the imaging of fine structure in lung tissue.

In this work, ex vivo cross-sectional imaging of isolated rat lungs was demonstrated using UHR-OCT. A 120 nm-wide, high-power, Gaussian-like supercontinuum (SC) was generated at wavelength of 0.8 um region and it was used as the light source in time domain UHR-OCT. An ultrahigh resolution of 2.1 um in tissue was obtained and the achieved sensitivity was 105 dB.

For the UHR-OCT imaging of trachea, the detailed structures of the tracheal cartilage and tracheal mucosa overlying the cartilage were observed clearly. The epithelium and lamina propria were also distinguishable.

For the imaging of visceral pleura and alveoli, when saline was instilled into the lung, the penetration depth was improved, and clear images of the fine structure of the lung, including alveoli, were observed owing to the index matching effect. The clear images of up to about 4 alveoli were observed below the visceral pleura. The shape of the alveolar septum was clearly observed, and the alveolar sac was clearly visible.

MEMS motor-based common-path endoscopic Fourier-domain OCT

R. Wang, Clemson Univ. (United States); X. Yuan, Nankai Univ. (China); B. Li, Clemson Univ. (United States); R. L. Goodwin, Univ. of South Carolina (United States); R. R. Markwald, Medical Univ. of South Carolina (United States); B. Z. Gao, Clemson Univ. (United States)

In conventional endoscopic OCT, bending of the sample-arm fiber leads to polarization mismatch between the reference and the sample beams, resulting in fading signals and artifacts. Common-path OCT (CPOCT) is able to remove this mismatch as both beams share the same physical path. Typical CPOCT uses a precisely aligned glass plate located near the beam waist in the sample arm to generate the reference beam through surface reflection. The reflection from the second surface of the glass plate must be carefully eliminated to avoid a ghost image. In a recent report of an in-fiber conical-tip endoscopic OCT, the distal end of the optical fiber was chemically etched to form a conical tip that served both as reference mirror and focusing lens. Because this conical-tip configuration requires that the sample is placed very close to the reference mirror, the configuration is not suitable when circumferential scanning is required, especially for large lumens such as the gastrointestinal tract.

A MEMS motor based common-path Fourier-domain OCT for endoscopic imaging is reported, which uses the distal-end surface of the fiber interfaced with an index match oil as the self-aligned reference mirror. The reference beam’s intensity can be easily tuned by altering the index of the match oil to optimize the signal to noise ratio of the system. A GRIN lens is used to focus the sample beam, and a right angle prism mirror, which is mounted on the shaft of a MEMS micromotor, is used to realize a circumferential OCT scan.

An external Michelson interferometer is used to compensate for the optical path difference and mismatch of dispersion due to index match oil and GRIN lens. This arrangement allows arbitrary probe fiber length and provides sufficient space for imaging optics and circumferential scan, and thus is suitable for OCT imaging of lumens of various sizes. Due to this common-path design, the OCT signal is immune to bending or handling of the catheter. This feature makes it suitable for clinic use. With high speed spectrometer, our system could achieve 20,000 lines/s scanning speed. A free space Ti:sapphire laser beam is coupled into a fiber optics and provides a spectrum with 35nm bandwidth centered at 830nm. As a result 7um axial resolution is achieved.
Endoscopic full-field optical coherence tomography system for cellular imaging

A. Latrived, C. A. Boccara, Ecole Superieure de Physique et de Chimie Industrielles (France)

Detecting pathologies such as cancer at an early stage requires an in-situ cellular diagnostic, which is currently provided through excisional biopsy procedures. Alternative solutions are appearing with minimally invasive imaging techniques such as Optical Coherence Tomography.

Here we present an endoscopic cellular imaging system adapted from the Full-Field OCT principle.

Our approach is to couple two different interferometers. A Michelson-type interferometer is placed after the light source and is used for spectral modulation [Teerney et col, 2005]. A Fizeau common-path interferometer is formed at the distal end of the probe [Tatem et col. 2007]. The probe has a fixed focialization depth within the sample, but a variable scan depth set by the processing interferometer. The advantage of this technique is that all moving parts are exterior to the entirely passive probe.

We have developed two different endoscopic probes compatible with our system. The first probe is a 2-mm-diameter rigid endoscope built with graded-refractive-index lenses: it could be used for imaging of oral and gynecological cavities, or during surgical operations. The second probe is a fiber-bundle-based flexible endoscope which is less than 1 mm in diameter, narrow enough for imaging of the respiratory or the digestive tract.

The system achieves an axial resolution of 1.3 µm and a lateral resolution of 3 µm. First images were acquired from a solid phantom made of polyurethane resin and scattering TiO2 particles. We also present images from ex vivo biological samples: Xenopus Laevis tadpole, cancerous and healthy human tissues.

Multimodal imaging for laser-guided treatment of post intubation tracheal stenosis

S. D. Murgu, H. G. Colt, Univ. of California, Irvine (United States); S. Lee, D. Yoon, M. Brenner, Beckman Laser Institute and Medical Clinic (United States)

Background and Objective: Certain therapeutic algorithms for complex post intubation tracheal stenosis include repeated laser assisted dilations prior to surgical resection. Recurrence after such bronchoscopic treatments is common. The purpose of this study was to demonstrate how multimodal bronchoscopic imaging techniques identify in-vivo airway wall changes before and after laser and dilation of tracheal stenosis.

Materials and Methods: High frequency endobronchial ultrasound (EBUS) and time-domain optical coherence tomography (OCT) systems were used to characterize airway wall microstructures in the area of hypertrophic tissue formation before and after treatment of post-intubation tracheal stenoses.

Results: EBUS revealed: 1) a homogeneous layer consistent with hypertrophic tissue overlying a hyperechogenic layer corresponding to tracheal cartilage and 2) a thick homogeneous layer indistinguishable from tracheal posterior membrane. OCT revealed homogeneous light backscattering layer and absence of layered microstructures within hypertrophic tissue. After laser ablation, OCT of charred tissue showed high-backscattering and shadowing artifacts; OCT of non-charred tissue showed a homogeneous, bright light-backscattering layer suggesting acute inflammation. EBUS revealed thinner but persistent hypertrophic tissue overlying the cartilage and unchanged thick posterior hypertrophic tissue. Stricture recurrence was documented on follow up bronchoscopy.

Conclusion: In-vivo findings of residual hypertrophic tissues and inflammation using EBUS and OCT help support current hypotheses for stricture recurrence after bronchoscopic laser-assisted dilation of tracheal stenosis. Further studies are warranted to determine how these technologies might be used to guide laser resection and potentially assist in therapeutic algorithms.
cellular infiltration was observed in both groups. Our objective was to assess the potential of spectroscopy in differentiating these two groups.

Methods: We previously demonstrated that in healthy smokers alveolar elastin backbone and tobacco tar contained in macrophages contribute to the observed signal. Each normalized spectrum was modeled as a linear combination of 3 components:

\[ \text{Sexp}(\lambda) = C_e \cdot \text{Se}(\lambda) + C_t \cdot \text{St}(\lambda) + C_g \cdot \text{SG}(\lambda) \]

where \( C_e, C_t, C_g \) are amplitude coefficients. \( \text{Se}(\lambda) \) and \( \text{St}(\lambda) \) are respectively the normalized elastin and tobacco tar emission spectra measured experimentally and \( \text{SG}(\lambda) \) a gaussian spectrum with tunable width and central wavelength. Levenberg-Marquardt algorithm determined the optimal set of coefficients.

Results: AIP patient autofluorescence spectra can be uniquely modeled by the linear combination of the elastin spectrum (\( C_e = 0.6 \)) and of a gaussian spectrum (central wavelength 550nm, width 40nm) attributed to amiodarone; the tobacco tar spectrum coefficient \( C_t \) is found to be zero. For healthy smoking volunteers, three spectral components are present: the tobacco tar component \( (C_t = 0.55) \), the elastin component \( (C_e = 0.44) \) and a weak gaussian component \( (C_g = 0.10) \) centered at 630nm of still unknown origin.

Conclusion: Spectral analysis is able to distinguish cellular infiltrated images from AIP patients and healthy smoking volunteers. It appears as a powerful complementary tool for FCFM.

7893-40, Session 8

An integrated fluorescence confocal and spectral-domain optical coherence tomography micro-endoscope

H. Makhlof, College of Optical Sciences, The Univ. of Arizona (United States); A. R. Rouse, A. F. Gmitro, The Univ. of Arizona (United States)

Optical biopsies are aimed at providing fast and thorough screening of biological tissues in vivo. Disease diagnosis is based on the morphological structures and biochemical features of tissues that can be sampled in situ with high cellular level image resolution. Some optical screening techniques, such as fluorescence confocal microendoscopy, provide a limited imaging depth due to the shallow penetration depth of visible light. Despite confocal microendoscopy’s high resolution and image quality, morphological changes that occur deeper in the tissue cannot be detected. Other imaging techniques, such as optical coherence tomography (OCT), are able to obtain image data at greater depth into tissue. A combination of fluorescence confocal and OCT into a single instrument capable of rapidly switching between these modalities, has the potential of providing complementary en face confocal images showing the morphologic features of cells within a surface layer, and cross-sectional images OCT showing tissue microarchitecture below the surface. The concept for such a dual system was presented last year, where the optical train of an existing confocal microendoscope was slightly modified into an effective spectral domain OCT system. Progress made on the implementation of this combined dual integrated imaging system is presented. A performance analysis, discussion of the limitations inherent to the use of an imaging fiber bundle, and recent imaging results are presented.

7893-41, Session 8

Multiple-functional endoscopic OCT for bladder and ureter

H. Wang, W. Kang, C. P. Fleming, G. MacLennan, H. Zhu, A. M. Rollins, Case Western Reserve Univ. (United States)

The development of endoscopic OCT is a key step to translate OCT from the bench to the bedside. Circumferential scanning and forward scanning are two main scanning mechanisms adopted by endoscopic OCT to image different organs. In Urology, OCT has been used to image bladder through forward scanning and ureter through circumferential scanning. Developing a single device to image both bladder and ureter will maximize the benefit of OCT in urological clinic. Although it is possible to simply integrate two scanning mechanisms into a single device through separated driving systems, it will be more convenient and advantageous if both scanning mechanisms can share the same driving system. We will demonstrate a multiple functional endoscopic OCT, which is capable of supporting both circumferential scanning and forward scanning. Both scanning mechanisms are driven by a fiber rotary joint. The circumferential scanning can obtain 3D comprehensive urethral image through a helical scanning pattern; the forward scanning can image bladder through a cone scanning pattern. The system has been tested by ex vivo imaging of porcine bladder and ureter. In the clinics, physician will be able to image both organs by simply connecting the corresponding fiber catheter to the platform. We expect that this device can be used as a powerful tool for early urological cancer diagnosis.

7893-42, Poster Session

In-vivo confocal imaging of human epitheliums: which contrast agent to use?

S. El Hallani, C. E. MacAulay, P. M. Lane, The BC Cancer Agency Research Ctr. (Canada)

PURPOSE: To compare the ability of fluorescent intravitral contrast agents to stain the cellular components of normal and tumoral epithelial cells.

DESIGN: Confocal imaging of cultured human cells (in vitro) and human surgical specimen (ex-vivo). METHODS: 5 clinically used intravital dyes, Methylene blue (1%), Toluidine blue (1%), Cresyl violet (1%), Fluorescein sodium (0.2%), and Acriflavine hydrochloride (0.02%), were topically applied onto live epithelial cells of normal (NBEC), dysplastic (HaCaT) and cancer (HTB-58; HeLa; SCC-15) origin. H2B-GFP expressing cells were generated to visualize chromatin in live cells. The labeled cells were imaged using a bench-top confocal microscope. Ex vivo imaging of freshly removed human oral mucosa was used for validation. RESULTS: Cresyl violet resulted in cytoplasmatic staining with high fluorescence yield and the nuclear morphology could be negatively visualized by exclusive cytoplasmatic enrichment. Toluidine blue and methylene blue stained both the nucleus and cytoplasm at first, but they were rapidly pumped out of the nucleus into the cytoplasmatic compartment leaving the nucleoli labeled. Fluorescein sodium showed non specific binding to all intracellular fractions. Acriflavine hydrochloride strongly stained the nucleus and nucleolus whereas the cytoplasm was slightly labeled enabling positive visualization of nucleus morphology. Cresyl violet and acriflavine hydrochloride were selected for ex-vivo validation and showed consistent results. CONCLUSION: Acriflavine hydrochloride and Cresyl violet are the suitable contrast agents for in vivo confocal imaging of human epitheliums.
7893-43, Poster Session

**Development of a combined ultrasound and photo-acoustic endoscopic probe**

W. Wei, Univ. of California, Irvine (United States); X. Li, Q. Zhou, K. K. Shung, The Univ. of Southern California (United States); Z. Chen, Univ. of California, Irvine (United States)

For in vivo medical applications, endoscopy shows great potential for its minimum invasive manner, flexibility and close-up imaging characteristic. A miniaturized imaging probe combining ultrasound and photoacoustic endoscopy has been developed. The free space 532 nm pulse laser beam was coupled into and delivered by a 200-micron-core multimode fiber. A 40 MHz ring shape ultrasound transducer was fabricated to receive pulse echo ultrasound and photoacoustic signals as well. The light-guiding optical fiber, the ring ultrasound transducer and a mirror-based reflection media for both the laser beam and ultrasound signal were integrated into the probe with a final packaged diameter of 2.6 mm. The performance of the probe was tested from imaging a graphite rod. The imaging ability of this dual-modality system was demonstrated by imaging the cross section of a rabbit aorta.
Smart pillow for heart-rate monitoring using a fiber optic sensor

Z. Chen, J. T. Teo, S. H. Ng, H. Yim, A*STAR Institute for Infocomm Research (Singapore)

The common method for real-time long-term heart rate measurement is the use of electrocardiogram (ECG) that requires electrodes with wires or wireless to be attached to the patient. The other method is to use photoplethysmography (PPG) to measure heart rate. Patients will feel uncomfortable by using these two methods for long-term monitoring. Acoustic sensing, Doppler radar sensor and sensor with electromechanical film are also used for heart rate measurement. A fiber optic heart rate sensor based on speckle patterns detection was proposed. The speckle sensing requires highly coherent light source. As for fiber Bragg grating sensor, the system is expensive. In this paper, however, we propose and demonstrate a new fiber optic microbending based heart rate sensor for in-bed non-intrusive monitoring. The key technological advantage of our system is its ability to measure heart rate with no preparation and minimal compliance by the patient. Although various microbending sensors have been explored for physical and chemical parameters detection with good sensitivity, to our knowledge, no publications have been found to use microbending sensor for heart rate monitoring. Heart rate measurement is based on ballistocardiography (BCG), which measures the recoil of human body due to the momentum of the blood that the heart is currently pumping. The light is modulated through microbending effect when heart beating. The sensor pad is embedded inside a commercially available pillow. An algorithm was developed to extract and report heart rate information of bedded patient. Our results agree with results from benchmarking device. We will also report field trial results.

Research on the FBG’s high-temperature sustainability influenced by the doping process

W. Huang, F. Tu, Yangtze Optical Fibre and Cable Co., Ltd. (China)

The numerous potential applications of UV-induced fiber Bragg gratings (FBGs) in fiber optic sensing and telecommunications have generated a significant interest in this field in recent years. However, two major factors—the photosensitivity of the fiber in which the grating is written and the thermal stability of the grating—are of prime importance in terms of choosing the most appropriate fiber to use and of the long-term functionality of the grating over a wide range of temperatures. B/Ge-codoped fiber has been reported to give a much higher level of photosensitivity when compared with other fibers, and the technique of hydrogen loading can further enhance this property of the fiber, but the gratings written in these fibers, with or without pre-treatment or post-treatment, are reported to have a much poorer high-temperature stability. Based on the plasma chemical vapor deposition (PCVD) process, the high Ge (Germanium) and Ge/B (Germanium/Boron) co-doped photosensitive fiber were developed. The photosensitive fibers with different doping composition and doping concentration have been studied. Based on the experimental results obtained from studies of several kinds of photosensitive fiber on both the photosensitivity and the temperature sustainability of the FBGs written into them, the so-called cation hopping model has been used to explain, in which the size of the cation responsible for the temperature sustainability.

Using modalmetric fiber optic sensors to monitor the activity of the heart

M. Zyczkowski, Military Univ. of Technology (Poland)

The paper presents the concept of the modalmetric fiber optic sensor system for human psycho-physical activity detection. A fiber optic sensor that utilizes intensity of propagated light to monitor a patient’s vital signs such as respiration cardiac activity, blood pressure and body’s physical movements. The sensor, which is non-invasive, comprises a multimode fiber proximately situated to the patient so that time varying acousto-mechanical signals from the patient are coupled by the singlemode optical fiber to detector. The system can be implemented in embodiments ranging form a low cost in-home to a high end product for in hospital use. We present the laboratory test of comparing their results with the known methods like EKG. Addition, the article describes the work on integrated system to human psychophysiology activity monitoring. That system including a EMFIT, microwave, fiberoptic and capacitive sensors.

Fe:ZnSe laser radiation transmission by hollow waveguide

M. Nemeč, H. Jelínková, J. ?ulc, Czech Technical Univ. in Prague (Czech Republic); M. Miyagi, K. Iwai, H. Takaku, Sendai National College of Technology (Japan); M. Doroshenko, T. Basiev, General Physics Institute (Russian Federation); V. Komar, A. Gerasimenko, Institute for Single Crystals (Ukraine)

A special type of COP/Ag hollow waveguide was used for delivery of 4.3µm laser radiation. This mid-infrared radiation having major significance in special lidar or spectroscopy applications was generated by new bulk Fe:ZnSe laser working at the room temperature in gain switched regime. The coherent pumping of Fe:ZnSe laser was performed by electro-optically Q-switched Er:YAG laser which wavelength (2.94µm) corresponds to the maximum of Fe:ZnSe absorption peak. The Er:YAG laser energy and pulse-length used was 11mJ and 300ns, respectively. The generated Fe:ZnSe laser output energy was reached 1.1mJ with the pulse-length 300ns. The aim of the presented project was to investigate the transmission possibility of 4.3µm mid-infrared Fe:ZnSe radiation by the COP and silver coated hollow glass waveguide. The inner waveguide diameter was 700µm and length 103cm. Mid-infrared laser radiation was focused into the guidance protector by the CaF2 lens with the focal length 55mm. After the coupling Fe:ZnSe radiation optimization, the maximum transmission of radiation through the waveguide reached 64%. The time evolution of the pulse was not changed by the delivery but space structure is changing essentially which follows from the transmission principle of the hollow waveguide. Bent waveguide transmission was also investigated and 60% was obtained. For the case of contact application the fused silica cap was performed. As conclude the compact delivery system for 4.3µm mid-infrared radiation with the short 300ns pulse length and transmitted power density 0.57MW/cm2 was successfully investigated and it can be used for the applications.
7894-06, Session 1

Real-time optical fiber dosimeter probe
A. Croteau, S. Caron, F. Roy-Moisson, INO (Canada); A. Rink, D. A. Jaffray, Princess Margaret Hospital (Canada); O. Mermut, INO (Canada)

There is a pressing need for a passive fiber-optic dosimeter probe for use in real-time monitoring of radiation dose delivered to clinical radiotherapy patients. A fiber optic probe using radiochromic material has been proposed. Unique advantages for this dosimeter are MR-compatible features, water-equivalent composition, and real-time readout. Various parameters for the plastic optical fibers and reflective ends, and methods of incorporating the radiochromic material onto the optical fiber and reflector into a single probe were investigated. Since the commercial radiochromic film is not appropriate for miniaturization, we designed, and fabricated the probe based on developing a thin film of the radiochromic material on the dielectric mirror through spin coating, and then assembling it onto plastic optical fibre. Thin films 2 - 20µm thick were studied. The absorbance spectra of the optical fiber dosimeter probes were measured before and after irradiation. After irradiation, the absorbance increase in the 585nm and 636nm bands. Measurements of the net optical density vs. time before, during, and after irradiation at a rate of 500cGy/minute to a total dose of 5Gy were performed. Net OD increased from 0.2 to 2.0 for radiochromic thin film thicknesses of 2 to 20µm, respectively. These results demonstrated that our optical fiber dosimeter probes are real-time sensitive to the radiation beam, and that the radiochromic material performed as expected, and as previously reported.

7894-07, Session 2

Sensor performance improvements for high-quality low-cost imaging systems in ophthalmic applications
A. Plaian, P. Griggio, M. D’Aguanno, CENTERVUE SpA (Italy)

Diagnostic imaging in human eye is safe and comfortable for patients when pictures are taken in low light exposure condition. When dealing with CMOS sensors it becomes necessary to adopt techniques to identify and subtract the fixed pattern noise that depends on sensor temperature and exposure. However, these well-known techniques do not fully solve the problem when high image quality is required. We investigated the efficiency of the noise subtraction as function of the sensor temperature in a range between +50°C and -50°C. Experimental set-up is made of a commercial low-cost 5MPixel CMOS sensor mounted on a fundus camera (Centervue SpA, Italy) equipped with a temperature regulation system. The temperature regulation system uses a dual stage Peltier cooler whose first side is in thermal contact with the sensor and the second side is maintained at 0°C by means of a tank full of water and melting ice. One of the main problem to be solved is the condense on the sensor surface for very low temperatures. To overcome the issue we sealed the volume between the sensor surface and the last optical element and used silica gel to absorb the water vapor. We measured the fixed pattern noise as function of the sensor temperature for a typical exposure time of about 20ms requested by the white LED based illumination system of the fundus camera. We also used fixed pattern noise subtraction to evaluate photos taken at different sensor temperatures on a phantom and on voluntary subjects.

7894-08, Session 2

A motion compensated fiber optic confocal microscope based on common-path optical coherent tomography distance sensor
Y. Huang, K. Zhang, C. Lin, J. U. Kang, The Johns Hopkins Univ. (United States)

A motion compensated fiber optic confocal microscope system is demonstrated using a combination of a common-path optical coherence tomography (CP-OCT) distance sensor and a high speed stepper motor. The confocal microscope is based on 0.4 mm diameter fiber bundle terminated with GRIN lenses. Using the peak detection of a 1-D A-scan data of CP-OCT, the distance deviation from the focal plane could be monitored in real-time. Once the distance deviation surpassed a certain threshold, the linear motor was used to drive the confocal microscope probe at a speed related to the deviation to maintain the distance deviation within a predetermined limit. As a primary result, motion compensation up to a frequency of 1 Hz and amplitude of 70 microns was achieved with an average distance error of 4 microns.

7894-09, Session 2

Measurement of complex-mode amplitudes in multimode fibers
B. A. Alvi, A. Israr, S. Azhar, M. Asif, Sir Syed Univ. of Engineering & Technology (Pakistan)

This paper describes the characterization of mode filling of a multimode optical fiber by the measurement of complex mode amplitudes. A simple optical technique involving the launching of a laser beam into the core of a low mode multimode optical fiber, which excites a mixture of normal modes. The mode pattern emerging from the fiber is then analyzed with optical analyzers. The analyzed mode patterns are stored. The problem of the phase retrieval from single intensity measurement has been solved by using a modified version of the Gerchberg-Saxton algorithm. The agreement between the absorbed modes and a reconstructed pattern is satisfactory.

7894-13, Session 2

Spatially resolved probing of biological phantoms by point-radiance spectroscopy
S. Grabtchak, T. Palmer, W. M. Whelan, Univ. of Prince Edward Island (Canada)

Interstitial fiber-optic based strategies for therapy monitoring and assessment rely on detecting treatment-induced changes in a light distribution in biological tissues. We present an optical technique to identify spectrally and spatially specific tissue chromophores in a highly scattering turbid media. Typical optical sensors measure non-directional light intensity (i.e. fluence) and require fiber translation (i.e. 3-5 positions), which is difficult to implement clinically. Point radiance spectroscopy is based on directional light collection (i.e. radiance) at a single point with a side-firing fiber that is rotated up to 360o. A side firing fiber accepts light within a well-defined solid angle thus potentially providing an improved spatial resolution. Experimental measurements were performed using an 800-micron diameter isotropic spherical diffuser coupled to a halogen light source and a 600 micron, ~43 degree cleaved fiber (i.e. radiance detector). The background liquid-based scattering phantom was fabricated using 10% Intralipid (i.e. scattering medium). Light was collected at 1-5 degrees increments through 360 degree segment. Localized scatters and absorbers were introduced into the liquid phantom both on the axis between source and detector and off-axis in a ~1-mm diameter capillary tube. Gold nanoparticles of varying size were used either as preferential scatterers or absorbers. Hemoglobin was also included in the study as one of biologically relevant chromophores. These localized optical inhomogeneities were detectable as angular-resolved variations in the radiance polar plots. Acquired radiance data was also used to determine the extinction coefficient of the absorber (i.e. chromophore). This technique is being investigated as a non-invasive optical modality for prostate cancer monitoring.
Fabrication of silver-coated hollow fiber with an inner diameter of 100 µm or less

K. Iwai, Sendai National College of Technology (Japan); M. Miyagi, Tohoku Univ. (Japan); Y. Shi, Fudan Univ. (China); Y. Matsuura, Tohoku Univ. (Japan)

Extremely flexible hollow fibers with 100-µm-bore size or less were developed for infrared laser delivery. Fabrication process and transmission properties of the ultrathin hollow fiber were discussed. The silver layer was inner-plated by using the conventional silver mirror-plating technique. Concerning the fabrication parameters used up to now for 320-µm-bore-sized fibers, the target flowing rate for plating solutions was 10 ml/min. Parallel bundles of silica capillary were used to increase the cross-sectional area. To achieve the target, bundles with 560 pieces, 1200 pieces, and 9600 pieces were used for the capillary with inner diameters of 100-µm, 75-µm, and 50-µm, respectively. The loss for the 50-µm bore size, 10-cm length silver hollow fiber was 10 dB at the wavelength of 1 µm.

Gas sensing in the human body by diode-laser absorption spectroscopy

M. Lewander, T. Svensson, A. Bruzelius, Lund Univ. (Sweden); S. Lindberg, R. Siemund, K. Svahnberg, Lund Univ. Hospital (Sweden); S. Svahnberg, Lund Univ. (Sweden)

We present a technique, GASMAS (GAs in Scattering Media Absorption Spectroscopy), that enables non-invasive monitoring of free gas inside the human body. It is the fundamental spectroscopic differences between gases (narrow absorption lines) and the tissue (broad absorption features) that make the gas distinguishable, although surrounded by heavily scattering and absorbing tissue. Within the optical window 600 nm to 1400 nm light can penetrate into the tissue and interact with gases like oxygen and water vapor. The unknown travelled path length through gas, a result of the scattering of the light, limits the direct evaluation of gas concentration. However, water vapor information serves as data of the probed gas volume due to saturated condition (100% relative humidity) and known temperature (37°C), and can be used for normalization. The sensing of gases in situ opens up for a variety of application, such as diagnosis of sinusitis or mastoiditis and monitoring of air in neonatal lungs. We will present data from a clinical trial of 40 patients where the GASMAS technique has been correlated to findings of sinus and mastoid disease in CT images. In addition we will provide results from a feasibility study of gas sensing of an in vitro neonatal lung model.

PPG motion artifact handling using a self-mixing interferometric sensor

R. Wijshoff, Technische Univ. Eindhoven (Netherlands); J. Veen, A. M. Van der Lee, Philips Research Nederland B.V. (Netherlands); M. Stijnen, S. Van Tuijl, HemoLab (Netherlands); R. M. Aarts, Technische Univ. Eindhoven (Netherlands)

In the near future photoplethysmograms (PPGs) are expected to be used to measure heart rate and blood oxygenation of ambulant patients. Since PPGs suffer greatly from motion artifacts, their applicability in ambulatory settings is currently limited. To improve motion robustness of photoplethysmography, this research aims at reducing the optical motion artifacts in PPGs caused by sensor movement with respect to the skin. In this research it is hypothesized that optical artifacts in PPGs correlate with sensor displacement with respect to the skin. In this case, a measure of sensor displacement can be used to detect/correct optical artifacts, which is considered to outperform artifact handling methods which do not measure displacement explicitly.

Correlation between sensor displacement and optical artifacts in PPGs is investigated in a laboratory setup containing a flow cell that mimics changes in blood volume underneath a diffuse scattering skin phantom. A laser diode illuminates the pulsatile flow through the cell to obtain a PPG. Actuator controlled sensor movement with respect to the flow cell is accurately determined using self-mixing interferometry (SMI) as measured by the laser diode’s internal photodiode. Using SMI is advantageous, since this self-aligning, compact and cheap method measures relative displacement of the light source itself, rather than displacement at a different sensor site or global sensor movement, as additional optical sensors or accelerometers do. Influence of the skin phantom on the correlation between optical artifacts and sensor displacement is investigated. A signal processing scheme to handle optical artifacts in PPGs is presented.
Simultaneously, the fiber has excellent UV transmission down to 200 nm comparable to standard high OH fibers. Additionally, the UV defect concentrations in this low OH fiber have been reduced significantly, such that the solarization degradation properties are close to UV optimized high OH fibers with high radiation resistance.

First results of spectral performance testing are given using different light sources, including deuterium lamp, tungsten-halogen lamp and laser-driven broadband light source. In addition, the test results evaluating UV solarization are reviewed. Finally, potential applications in the medical and industrial fiber sensing field will be discussed.

7894-19, Session 4

**In-vivo diffuse reflectance spectroscopy detected absolute oxygen saturation and total hemoglobin concentration in malignant and benign oral tumors to compare with molecular markers**

P. Chen, H. Wang, National Yang-Ming Univ. (Taiwan); L. Lee, J. Wang, C. Chang, Y. Wong, Taichung Veterans General Hospital (Taiwan)

Tumor physiological properties play an important role in cancer diagnosis and treatments. Near-infrared diffuse reflectance spectroscopy (DRS) provides several unique measurable parameters for characterization of a wide variety of tissue samples. We accessed tissue physiological properties including total hemoglobin concentration (THC) and oxygen saturation (StO2) using DRS in 22 patients with squamous cell carcinoma (SCC), 6 patients with hyperkeratosis, and 11 patients with hyperplasia. In each patient, 2-3 and 6-9 DRS measurements were performed over adjacent normal and disease sites, respectively. After DRS measurements, ex vivo tissue sections of the disease site were performed for blood vessel (CD 31, CD 34, angiogenesis factor 8), and HIF-1α staining. Hyperkeratosis and hyperplasia are benign tumors that hyperkeratosis is a thickening of the outer layer of the skin and hyperplasia is an abnormal increase in the number of cells in an organ or a tissue with consequent enlargement. We hypothesized higher blood vessel content in SCC than benign tumors. Surprisingly, blood vessels in both SCC and benign tumors were highly stained in all of 3 blood vessel staining methods used. From DRS measurements, the data indicated that higher THC in SCC, hyperkeratosis and hyperplasia sites than their corresponding normal sites. In StO2 measurements, normal, benign, and malignant oral tissues were well oxygenated with no significant difference although there is a trend that StO2 at the disease site decreases gradually from hyperkeratosis to hyperplasia and SCC patients. HIF-1α stains show no difference between SCC and benign tumor sites. Further statistic analysis will be performed to confirm these trends.

7894-20, Session 4

**Modification of a long-period grating based fiber optic for DNA biosensing**

M. Sozzi, A. Cucinotta, R. Corradini, R. Marchelli, Univ. degli Studi di Parma (Italy); M. Konstantaki, S. Pissadakis, Foundation for Research and Technology–Hellas (Greece); S. Selleri, Univ. degli Studi di Parma (Italy)

Long period fiber gratings (LPFGs) are devices that can be exploited for several sensing applications, while exhibiting great potential in the detection of biomolecules and different chemical agents. In fact, the presence of a layer of biomolecules involves a change in the refractive index and/or loss of the medium surrounding the LPFG and a consequent shift of the grating resonance wavelength, which can be exploited to perform an accurate detection, without the need of labeling. Microstructured fibers are very promising platforms for optical sensing. Previous analyses have already shown that it is possible to functionalize and hybridize the surface of microstructured optical fibers.

In the present work a LPFG has been inscribed on a microstructured fiber. Subsequently the surface of the microstructured optical fiber, has been modified with peptide nucleic acid (PNA) probes. DNA molecules, matched with the PNA probes, have subsequently been captured using the fiber modified surface. The feasibility of label-free detection of DNA molecules using LPFGs in microstructured optical fiber will be demonstrated providing evidence of the wavelength shift and the related sensitivity. The possibility to reuse the sensor for multiple measurements will also be discussed.

7894-22, Session 4

**Double-cladding fiber-based detection system for intravascular mapping of fluorescent molecular probes**

R. N. Razansky, M. S. Mueller, N. C. Delilanos, Technische Univ. München (Germany); F. A. Jaffer, Massachusetts General Hospital (United States); A. W. Koch, V. Ntziachristos, Technische Univ. München (Germany)

Early detection of high-risk coronary atherosclerosis remains an unmet clinical challenge. We have previously demonstrated a near-infrared fluorescence catheter system for two-dimensional intravascular detection of fluorescence molecular probes [1]. In this work we improve the system performance by introducing a novel high resolution sensor. The main challenge of the intravascular sensor is to provide a highly focused spot at an application relevant distance on one hand and a highly efficient collection of emitted light on the other.

We suggest employing a double cladding optical fiber (DCF) in combination with focusing optics to provide a sensor with both highly focused excitation light and highly efficient fluorescent light collection. The excitation laser is coupled into the single mode core of DCF and guided through a focusing element and a right angle prism. The resulting side-fired beam exhibits a small spot diameter (50 μm) throughout a distance of up to 2 mm from the sensor. This is the distance determined by an average human coronary artery diameter. At the blood vessel wall, an activatable fluorescence molecular probe is excited in the diseased lesions and light of slightly shifted wavelength will be emitted only in the places of the inflammations, associated with dangerous plaques [2]. The emitted light is collected by the cladding of the DCF, with a large collection angle (NA=0.3). The double-cladding acts as multimodal fiber and guides the collected light to the photo detection elements. The sensor automatically rotates and is being pulled-back, while each scanned point is mapped according to the amount of detected fluorescent emission. The resulting map of fluorescence activity provides a valuable tool to associate the atherosclerotic plaques with the inflammation process. This can help to differentiate the atherosclerotic plaques based on their biological activity and identify the ones that prone to rupture and require more medical attention.


7894-23, Session 4

**Determination of absorber concentration in turbid media free from scattering complications**

C. Fang, Y. Liu, D. Brokil, Univ. of Pittsburgh Medical Ctr. (United States)
The in vivo quantification of absorber concentrations at a consistent tissue depth in biological tissue is important for many biomedical applications, such as monitoring the response of therapeutic agents and quantitative physiology via in vivo assessment of hemoglobin concentration, oxygenation and melanin content. Optical spectroscopy is an established non-invasive technique to quantify various scattering and absorption properties in biological tissue, often implemented by a portable, compact fiber optic probe with well-controlled source-detection fiber separations. However, the determination of absorber concentration and probing depth in turbid media is often complicated by their scattering properties (i.e., scattering coefficient and anisotropy factor). In this work, we present a novel design of a fiber-optic probe for in vivo assessment of the absorber concentration at a constant tissue depth, free from complications of scattering properties. Our probe is optimized by controlling the light delivery and collection geometry with a coupled high-index ball lens. We validated the performance of the fiber-optic probe by both Monte Carlo simulation and experimental studies. Our results show that within a wide range of tissue-relevant scattering coefficient and anisotropy factor, the quantification of absorber concentration remains unaffected. Additionally, its probing depth stays nearly constant in various tissue scattering properties. Our probe shows a great promise for accurate in-vivo quantification of absorber concentration in biological tissue without the distortion of various tissue scattering properties.

7894-24, Session 4

Optimization of radial angular filter arrays for detecting the angular distribution of light
Y. Zhang, F. Vasefi, M. Najimnaini, B. Kaminska, Simon Fraser Univ. (Canada); J. Carson, Lawson Health Research Institute (Canada)

Radial Angular Filter Array (RAFA) is a novel silicon micro-machined optical filter for real-time high resolution measurement of the angular distribution of scattered or diffused photons. It includes a radial-distributed series of micro-channels, facing a focal point which is several millimeters away from the edge of the device. Four RAFA designs have been proposed in this study that enhance the angular resolution, while lessening the complexity of the output coupling. These new RAFA designs solve issues discovered with a prototype device, including signal loss in high angle channels and light leakage beyond the acceptance angle range. Multiple output interface designs have been explored and compared for coupling the RAFA to line cameras and other detectors. Typically, channels in the RAFA are 80 µm deep with the minimum length of 10 mm. The effective channel length and width define the aspect ratio of the channel, which determines the Signal to Noise Ratio (SNR) of the device. The proposed RAFAs are partially coated with a thin Aluminum layer, which helps to increase the light output intensity, particularly from high angle channels. A characterization system was constructed from a collimated He-Ne laser, diffusers or trans-illuminated turbid samples, RAFAs with output coupling accessories, and a linear camera. The test results identified optimized design configurations, including the preferred RAFA aspect ratio, output interface design and other geometrical parameters, and demonstrated that the RAFA could be a useful tool for tissue surface roughness measurements and angularly-resolved tissue imaging.

7894-25, Session 5

Small-volume cavity cell using hollow optical fiber for Raman scattering-based gas detection
Y. Okita, T. Katagiri, Y. Matsuura, Tohoku Univ. (Japan)

The highly sensitive Raman cell based on the hollow optical fiber suitable for the real-time breath analysis is reported. Hollow optical fiber with inner coating of silver is used as a gas cell and a Stokes light collector. A very small cell whose volume is only 0.4 ml or less enables the fast response and real-time measurement of trace gases. In the present report, sensitivity improvement of the cell is examined. To increase the interaction length the cell is arranged in the cavity which consists of a long-pass filter and a high reflective mirror. The excitation laser light is launched into the cavity through the 0.3 mm pinhole formed on the center of the long-pass filter. Stokes light collected in the cell passes through the long-pass filter and is detected by the multichannel Raman spectrometer. The cell in the cavity has more than ten times the sensitivity of the cell without cavity.

7894-27, Session 5

Extended-range multiwavelength all-fiber interferometer
A. Prabhakar, A. A. Kulkarni, S. Bhattacharya, Indian Institute of Technology Madras (India)

Interferometers are often used to determine the thickness of biological samples. The all fiber interferometer (AFI) discussed in this paper is a wavelength multiplexed Michelson’s interferometer; one wavelength being used for stabilization against error sources and the other one for measurements. In addition, the use of a mutli-wavelength fibre ring laser allows us to extend the measurement range of the instrument. The paper discusses the design procedure for a low cost, compact AFI and its associated electronic control. We present a systematic way to estimate the effect of different error sources on the measurements and hence to calibrate the system with respect to the maximum error and the resolution. The interferometer was built into two stages. The first stage used one wavelength (1546 nm) to measure samples with step heights in hundreds of nanometers, with improvements in accuracy through averaging of data. The next stage included two measurement wavelengths (1546 nm and 1549.3 nm) to extend the measurement range to a few hundreds of micrometers. We verified the successful working of the instrument by measuring a height of 423 nm for a 420 nm structure in the first stage and a height of 95 nm for a 100 µm structure in the second stage. Since the electronics permits us to collect as many as 3000 samples each second, we can use least square minimization techniques to determine the phase delay between the two arms of the interferometer. This offers several improvements over the more common three phase measurement algorithm.
On-chip integrated lensless fluorescence microscopy/spectroscopy module for cell-based sensors

W. Li, A. Sossalla, T. Knoll, H. Büth, H. Thielecke, Fraunhofer-Institut für Biomedizinische Technik (Germany)

The integration of a fluorescence microscopy/spectroscopy module in cell-based lab-on-a-chip systems is of high interest for applications in cell-based diagnostics and substance evaluation in situ. In this study, we present an on-chip integrated lensless fluorescence imaging module based on the principle of contact/proximate optical lithography. It can be implemented comparable with a 4 × objective microscope for both the morphology and fluorescence imaging of mammalian cells (15-20 µm) down to singe cell level, as well as used as a fluorescence spectrometer testing the concentration of a substance. A white LED and a spectrally tailored blue LED together with a collimating waveguide are used for the illumination or excitation. Biological samples or solutions are sustained and interfaced by a disposable microfluidic chip with a 1 µm thick silicon nitride (Si3N4) substrate onto the surface of a filtered 5 megapixel CMOS image sensor array with 1.75 µm pixel size. The chip system is formed by integrating a MEMS cavity chip with a transparent polymer microfluidic interface supportive of cell suspension and reagents delivery. As for the detector part, the surface of the CMOS image sensor is planarized using SU-8 photoresist spin-coating, and then a commercial grade interference filter (optical density ≥ 5) is coated by Plasma-Ion Assisted Deposition. Parameters involved in this fabrication process that could cause the variation of designed spectral response are discussed and evaluated. The function of the system is characterized and demonstrated by imaging the non-/fluorescent mammalian cells/microspheres as well as identification the concentrations of FITC solutions.

High-power diode lasers as all rounders in medical applications for soft tissue treatment

A. Grütz, J. Meinschien, LIMO Lissotschenko Mikrooptik GmbH (Germany)

Meanwhile, laser applications are part of the daily medical practice. The area of applications ranges from dental applications or surgical, to beauty treatments. The most important advantages of diode lasers which qualified to a status of an all-rounder are the wide wavelength range, many equipped features in combination with compact design, scalable output power and the long lifetime. Starting in the 90th wavelengths of 808nm and 980nm, wavelengths which were pushed from the telecom market, are used for dental or surgical applications. In the meantime, “new” and longer wavelengths are available and bring the different applications in the position to achieve higher treatment speed with lower output power, based on much better absorption coefficient of these wavelengths in water. The result of such laser modules, which combines the well established wavelength 808 and/ or 980nm with one of the new wavelengths 1470nm until 2000nm, is a higher comfort for patients. Furthermore, diode lasers with wavelengths of 1470nm until 2000nm open the door to discuss the possibility to replace other laser technologies like holmium lasers in the urology field.

In this discussion further big advantages of diode lasers are the features like pilot laser, monitor diode, fibre contact switch and exchangeable projection window combined in a compact housing, the basic prerequisite for a cost optimised product. Finally, the long lifetime and the fact that diode lasers are maintenance free provide a good future in medical applications.

Single-fiber optical imaging device using solid etalon

H. J. Ma, S. S. Lee, Y. J. Shin, E. Choi, Chosun Univ. (Korea, Republic of)

We demonstrate optical imaging system, which consists of single fiber as waveguide and non-mechanical scanner. Without conventional scanning process, the proposed system can perform two-dimensional optical imaging with utilizing solid etalon where it works diffractive optical element. Solid etalon stretches broadband spectrum spatially and each spectral components incident on specimen represents different positions. This mapping process can distinguish different position of the specimen through each spectral component without any mechanical scanning. We utilized wavelength sweep light source that means wavelength tuning at fixed repetition rate carry out spectral scanning on the specimen. The combination of solid etalon and swept source provides scanningless optical imaging of the specimen. In addition, the spectrally distinguished position information is conveyed by single optical fiber. Conventional optical imaging system, for example, confocal microscope uses bulk optics to secure large field of view. This cannot be replaced with fiber optics. When we apply the proposed scheme to optical imaging system, single optical fiber is good alternative for large field of view imaging. The field of view of an interesting object is determined by the dispersion performance of the solid etalon. If we add additional diffractive elements after solid etalon, whole field of view can be covered fully. In the experiment, we use wavelength swept source (105-nm broadband source centered 1310 nm). With specially designed etalon, we obtain two-dimensional images of biomedical samples and prove the feasibility of the proposed scheme.

Fiber-based surface plasmon resonance sensor for neural recording from rat brain cortex

S. Kim, H. W. Moon, Seoul National Univ. (Korea, Republic of); J. Lee, Harvard Medical School (United States); K. M. Byun, Kyung Hee Univ. (Korea, Republic of); S. J. Kim, Seoul National Univ. (Korea, Republic of)

In vivo neural recording is a key technique in neuroscience and neural engineering fields. The most common method for the in vivo recording is to extracellularly detect the neural activity using implanted microelectrodes. However, electrical recording is very sensitive to electrical noise and the recorded signals are often deteriorated by the artifacts arising from electrical stimulation. Optical recording can be an alternative circumventing these drawbacks. Among various optical techniques for neural recording, label-free optical recording is minimally invasive and thus suitable for in vivo studies since it is free from any phototoxic dyes. We proposed a fiber-based surface plasmon resonance (SPR) system which permits sensitive and label-free optical detection of neural signals. The shape of the fiber tip was optimized for the detection of neural responses using SPR. The fiber tip was coated by a thin gold layer and implanted to the somatosensory cortex of an anesthetized rat. Following electrical stimulations on a forepaw over the threshold level, evoked neural responses were successfully measured from the rat cortex without artifact noises. Simultaneous electrical recording showed that the optical responses were highly correlated with electrical neural activities. The optical signal was proportional to the stimulation amplitude and the response disappeared in the presence of tetrodotoxin, confirming that the optical signals measured by our system were originated from the neural activity. This study showed that a fiber-based SPR system can be used for intrinsic optical detection of neural activity.
Hollow fiber-based Raman tweezers

T. Katagiri, Y. Morisaki, Y. Matsuura, Tohoku Univ. (Japan)

The optical trapping is useful to suppress random motions of particles when one analyzes a single particle in solutions. Recently, this technique has been combined to Raman spectroscopy and applied for the analysis of bio-particles such as cancer cells, erythrocytes and lymphocytes, micro-organisms and sub-cellular components. Although commonly used Raman tweezers systems consists mainly of laser source, spectrometer and microscope, applications in turbid biological media present significant challenges since it is difficult to achieve a tightly focused laser beam necessary for optical trapping. One of the solutions of this problem is to utilize optical fibers to carry the light to the particle which one wish to analyze. In this report, we describe a fiber-optic Raman tweezers for analysis of bio-particles in turbid biological media. The Raman tweezers consists of the single hollow optical fiber which is suitable for Raman spectroscopy and specially designed SrTiO3 lens. The Raman spectra of polystyrene particles dispersed in NaCl aqueous solution were successfully observed. The new design of lens shape for improvement in axial trapping efficiency is examined.

In-vivo stability and accuracy of oxygenation, total hemoglobin concentration, and reduced scattering coefficient measurements of forearm muscle during artery occlusion using broadband steady state diffuse reflectance spectroscopy

C. Huang, H. Wang, National Yang-Ming Univ. (Taiwan)

Near infrared reflectance spectroscopy (NIRS) is widely used in exercise medicine where the measurement of blood flow and oxygen consumption in skeletal muscle have relevant applications. NIRS can provide total hemoglobin concentration (THC) and oxygen saturation (StO2) that enable the calculation of blood flow and oxygen consumption. Majority of commercially available NIRS are continuous wave (CW). To obtain absolute StO2, non-spatially resolved CW-NIRS needs to assume a constant tissue scattering property; spatially resolved CW-NIRS required a sophisticated computational technique such as diffusion model. We developed a broadband spatially resolved CW-NIRS or diffuse reflectance spectroscopy (DRS) system at a minimal (i.e., two) source-detector separations capable of reconstructed reduced scattering coefficient (μs’), THC, and StO2 simultaneously using diffusion model. We validated our system and analysis algorithm using tissue phantoms of bio-particles such as cancer cells, erythrocytes and lymphocytes, micro-organisms and sub-cellular components. We demonstrate the capability of this system on clinically long term monitoring of absolute μs’, THC, and StO2.

Development of hollow-core fibre beam delivery systems for surgical applications

A. Urich, T. Delmonte, R. R. J. Maier, D. P. Hand, J. D. Shephard, Heriot-Watt Univ. (United Kingdom); J. C. Knight, Univ. of Bath (United Kingdom)

At present there is no truly flexible delivery system for medical lasers (λ = 2.94 µm) which allows surgeons to work unrestricted due to the drawbacks of the state-of-art technologies. Currently, either a relatively inflexible articulated arm or multi-mode fibre, limited to large bend radii, must be used. One proposed solution is the use of novel types of hollow core - band gap optical fibre rather than more traditional large area solid core fibres. In these silica based fibres material absorption and damage limitations are overcome, the photonic band gap structure confines radiation to lower order modes in a smaller diameter fibre resulting in a smaller mechanical bend radius and higher laser power damage threshold. However, there are many practical hurdles that must be overcome before a robust system can be developed. One of the main problems is that as the fibre structure is hollow ingress of dust, vapour, fluids and other contaminants needs to be prevented to ensure safe usage in-vivo. Additionally, such contamination will degrade the damage resistance of the fibre leading to catastrophic failure. The development of a robust and hermetically sealed end cap for the fibre, without adversely affecting beam quality or damage threshold is an essential prerequisite for the safe and efficient use of such fibres in surgery. In this paper we report on progress for implementation of hollow fibres for surgical applications and describe novel methods of sealing different hollow core fibres suitable for a wide wavelength range demonstrating that the use of these superior fibres for guidance at 2.94 micron will be feasible.

Photothermal imaging through coherent infrared bundles

Y. Milstein, M. Tepper, M. Ben-David, Tel Aviv Univ. (Israel); J. Harrington, Rutgers, The State Univ. of New Jersey (United States); I. Gannot, Tel Aviv Univ. (Israel) and Johns Hopkins Univ. (United States)

Thermal imaging is a powerful tool to record skin temperature maps at the surrounding of a lesion. It is non- ionizing thus safe for clinical use. The improvement of thermal cameras technologies in spatial resolution; thermal resolution and size makes this technology attractive for medical application. However the signal collected is non-specific and can relate to inflammation benign or cancerous tumors. It is also a bit slow since temperature reading is done only when reading is stabilized.

Photothermal imaging makes the thermal reading specific and faster. The temperature map collected is related to specific absorption of a select number of wavelengths in the near infrared spectral range. The temperature map is a function of the ratio of different materials within area of interest. In our case, we are looking at the ratio of Oxy and De-Oxy hemoglobin in suspected lesion surrounding. The faster pace is achieved by looking at the temperature rise (first derivative), thus there is no need to reach stability.

An additional powerful tool that we use in this system is a coherent bundle in the mid-IR. This bundle is developed in a parallel project in our laboratory. The bundle is optimized to the 8-12 m spectral range, a range were the thermal camera is sensitive to. It is also the range were body temperatures are expected. The number of elements in the bundle is an optimization diameter size and energy collection need as well as needed resolution.

The theoretical model was tested with and without the bundle on a large set of tissue like phantoms with chemical compounds (Methylene Blue ICG) and that have a similar spectral behavior as oxy and de-oxy hemoglobin. We further tested the system on blood samples mixed with B human blood and the oxygenation level of the blood was determined by adding Sodium dithionite (Sigma Aldrich, Israel) in varying conventions ranging from 0.2 mg/g to 2.7 mg/g blood.

The theory; experiments and results will be discussed as well as the current plan to move to clinical trials on oral lesions.
7894-36, Session 7

Performance of low-mode and single-mode optical fibers for high-peak-power 355-nm laser radiation

G. Hillrichs, R. Wandschneider, Hochschule Merseburg (Germany); K. Klein, C. P. Gonschior, Fachhochschule Giessen-Friedberg (Germany)

For applications of fiber guided pulsed UV-laser radiation in biomedical optics, laser material processing or laser spectroscopy which need of a good beam quality low mode or single mode optical fibers are required. We investigated the fiber properties at 355 nm wavelength with pulse energies up to several µJ, pulse durations shorter than 2 µs, peak powers up to 26 GW/cm² and repetition rates up to 25 kHz. It turns out, that degradation or microstructural damage in the fiber core plays a minor role in long term transmission. Single mode UV laser beam guiding is possible, but the coupling conditions have to be carefully chosen. The upper intensity limit is given by the surface damage threshold. UV beam guiding at high repetition rate, moderate peak power will be compared with that of moderate repetition frequency, high peak power lasers.

7894-37, Session 7

Novel evanescent-field-sensor using selectively excited modes in step-index fibers

K. Klein, C. P. Gonschior, P. Dahal, Fachhochschule Giessen-Friedberg (Germany); G. Hillrichs, Hochschule Merseburg (Germany)

A novel ATR-sensor for medical and analytical applications will be introduced, based on a controlled interaction of the evanescent field with the compounds of interest, surrounding the core and the cladding region without a coating layer.

In previous studies, meridional and skew modes in multimode step-index fibers have been studied in detail, using laser excitation only. Using a special excitation, high-order skew modes could be excited. The most surprising results for these skew modes are: large input angles in comparison to NA and high bending stability.

Combining the selective mode-excitation with new powerful broadband light-sources, the spectral light-guidance of skew modes in different optical fibers has been studied in more details. The results of the optical fiber properties in relationship to the new sensor and light-sources will be shown and discussed. In addition, the ATR-sensor properties and possible applications will be presented.

7894-38, Session 7

Opto-electrophoretic detection of biomolecules using conducting chalcogenide glass sensors

P. Lucas, Z. Yang, K. A. Reynolds, The Univ. of Arizona (United States); M. Anne, B. Bureau, Univ. de Rennes 1 (France)

Novel telluride glasses with high electrical conductivity, wide infrared transparency and good resistance to crystallization are used to design an opto-electrophoretic sensor for detection and identification of hazardous microorganisms. The sensor is based on an attenuated total reflectance element made of Ge-As-Te glass that serves both as an optical sensing zone and an electrode for driving the migration of bio-molecules within the evanescent wave of the sensor. An electric field is applied between the optical element and a counter electrode in order to induce the migration of bio-molecules carrying surface charges. The effect of concentration and applied voltage is tested and the migration effect is shown to be reversible upon switching the electric field. The collected signal is of high quality and can be used to identify different bacterial strain through statistical spectral analysis. This technique therefore provides the ability to detect hazardous microorganism with high specificity and high sensitivity in aqueous environments. This has great potential for online monitoring of water quality.

7894-39, Session 7

Laser beam uniformity and stability using homogenizer based fiber optic launch method: square core fiber delivery

T. E. Lizotte, Hitachi Via Mechanics (USA), Inc. (United States)

Over the years, technological achievements within the laser medical diagnostic, treatment, and therapy markets have led to ever increasing requirements for greater control of critical laser beam parameters. Increased laser power/energy stabilization, temporal and spatial beam shaping and flexible laser beam delivery systems with ergonomic focusing or imaging lens systems are sought by leading medical laser system producers. With medical procedures that utilize laser energy, there is a constant emphasis on reducing adverse effects that come about by the laser itself or its optical system, but even when these variables are well controlled the medical professional will still need to deal with the multivariate nature of the human body. Focusing on the variables that can be controlled, such as accurate placement of the laser beam where it will expose a surface being treated as well as laser beam shape and uniformity is critical to minimizing adverse conditions. This paper covers the use of fiber optic beam delivery as a means of defining the beam shape (intensity/power distribution uniformity) at the target plane as well as the use of fiber delivery as a means to allow more flexible articulation of the laser beam over the surface being treated.

The paper will present a new concept of using a square core fiber beam delivery design utilizing a unique micro lens array (MLA) launch method that improves the overall stability of the system, by minimizing the impact of the laser instability.

7894-40, Session 8

Development of optical fiber head probes for infrared endoscopic medical diagnosis

M. Anne, Univ. de Rennes 1 (France); P. Houizot, Univ de Rennes I (France); B. Bureau, Univ. de Rennes 1 (France); O. Loréal, INSERM (France); V. Monbet, Univ. de Bretagne Sud (France); C. Boussard-Plédel, J. Lucas, Univ. de Rennes 1 (France)

Thanks to the development of chalcogenide glass fiber, transmitting light in the infrared range, the infrared spectroscopy can be carried out in situ by Fiber Evanescent Wave Spectroscopy, so-called FEWS. FEWS has proved to be a powerful technology for the study of biomedical samples, in particular for the detection of foodborne pathogens in the food processing industries and for non invasive diagnosis in the medical domain. Recent important progress in the quality and the design of chalcogenide fibers have enabled to obtain optical fiber with a miniaturized head-probe which can be used for endoscopic measurements. FEWS coupled to chemometric methods, such as PCA and PLS, allowed to differentiate between, for instance, healthy and non healthy tissue on human body biological liquid. Thus, FEWS carried out with chalcogenide optical fiber is becoming an efficient tool for physician.
7894-41, Session 8

Delivery of single-mode and multi-mode therapeutic laser light using a single and dual cladding optical fiber for a scanning fiber endoscope

M. R. Kirshenbaum, E. J. Seibel, Univ. of Washington (United States)

The integration of thermal and photodynamic therapy into a scanning fiber endoscope (SFE) for the purpose of pixel accurate laser therapy during an endoscopic procedure has been examined using two distinct methods: combining R-635nm, G-532nm, B-440nm laser light for imaging with high-power 405nm single-mode or multi-mode laser light for therapy. The single-mode system utilizes a SIFAM fused fiber 405/635nm WDM device to combine single-mode 405nm and 635nm laser light into the core of a single output fiber allowing therapy to be directed at any point in an image. The multi-mode system uses a custom combiner (Lightel Technologies) to guide single-mode RGB light from one fiber into the core of a custom single-mode dual cladding fiber (DCF, Coherent) and places multi-mode 405nm light from a second input optical fiber into the inner cladding of the DCF. This multi-mode system has a higher output power measured at 364-mW compared to the single-mode system measured at 29.2-mW before the addition of combiners. A lens system from the SFE at the distal tip of each therapeutic system was used to focus the light without scanning. The resulting minimum spot sizes were 70.9-microns and 310.8-microns for the single-mode and multi-mode systems respectively, which translates into an optical density around 3 mm. The hollow-fiber bundle enables observation of the surface temperature imaging with high temperature resolution of 1 degree C for tissues produced slightly smaller value due to the compression of soft tissue surface. We believe the new concept is feasible for the non-contact thickness measurement of objects with any thickness and transparency as well as the ability to use wide-field fluorescence imaging with 405nm excitation.

7894-42, Session 8

Hollow waveguides for the transmission of quantum cascade laser (QCL) energy for spectroscopic applications

J. A. Harrington, C. M. Bledt, Rutgers Univ. (United States); J. M. Kriesel, Opto Knowledge Systems, Inc. (United States)

The development and evaluation of hollow glass waveguides (HWGs) for use in Long-Wave Infrared (LWIR) spectroscopy systems is described. The LWIR wavelength region (8 to 12 microns) is useful for detecting trace chemical compounds indicative of weapons of mass destruction (WMD). Fiber optics that can operate in the LWIR region (8 to 12 microns) are useful for detecting trace chemical compounds indicative of weapons of mass destruction (WMD). Fiber optics that can operate in the LWIR region is described. The LWIR wavelength region (8 to 12 microns) is useful for detecting trace chemical compounds indicative of weapons of mass destruction (WMD). Fiber optics that can operate in the LWIR region is described.

7894-43, Session 8

Single-crystal YAG fiber optics for the transmission of high energy laser energy

J. A. Harrington, X. Zhu, B. T. Laustsen, Rutgers Univ. (United States); L. G. DeShazer, Lightwave Energetics LLC (United States)

Single-crystal (SC) YAG fibers have the potential of delivering extremely high laser energies. Sapphire fibers have been the most commonly studied SC fiber and the losses for sapphire fibers have been as low as 0.4 dB/m for a 300-micron core-only fiber at 3 microns. In this study we report on the growth of SC yttrium aluminum garnet Y3Al5O12 (YAG) fibers from undoped SC source rods using the Laser Heated Pedestal Growth (LHPG) technique. The advantage of YAG over sapphire is the slight improvement in IR transmission of YAG. The IR transmission of bulk YAG has been shown to extend to 5-m having an absorption coefficient of 0.6 cm-1. The garnet family of crystals is one of the most commonly used oxide crystal hosts for the lasing ion in high power solid-state lasers, with the most commercially common laser host being YAG. Thus, it is reasonable to assume that YAG fibers will have high laser damage thresholds. The optical losses for 400-micron diameter YAG fibers will be presented. While the current SC YAG fibers do not losses as low as the SC sapphire fibers, they are a good candidate for high energy laser transmission.

7894-44, Session 8

A thickness measurement method for biological samples using lensed-fiber sensors

D. Kim, I. K. Ilev, U.S. Food and Drug Administration (United States); Y. Han, Hanyang Univ. (Korea, Republic of)

To maintain the optical and physical quality of the test material surfaces, the usage of noncontact methods for thickness measurement is essential for some materials such as biological tissues, gel-type layered materials, liquid contained in a shallow dish, etc. The key issue here is to detect the boundary of test samples without contacting their surfaces. In this work, we presented a simple noncontact thickness measurement method using two fiber-optic confocal microscope heads. A novel reference comparison method was adapted to minimize the experimental error inevitable for confocal defocus detection. This method can be used for thickness measurement of objects with any thickness and transparency as well as for refractive index measurement of transparent objects.

We have adapted confocal optical fiber sensors to replace the bulk-optic confocal microscope heads. Focusing lens was fabricated at the tip of single-mode fiber and multi-mode fiber using fiber fusion splicer. By using different parameters for splicing, the size and curvature of the melted tip could be precisely controlled. Different curvatures of the melted tip provided different focal lengths of lensed-fiber sensor. Two lensed-fiber sensors were attached to a mechanical thickness gauge to measure the thickness of soft tissue without contacting their surface. The measurement results from the novel fiber sensor design were compared with the results from mechanical gauges. The results from mechanical gauges produced slightly smaller value due to the compression of soft tissue surface. We believe the new concept is feasible for the non-contact thickness measurement.

7894-45, Session 8

Flexible hollow-fiber bundle for body temperature imaging

Y. Matsuura, K. Naito, Tohoku Univ. (Japan)

A flexible and coherent bundle of hollow optical fibers was fabricated for infrared thermal imaging. For acquisition of thermal images, differences in the transmission efficiency among the fibers were numerically compensated to obtain high temperature resolution of 1 degree C for measuring body temperature. In a lens system with 10-fold magnification and hollow fibers of 320-micron inner diameter, the spatial resolution is around 3 mm. The hollow-fiber bundle enables observation of the surface temperature of inner organs and blood flow of the surfaces when the bundle is introduced into the human body with an endoscope.
K-domain linearization of wavelength swept laser for optical coherence tomography

B. C. Lee, Chungnam National Univ. (Korea, Republic of); T. Eom, Gwangju Institute of Science and Technology (Korea, Republic of); M. Y. Jeon, Chungnam National Univ. (Korea, Republic of)

We propose a new method for k-domain linearization based on fiber Bragg gratings (FBGs) in a wavelength swept source for optical coherence tomography (OCT). The wavelength swept source with a scanning fiber Fabry-Perot tunable filter is constructed on the basis of the conventional ring laser cavity. The five FBGs are used to recalibrate the nonlinear response from the wavelength swept source. We achieve a good quality sample imaging using the k-domain linearization algorithm based on FBGs. The sensitivity at 2 mm is improved more than 10 dB after k-domain linearization.
Miniaturized fiber raster scanner for endoscopy
D. R. Rivera, D. Kobat, C. Xu, Cornell Univ. (United States)

A miniaturized scanning mechanism is a crucial component in the creation of endoscopes for microscopic imaging. Several groups have developed resonant scanners (e.g., spiral or Lissajous scan pattern), but these suffer from limitations in non-uniform spatial coverage and sampling time, in comparison to a raster scanner. Additionally, a resonant scanner lacks the ability to perform line-scan imaging, a crucial capability in measuring a variety of fast, dynamic physiological phenomena (e.g., blood flow, molecular diffusion, etc.). However, current miniaturized raster scanners are limited in terms of their physical dimensions and scan speed. We demonstrate a novel hybrid resonant/non-resonant miniaturized raster scanner, fabricated by mounting a double clad fiber onto two perpendicularly oriented piezo bimorphs. The fiber scanner has a total length of 2.6cm, a width/thickness ≤1mm, achieves >800µm fiber tip deflection for both the resonant and non-resonant axes, and allows for imaging at approximately 4 frames per second (512 lines per frame). An essentially uniform spatial coverage and sampling time can be achieved by utilizing the middle portion (e.g., middle 500 µm) of the resonant scanning range. The small size allows for the fiber scanner to be easily packaged along with miniaturized lenses to form an endoscope for microscopic imaging. We bonded a stiffening fiber alongside the vibrating fiber to break its cylindrical symmetry. Thus, only one vibration mode is excited, generating a purely linear spatial motion. In order to demonstrate the fiber scanner’s imaging capabilities we have taken transmission and fluorescence images, in which the double clad fiber’s inner clad is used for fluorescence collection.

Dynamics of hybrid amoebae proteus ingesting Zoochlorellae studied using fluorescence spectroscopy

The native fluorescence spectra from several hybrids animal-plant composite chemotrophs of amoebae proteus and phototrophs cells including amoeba proteus ingesting Zoochlorellae were measured. The changes of fluorescence spectra with time evolutions were observed. Under the microscopic images of the control, microinjected amoebae proteus and the mean generation time were recorded. Both observations were consistent, which supports a newly proposed evolution model. A new model is proposed for the chemical evolution of life on Earth where first a composite hybrid heterotrophs and autotrophs formed functional cells along with cells in the chemical soup instead of heterotrophs.

Trimodal spectra for high discrimination of benign and malignant prostate tissue
M. Al Salhi, King Saud Univ. (Saudi Arabia); V. Trinka, Thendrel Inc (United States); V. Masilamani, D. Rabah, M. R. Turki, King Saud Univ. (Saudi Arabia)

Cancer of prostate occurs more frequently for older men and for many of them the malignancy is not aggressive. So a non-invasive technique of spectral scaling of virulence of malignancy is our ultimate goal. The first step is spectral discrimination of benign from malignant tumor.

In this paper, we have done fluorescence emission spectrum (FES), Stokes’ shift Spectrum (SSS) and reflectance spectrum (RLS) of excised benign and malignant tumor tissues (N = 15 each).

The tissues were minced, washed five times and dried before loading into the quartz cuvette for spectral analysis. The FES was done with excitation at 325nm only; SSS with Δλ = 70, and Δλ = 0, the latter being equivalent to reflectance spectra.

Of the three modes of spectra, SSS with Δλ =70nm showed the best discrimination. There were four important bands, one at 280nm (due to tryptophan); 320nm (due to elastin & tryptophan); 355 and 385 (due to NADH) and 440nm (due to flavin). From the relative intensities of these bands, three ratios were evaluated. Similarly another two ratios were obtained from reflectance spectra and two more from FES. Thus, there are 7 ratio parameters which represent the relative concentration of tryptophan, elastin, NADH and flavin. A statistical analysis showed that benign and malignant tissues could be classified with accuracy greater than 90%.

This report is only for in vitro analysis; but employing optical fiber, this can be extended to in vivo analysis too, so that benign tumor could be distinguished without surgery.

SUMMARY: In this paper, we have done fluorescence emission spectrum (FES), Stokes’ shift Spectrum (SSS) and reflectance spectrum (RLS) of excised benign and malignant tumor tissues (15 each).

Of the three modes of spectra, SSS showed the best discrimination. There were four important bands, one at 280nm (due to tryptophan); 320nm (elastin); 355 and 385 (NADH) and 440nm (flavin). From the relative intensities of these bands, three ratios were evaluated. Similarly another four ratios were obtained from reflectance spectra and FES. A statistical analysis of these ratios showed that benign and malignant tissues could be classified with accuracy of 90%.

Depth-resolved measurement of blood supply using low-coherence enhanced backscattering spectroscopy (LEBS)
A. J. Radosevich, V. M. Turzhitsky, N. N. Mutyal, J. D. Rogers, V. Backman, Northwestern Univ. (United States)

It is well known that during the development of cancer, an increase in blood supply is seen as the metabolic demands of the diseased tissue increases. While much of the research in this area focuses on increases in blood supply at the site of the neoplasia itself, our group has demonstrated that an increase in mucosal microvascular blood content also occurs in histologically normal-appearing tissue from an organ harboring a cancerous lesion, a hallmark of the field effect of carcinogenesis. Understanding the location and origin of this increase in blood supply is necessary for developing optical cancer prescreening techniques that can optimally target these changes. In order to better understand these changes, we acquired depth-resolved measurements of the optical absorption profile of hemoglobin using Low-coherence Enhanced Backscattering Spectroscopy (LEBS). LEBS is a novel self-interference interference phenomenon that uses partial spatial coherence illumination to measure the reflectance profile of backscattered light at length scales smaller than the transport mean free path (λt). As such, LEBS can be used to interrogate the optical scattering and absorption properties of light that has traveled only through the superficial depths of tissue where epithelial cancers originate. Using a post-processing reconstruction algorithm, we isolate the hemoglobin absorption signal from within a continuous distribution of different coherence volumes using a single spectroscopic LEBS measurement. The reconstruction algorithm is first confirmed using a liquid intralipid tissue phantom with different concentrations of hemoglobin and then applied towards measuring the blood supply profile in cancerous tissue specimens.
7895-02, Session 1

Translating ultraviolet autofluorescence microscopy toward clinical endomicroscopy

S. G. Demos, Lawrence Livermore National Lab. (United States); B. Lin, S. Urayama, R. M. G. Saroufeem, D. L. Matthews, UC Davis Medical Ctr. (United States)

Non-invasive autofluorescence endomicroscopy is an emerging application in the medical field that has the potential to reduce sampling error and time delay for real-time pathology diagnosis. We explore the translation of real-time ultraviolet autofluorescence microscopy towards endomicroscopy for in vivo visualization of epithelial tissue microstructure and organization in a clinical setting. Our approach enables short ultraviolet photon penetration depth to optimize autofluorescence acquisition without exponential signal decrease associated with tissue depth. Two prototype endomicroscopy systems were tested, a stand-alone Olympus Medical Systems Corporation flexible clinical prototype and a custom built bench-top rigid fiber conduit prototype. These flexible and rigid systems were used to collect UV AF images of ex vivo murine kidney and human gastrointestinal biopsy specimens, and to further explore design parameters. Both systems entailed compact laser sources operating at 266 nm and/or 325 nm. Preliminary results demonstrate that distinct microstructures of murine kidney and normal human mucosal epithelium of fresh, unprocessed ex vivo specimens were clearly visible using both the flexible and rigid prototypes. These features were immediately familiar to the histology gold standard. Adaptation of this non-contact AF imaging technology can be translated towards in vivo application to address the need for real-time histology and determine the basic design parameters for such instrumentation. This approach could provide a powerful tool for early detection of disease by providing cellular level resolution imaging into an endoscope probe for real-time in vivo histopathology without the use of contrast agents, sectioning methods, or tissue preparation.

7895-04, Session 1

OCT-based system for breast biopsy guidance

N. V. Iftimia, M. Mujat, A. J. Hicks, R. D. Ferguson, D. X. Hammer, Physical Sciences Inc. (United States)

We present the development and preliminary animal testing of a breast biopsy guidance system based on optical coherence tomography (OCT). The OCT signal is analyzed and the information about the tissue-type present at the needle tip is conveyed in real-time to biopsy physician. Preliminary data suggest that this technology could help to increase the diagnostic yield of current fine and core needle biopsy procedures. The detailed description of the instrument and its preliminary testing on animal models of breast cancer will be discussed.

7895-05, Session 1

Quantification of the optical properties and hemoglobin of tissue phantom using a hyperspectral imaging based system

C. Chen, T. Tseng, K. Sung, National Taiwan Univ. (Taiwan)

We present a method for the analysis of diffuse reflectance spectra obtained by using a home-made hyperspectral imaging system. The goal is to quantify the scattering coefficient, oxygen saturation and hemoglobin concentration in vivo. Spatially-resolved reflectance spectra of tissue phantoms were measured through optical fibers by using a hyperspectral imaging system based on Fourier transform spectrometry. A scaling Monte Carlo method was implemented to speed up forward simulations of reflectance spectra. Inverse modeling was performed with the Levenberg-Marquardt algorithm to estimate the absorption and reduced scattering coefficients from the measured spectra. We combined wavelength dependent and spatially resolved information to extract optical coefficients of a two-layer tissue model. Using simulated reflectance spectral data, we demonstrated that this method is more accurate in quantifying the optical coefficients than using only the wavelength dependent information from a single detection channel. The average errors in predicting the absorption and reduced scattering coefficients of the bottom layer were 5.35% and 1.89%, respectively. We also found that using more wavelengths resulted in higher accuracy. Our method has been validated on solid tissue-simulating phantoms which are made of agarose, human hemoglobin and polystyrene microspheres. We used a PDMS container for easy fabrication of two-layer phantoms and control of the thickness of the upper layer. The average errors in predicting the hemoglobin concentration and oxygen saturation of the bottom tissue-phantom layer were 0.27% and 8.59%, respectively. The average errors in quantifying the reduced scattering coefficient's proportional constant and power term were -10.09% and -12.71%, respectively.

7895-06, Session 1

Feasibility of minimally invasive fiber-based evaluation of chondrodystrophy canine intervertebral discs by light absorption and scattering spectroscopy

Y. Jiang, K. L. McKeirnan, K. E. Bartels, D. Piao, Oklahoma State Univ. (United States)

The chondrodystrophy intervertebral disc is a common, frequently debilitating and painful disease in the dog. A similar condition of intervertebral disc degeneration is also common to human patients. The chondrodystrophic intervertebral disc is associated with loss of water content, increase of collagen, and deposit of calcified mineral in the nucleus pulposus in a disc that is relatively avascular. Current diagnostic methods have many limitations for providing accurate information in situ prior to surgical intervention. The study investigates the feasibility of using fiber-needle sensor to analyze the chemical compositions involved in the chondrodystrophic condition of the canine intervertebral disc. The nucleus pulposus, wherein the degeneration develops, is approximately 2mm in thickness and 5mm in diameter. It lies in the center of the disc, surrounded by the annulus fibrosus and is enclosed by the vertebræ on cranial and caudal sides. This “shallow-and-small-slab” geometry limits the configuration of a fiber probe to sense the disc tissue volume without interference from the vertebræ. A single-fiber sensor is built into a 20 gauge myelographic spinal needle for inserting into the disc in situ and connecting via a bifurcated fiber to the light source and a spectrometer. A tungsten light source and a 940nm light-emitting-diode are combined for spectral illumination covering VIS/NIR with improved sensitivity to water. The reflectance spectra are processed to estimate the scattering and absorption compositions. This fiber-needle based sensing configuration may be feasible for integrating the evaluation of water content/ calcification into the holmium:YAG laser-ablation work-flow for in-line detection and monitoring.

7895-07, Session 2

Reflectance confocal microscopy of shave biopsy wounds in human skin: feasibility of intra-operative mapping of tumor margins

A. Scope, D. S. Gareau, K. S. Nehal, M. Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Reflectance confocal microscopy (RCM) images human skin with nuclear-level resolution and detects basal cell carcinomas (BCCs) in vivo with sensitivity of 92% and specificity of 97%. Here, we report the feasibility of RCM for imaging residual BCCs in shave biopsy wounds, using aluminum chloride as a contrast agent. The residual wound following a
shave biopsy is an excellent test model for the surgically exposed tissue conditions to be expected during surgery. Moreover, aluminum chloride of concentrations 20-35% is routinely used for hemostasis, following skin excisions. In a study on surgically excised skin tissue ex-vivo, staining with aluminium chloride was optimized in terms of concentration versus application time. Topical application of 35% aluminum chloride for 1 minute was found to brighten nuclear morphology, by increased light backscatter due to compaction of chromatin, and enhance contrast in RCM images. Subsequently, RCM imaging with aluminum chloride staining was tested in 50 patients undergoing shave biopsies. Retrospective comparison to the corresponding pathology revealed three consistent patterns of BCC tumor margins in the images by increasing depths: epidermal margins, peripheral dermal margins and deep dermal margins. BCC tumor nests were identified at peripheral or deep dermal margins, correlating on pathology with aggregates of neoplastic basaloid cells. Atypical cobblestone or honeycomb pattern were identified at the epidermal margins, correlating with a proliferation of atypical keratinocytes extending to biopsy margins. The results demonstrate the future possibility of RCM imaging for intra-operative mapping of tumor margins to potentially guide surgery.

7895-08, Session 2

Intra-operative ex-situ and in-situ cellular optical tomography

A. Latrive, A. Burcheri, Ecole Supérieure de Physique et de Chimie Industrielles (France); B. de Poly, F. Harms, LLTECH SAS (France); C. A. Boccara, Ecole Supérieure de Physique et de Chimie Industrielles (France)

During cancer surgery it is of critical importance for both surgeons and pathologists to ensure that a suitable diagnostic has been achieved within a few minutes. For example in the field of breast-conserving surgery a reoperation is unfortunately required for 36% of the patients because of an incomplete diagnostic during the first procedure.

In collaboration with different hospitals in France and the USA we have started a program that uses Light-CT (L-CT) technology, an improvement of Full Field OCT (FFOCT), to reach a better intra operative diagnostic. For this purpose two setups are used:

- For ex-situ imaging a compact Linnik microscope with an internal path length modulation and a refractive index correction has been built and used in particular for breast tumors. The images obtained with a 3-D isotropic resolution of 1 micrometer show a very good correlation with histological slices as large as 1 cm².
- For in-situ imaging we have developed a new endoscopic full field system with a needle probe. Two interferometers are coupled: an external illumination one and an imaging one that uses a 10 cm long, 2 mm in diameter needle probe. The axial sectioning ability and lateral resolution are 1 and 3 micrometers respectively. Such a rigid endoscope allows the imaging of various suspicious areas during operation.

We will show 2-D and 3-D L-CT images of human tissues as well as corresponding histological sections.

We hope that both imaging systems will be helpful diagnostic tools for the pathologists and the surgeons, decreasing patient pain and health costs.

7895-09, Session 2

Multiphoton imaging and quantification of tissue glycation

A. Ghazaryan, J. Tseng, W. Lo, Y. Chen, V. A. Havhannisyan, National Taiwan Univ. (Taiwan); S. Chen, National Cheng Kung Univ. (Taiwan); H. Tan, Chang Gung Memorial Hospital (Taiwan); C. Dong, National Taiwan Univ. (Taiwan)

The age-related complications caused by tissue glycation can lead to renal failure, blindness, nerve damage and vascular disease. The accumulation of advanced glycated end products (AGEs) also leads to diabetes-related organ injury. Recent studies have shown that optical spectroscopy offers one potential avenue of early, non-invasive detection of AGEs in vivo. However, the traditional one-photon technique lacks tissue-specificity in identifying the origin of autofluorescence intensity. The present study utilizes the distinct advantages of multi-photon microscopy to improve the reliability of diagnosis. Specifically, the characteristic features of multi-photon autofluorescence (MPAF) and second harmonic generation (SHG) images as well as MPAF spectra of glycated tissues will be presented. In addition, spectral features of glycated tissues will be used to characterize the extent of tissue glycation. Our study shows that multiphoton imaging is capable of providing qualitative and quantitative information of the extent of tissue glycation and that this approach has potential for monitoring AGE formation in the clinical setting.

7895-10, Session 3

Detection of cervical cancer by fluorescence and Stokes’ shift spectra of blood and urine

V. Masilamani, King Saud Univ. (Saudi Arabia); V. Trinka, Thendrel Inc. (United States); M. Al Salhi, King Saud Univ. (Saudi Arabia); K. Govindaraj, GVH Cancer Hospital (India); A. P. Vijaya Raghavan, King Saud Univ. (Saudi Arabia); R. Rai, Rai Memorial Cancer Hospital (India)

A number of non-invasive techniques are being tried for detection of cancer and among them spectroscopic approach is promising. In this paper we present results of fluorescence emission spectra (FES) and Stokes’ Shift Spectra (SSS) of blood components and urine of subjects consisting of confirmed cervical cancer patients (N = 50) and age adjusted normal controls (N = 50).

5.0ml of intravenous blood and 5.0ml of first voided urine were collected from each subject. The blood was drawn into an EDTA vial and centrifuged and the supernatant plasma was subjected to SSS with an offset, Δλ, of 70 nm; The formed elements containing cellular fraction was lyzed with acetone, centrifuged and the supernatant containing the fluorescent biomolecules was subjected to FES with excitation at 400 nm. The untreated urine sample was carried through FES and SSS as mentioned above.

For SSS of plasma, the ratio between intensities of two bands of tryptophan and NADH (I280/I350) is 4.2 for normal and is 8.5 for cancer samples; that is tryptophan is elevated twice more. For FES of acetone extract of cellular components, the ratio of intensities of bands of two forms of porphyrin (I630/I580) is 2 for normal control and 3 for cancer patients; that is 630nm band is elevated more than 1.5 times for cancer patients. Similarly FES and SSS of urine samples showed twice more elevation of flavin for cancer patients.

A discriminate analysis combining all three parameters showed sensitivity of 80% and specificity of 85% for this technique.

7895-11, Session 3

Time-resolved polarization spectroscopy and near-infrared imaging enhanced by receptor-targeted contrast agents for prostate cancer detection

Y. Pu, W. Wang, R. R. Alfano, The City College of New York (United States); S. Achilefu, Washington Univ. in St. Louis (United States)

Time-resolved polarization of free and tissue cell-bonded contrast agents to cells are investigated in cancerous and normal prostate tissues. The dynamics of polarized fluorescence of the receptor-targeted Cybesin (Cyate) contrast agents, in prostate tissues was studied using
time-resolved spectroscopy. Time-resolved fluorescence polarization spectroscopy of Cybesin (Cytate) in solution, and in cancerous and normal prostate tissues was measured. It was found that more Cybesin (Cytate) was uptaken in the cancerous prostate tissue than those in the normal tissue. The different kinetics in the free and bonded states of Cybesin (Cytate) in cancerous and normal prostate tissue is due to changes of the micro-structures of tissue with the evolution of tumor. The preferential uptake of Cybesin (Cytate) in cancerous tissue was used to image and distinguish cancerous areas from the normal tissue. To investigate rotational dynamics and fluorescence polarization anisotropy of the contrast agents in prostate tissues, an analytical model was applied to extract the rotational times and polarization anisotropies. Higher values of fluorescence polarization anisotropy and longer rotational time for Cybesin (Cytate)-stained cancerous prostate tissue was observed in comparison with the normal tissue. These reflect the change of microstructures of cancerous tissues compared with normal tissues and their different bound affinity with contrast agents.

The results indicate that the use of time-resolved spectroscopy and imaging combined with receptor-targeted contrast agents is a potential clinical tool to study micro-structure changes in tissue, and to detect prostate cancer in early stage.

7895-12, Session 3

**Time-domain diffuse optical spectroscopy up to 1700 nm using an InGaAs/InP single-photon avalanche diode**

I. Bargigia, A. Tosi, A. Farina, A. Bassi, P. Taroni, A. Bahgat Shehata, Politecnico di Milano (Italy); A. Della Frera, Micro Photon Devices S.r.l. (Italy); A. Dalla Mora, F. Zappa, R. Cubeddu, A. Pifferi, Politecnico di Milano (Italy)

Time domain diffuse optical spectroscopy provides basic information on the absorption and scattering properties of biological tissues both ex vivo and in vivo. Industrial applications on diffusive media are also growing (e.g. agricultural produce and food products, wood industry, pharmaceuticals). Few measurements are presented up to 1100 nm, sparing ones at discrete wavelengths beyond 1100 nm, and just one time-domain continuously tunable system operating up to 1400 nm. In this paper we extend the applicability of time-domain broadband diffuse optical spectroscopy up to 1700 nm. For illumination we used a supercontinuum source coupled to an acousto-optics tunable filter, providing 40 MHz light pulses in the 900-2000 nm range. For detection we exploited an InGaAs-InP Single-Photon Avalanche Diode (SPAD) driven by a custom gated electronics with sensitivity in the 900-1700 nm range. Smooth and automated operation of the full system was demonstrated in the 1100-1700 nm range with a spectral resolution of 15 nm, a temporal resolution of 150 ps (FWHM) and a background of 6000 counts/s. Furthermore, a first example of application on the optical characterization of collagen powder in the whole spectral range is given, revealing absorption bands around 1200 and 1500 nm. Besides the extended spectral coverage, the new proposed technology is particularly appealing due to aptness of InGaAsInP SPADs to be engineered in compact devices with no need for external cooling and well suited for clinical or industrial implementation.

7895-13, Session 3

**Autofluorescence image analysis of lesions in the oral cavity**

C. E. MacAulay, C. F. Poh, P. M. Lane, M. Rosin, The BC Cancer Agency Research Ctr. (Canada)

Direct fluorescence visualization is increasingly being used by dentists and other oral specialists to screen for oral cancers and pre cancers. Common reactive lesions (cofounders), such as lichen planus, can sometimes mimic high-risk lesions, which are not common in community settings.

Using 405nm illumination 152 autofluorescence digital images of the oral cavity were acquired from 84 patients. These were divided into two sets of images; 1) 100 Abnormal consisting of 39 SCC/CIS/Severe dysplasias, 12 Moderate dysplasias, 31 mild dysplasias, 1 erythroplakia, 17 leukoplakia and 2) 52 normal confounders consisting of 36 lichen planus/pigmentation/geographic tongue and 16 sites of trauma. For each lesion a clinician experienced in fluorescence visualization traced the lesion boundary. From these 24 digital image features were calculated: the gradient of the colour ratios (R/G, R/B, G/B, G/R, B/R, B/G), colour intensities (R, G, B) and colour Hue across a 100 pixel line normal to the lesion boundary averaged over the boundary (see adjacent figure) as well as the average ratios and hue inside and outside the boundary. These 3 of these features were used classify the lesions with an accuracy of 72%.

7895-14, Session 3

**Use of Mueller polarimetric imaging for the staging of human colon cancer**

A. Pierangelo, Ecole Polytechnique (France); A. Benali, Institut Mutualiste Montsouris (France); M. R. Antonelli, T. Novikova, Ecole Polytechnique (France); P. Validire, B. Gayet, Institut Mutualiste Montsouris (France); A. De Martino, Ecole Polytechnique (France)

Analysis of ex vivo healthy and cancerous human colon tissues was performed using a multispectral imaging Mueller polarimeter. The studied tissues turn out to be almost pure depolarizers, whose depolarizing power is plotted in the polarimetric images. With this imaging technique, the studied samples demonstrate significant contrast between healthy and diseased parts. Moreover, different stages of cancer development can be differentiated. Schematically, in the first stage the cancer is a burgeoning proliferation of tumoral cells at the (intraluminal) surface of the sample. Then the tumor progressively invades the underlying layers (mucosa, submucosa, musculara, serosa), while its thickness typically decreases (ulceration). At the first stage we observe a decrease of the depolarization power at all investigated wavelengths (500 to 700 nm). Then, the response of the deeper ulcerated parts is similar to that of the burgeoning parts in the green while in the red this response is close to that of the healthy parts. This behaviour can be qualitatively understood in terms of light penetration depth, which is smaller in the green than in the red: the former is essentially sensitive to the nature of the top layers, while the latter hardly “sees” them. Finally, when the tumor reaches the serosa, its response becomes more depolarizing than all other tissues in the whole spectral range, certainly as a result of the strong depolarizing power of the serosa. These results pave the way to the development of an optical tool for a quick staging of surgical samples by optical means.

7895-15, Session 3

**Synchronous luminescence spectroscopic characterization of blood elements of normal and patients with cervical cancer**

K. Muthuvelu, Stanley Medical College & Hospital (India); S. Shanmugam, Anna Univ. Chennai (India); D. Koteeswaran, Meenakshi Ammal Dental College & Hospital (India); S. Srinivasan, P. Venkatesan, Tuberculosis Research Ctr. (India); P. Aruna, S. Ganesan, Anna Univ. Chennai (India)

As many metabolic changes in the body will be indirectly reflected in blood, which has many intrinsic fluorophores, native fluorescence spectroscopy of blood and its constituents has emerged as one of the complementary techniques in analytical hematology. In this regard, we analyze the blood formed elements using synchronous fluorescence spectroscopy (SFS). In this study the diagnostic potential of synchronous fluorescence spectroscopy technique for the characterization of
normal and different pathological condition of cervix viz., moderately differentiated squamous cell carcinoma (MDSCC), poorly differentiated squamous cell carcinoma (PDSCC) and well differentiated squamous cell carcinoma (WDSCC). Synchronous fluorescence spectra were measured for 70 abnormal cases and 30 normal subjects. Characteristic, highly resolved peaks and significant spectral differences between normal and abnormal cases were observed. Statistical analysis of normal and abnormal cervical cancer patients were subjected to synchronous luminescence spectroscopy. The synchronous luminescence spectra of formed elements obtained. The synchronous luminescence spectra of formed elements of normal and abnormal cervical cancer patients were subjected to statistical analysis. Synchronous luminescence spectroscopy provides 90% sensitivity and 92.6% specificity.

7895-16, Session 3
A hyperspectral fluorescence lifetime fibre probe spectrometer for use in the study and diagnosis of skin cancer and osteoarthritis
A. J. Thompson, H. B. Manning, Imperial College London (United Kingdom); M. Blydegaard, Lund Univ. (Sweden); S. Coda, G. T. Kennedy, R. Patalay, Imperial College London (United Kingdom); U. Wiertzen-Braemming, Lund Univ. Hospital (Sweden); P. A. De Beule, M. A. Neil, Imperial College London (United Kingdom); S. Andersson-Engels, S. Svanberg, Lund Univ. (Sweden); Y. Itoh, Imperial College London (United Kingdom); N. Bendsoe, Lund Univ. Hospital (Sweden); C. W. Dunsby, Imperial College London (United Kingdom); K. Svanberg, Lund Univ. Hospital (Sweden); P. M. W. French, Imperial College London (United Kingdom)

We present two time-resolved spectrofluorometers (TRS) and a compact multichannel spectrometer (MCS) - combining measurements of spectrally resolved fluorescence and diffuse reflected light - which have been developed for in-vivo diagnostic applications, together with data from studies of skin cancer and osteoarthritis. Both TRS utilize a fibre bundle for the delivery of excitation light and the collection of fluorescence. The fluorescence collected by the fibre bundle is then imaged onto a spectrograph and a 16 channel multianode photomultiplier readout which uses Time Correlated Single Photon Counting (TCSPC) to record fluorescence decays in each spectral channel. In the first TRS, pulsed excitation is provided by a diode laser at 435nm and a frequency-tripled Yb:fibre laser at 355nm. The second TRS offers tunable (visible-infrared) excitation via a supercontinuum source as well as 355nm excitation from a frequency tripled Nd:Vanadate laser. The MCS provides excitation at 355, 375 and 395nm and measures reflection between 400 and 850nm.

The first TRS and the MCS were applied to a skin cancer study at Lund University. 27 lesions on 25 patients were investigated in-vivo before surgical excision of the irradiated region. Preliminary data analysis has shown that there is a statistically significant decrease in the fluorescence lifetime of basal cell carcinomas (BCCs) compared to neighbouring healthy tissue, which is in agreement with previous measurements performed on ex-vivo samples. The second TRS has been used to study autofluorescence signals associated with the onset of osteoarthritis, for which we have detected changes associated with collagen degradation and loss of aggrecan.

7895-17, Session 3
Prostate precancer detection by Stokes shift spectroscopy
E. Jeyasingh, Jamal Mohamed College (India); Y. Pu, W. Wang, G. Tang, C. Liu, R. R. Alfano, The City College of New York (United States)

Stokes Shift Spectroscopy (SSS) has emerged as a promising modality in the discrimination of normal from different pathological prostate tissues. Stokes shift (SS) spectra is measured by simultaneously scanning both the excitation and emission wavelengths while keeping a fixed wavelength interval Δ=20 nm between them. Characteristic, highly resolved peaks and significant spectral differences between normal and different pathological prostate tissues were observed. The SS spectra of normal, hyperplasia and malignant tissues shows the distinct peaks around 300, 345, 440 and 510 nm is attributed to tryptophan, collagen, NADH and flavin respectively. To quantify the spectral differences between normal and different pathological prostate tissues are verified by statistical analysis.

7895-18, Session 4
Optical biopsy of the prostate: can we TRUST (trans-rectal ultrasound-coupled spectral tomography)?
D. Piao, J. Zhen, K. E. Bartels, G. R. Holyoak, J. W. Ritchey, C. L. Ownby, K. Rock, C. F. Bunting, Oklahoma State Univ. (United States); G. Slobodov, The Univ. of Oklahoma Health Sciences Ctr. (United States)

Needle-based core-biopsy to locate prostate cancer relies heavily upon trans-rectal ultrasound (TRUS) imaging guidance. The prevalence of isoechic or nearly invisible prostate cancers on ultrasonography ranges from 25 to 42%. As a result, TRUS is useful and convenient to direct the needle trajectory following a systematic biopsy sampling template rather than to target only the potentially malignant lesion for focal-biopsy. To address this deficiency in prostate cancer detection, a TRUS-coupled spectral tomography (TRUST) approach is being developed to non-invasively resolve the likely optical signatures of prostate malignancy. The approach has evolved from optical measurements at one NIR wavelength to two NIR bands, and recently to three NIR bands. The concept has been evaluated on one normal canine prostate and three dogs with implanted prostate tumor developed as a model. In the initial works of detecting the neoplastic canine prostate the TRUST technology has been demonstrated with providing the following diagnostic outcome: (1) finding the onset of prostate tumor earlier than by using TRUS alone; (2) quantifying changes of blood concentration and the indicated mass-volume of a rapidly growing prostate tumor; (3) differentiating different characteristics of necrotic tumor and cystic lesion; and (4) indicating tumor metastasis to inguinal or pelvic lymph nodes. Despite these encouraging results, intensive technologic development is necessary for translating the approach to clinical practice, wherein the ultimate utility is not possibly to eliminate needle-biopsy but to perform focal-biopsy that is only necessary to confirm the cancer, as well as to monitor and predict treatment responses.

7895-19, Session 4
Pancreatic tumor margin detection by oblique incidence diffuse reflectance spectroscopy
A. Garcia-Uribe, Washington Univ. in St. Louis (United States) and Texas A&M Univ. (United States); C. Chang, J. Zou, Texas A&M Univ. (United States); B. Banerjee, J. Kuczynski, The Univ. of Arizona (United States); L. V. Wang, Washington Univ. in St. Louis (United States)

In surgical treatment of pancreatic cancers, the effectiveness of the procedures largely depends on the ability to completely and precisely remove the malignant tumors. However, the determination of a cancer free margin is difficult and time consuming, with an unmet need for rapid determination of tumor margin at surgery. We present the ex-vivo use of oblique incidence diffuse reflectance spectroscopy (OIRDS) to detect and differentiate normal from neoplastic tissue. A side-view OIRDS probe has been constructed to provide scattering and absorption information of the pancreatic tissue. This technology requires no sample preparation, and does not employ the use of hazardous agents for tumor detection. To reveal the physiological origin of the difference in these optical
signatures, the optical scattering coefficients were extracted along the pancreatic duct with 1-cm spacing. Experimental results show that OIDRS was able to successfully determine the tumor margins based on the higher optical scattering on malignant tissue.

7895-20, Session 4

Three-dimensional localization of objects in tissue using independent component analysis in backscattering scanning optical polarization imaging

Y. Pu, W. Wang, G. Tang, Y. Budansky, R. R. Alfano, The City College of New York (United States); M. Xu, Fairfield Univ. (United States)

Three-dimensional (3D) localization of objects in human prostate and animal tissue using backscattering scanning polarization imaging and independent component analysis (ICA) was demonstrated. Backscattering 2D images of a tissue sample illuminated by a raster scanning laser beam were recorded using a CCD camera to obtain multiple angular views of the object embedded inside the tissue medium. The signals from different targets in the sample can be separated by independent component analysis.

The retrieved independent component corresponds to the projection of the Green’s function of light propagating from the object on the boundary of the tissue medium. The difficulty arises in reflection geometry because the incident beam profile and the surface property of the sample affect appreciably the spatial distribution of the backscattering light. Such issues are relative easy in transmission geometry. We address this challenge by numerically marching the propagation of the scattered light from target to the surface until matching the retrieved independent component, incorporating both the beam profile and the surface property of the sample.

Backscattering imaging experiments were carried out on biological tissue with a thickness up to ~7 times larger than the transport mean-free path of the tissue medium. The retrieved 3D location is in good agreement with the position of the embedded object. The approach is applicable to different medium geometries, and can be used with any suitable photon propagation model and be amenable near-real-time imaging application.

7895-21, Session 4

Optical properties of neonatal skin measured in vivo as a function of age and skin pigmentation

N. Bosschaart, R. Mentink, J. H. Kok, T. G. van Leeuwen, M. C. Aalders, Jr., Univ. van Amsterdam (Netherlands)

Light tissue interactions are the basis of many experimental and routinely used therapeutic and diagnostic procedures at the neonatal intensive care - e.g. in transcutaneous bilirubinometry, pulse oximetry, NIRS brain monitoring and phototherapy in jaundiced neonates. Despite the dependency of these techniques on the absorption and scattering coefficients of neonatal skin, very little can be found in literature on these optical properties. In addition, the values for the published optical properties were measured in vitro. It is well known that these values may differ from in vivo measurements due to sample preparation procedures. We will show the first in vivo measurements of the optical properties of neonatal skin, as a function of age and skin pigmentation.

The absorption and reduced scattering coefficients of neonatal skin from 450 - 600 nm were measured using a spatially resolved, steady state diffuse reflectance spectroscopy setup combined with a spatially resolved diffusion model. The optical coefficients were measured on the skin at four different body locations of 47 preterm neonates with varying gestational maturity, age and skin pigmentation. The method was validated on phantoms with known values for the absorption and reduced scattering coefficient.

Our results show an increase of the reduced scattering coefficient with age and an increase of the absorption coefficient with skin pigmentation. From the measured absorption coefficient, we extracted the bilirubin concentration in the skin, which correlated very well with the serum bilirubin concentration ($r = 0.88$).

7895-22, Session 4

Site-dependant redox ratio in healthy oral cavity

S. Shanmugam, Anna Univ. Chennai (India); D. Koteeswaran, Meenakshi Ammal Dental College & Hospital (India); P. Aruna, S. Ganesan, Anna Univ. Chennai (India)

The metabolic coenzymes FAD (Flavin Adenine Dinucleotide) and NADH (Nicotinamide adenine dinucleotide (reduced)) are the primary electron acceptor and donor, respectively, in oxidative phosphorylation. These coenzymes can be monitored nondestructively using their native fluorescence characteristics. These endogenous fluorophores which are present in the cells and tissues gives rise to different emission/excitation fluorescence characteristics between the normal and different diseased conditions. In the recent years, finding the optical redox ratio i.e., the ratio of the fluorescence intensity of FAD and NADH, gives the relative change in the oxidation-reduction state of the cells. Unlike other organs oral cavity has lined with variety of mucosal types and hence there may be different electron chain transport mechanisms at different location of the oral cavity. In this context, it is aimed to investigate the optical redox ratio at four different anatomical locations of oral cavity viz., cheek mucosa, vermilion border of the lip, Hard palate, dorsal side of the tongue of healthy oral cavity, 20 healthy volunteers were subjected in this study and the statistical analysis reveals that there is a considerable variation in the redox state and this may also be considered in the optical biopsy of tissue discrimination.

7895-23, Session 4

Near-infrared pulsed light to guide prostate biopsy

J. Boutet, A. Laidevant, L. Hervé, M. Deboudeau, Commissariat à l’Energie Atomique (France); D. Vray, CREATIS-LRMN INSIA (France); J. Dinten, Commissariat à l’Energie Atomique (France)

The protocol for prostate cancer diagnosis, currently based on ultrasound guided biopsy, is limited by a lack of relevance. To improve this protocol, a new approach was proposed combining optical and ultrasound measurements to guide biopsy specifically to the tumors. Adding an optical measurement modality into an already existing ultrasound probe is challenging as the overall size of the system should not exceed a given dimension so as to fit the operative environment. Moreover, examination should not take more than 15 min to avoid any complication.

A combined ultrasound and optical endorectal probe was designed to comply with the constraints of the sterilization protocols, the examination duration and required compactness. Therefore a totally innovative pulsed laser source has been designed to meet compactness requirements while providing accurate time-resolved measurements. A dedicated multi-channel photon counting system was optimized to decrease the examination duration. A fast reconstruction method based on the analysis of the intensity and time of flight of the detected photons has been associated to provide 3D localization of fluorescent dots almost immediately after acquisition.

The bi-modal probe was capable of withstanding the sterilization procedures. The performance of the compact laser source has been shown at the same level as that of a standard laboratory Ti:Sa laser. The dedicated photon counting solution was capable of acquiring optical data in less than one minute. To evaluate the overall performance of the
system in dealing with a realistic background signal, measurements and reconstructions were conducted on mice bearing ovarian and prostatic tumors.

7895-24, Session 5
Developing a new toolbox for analysis of warrior wound biopsies: vibrational spectroscopy
N. J. Crane, E. A. Elster, Naval Medical Research Ctr. (United States)

The management of modern traumatic war wounds remains a significant challenge for clinicians. This is a reflection of the extensive osseous and soft-tissue damage caused by blasts and high-energy projectiles. The ensuing inflammatory response ultimately dictates the pace of wound healing and tissue regeneration. Consequently, the eventual timing of wound closure or definitive coverage is often subjectively based.

Additional wound complications include wound infection and biofilm formation and heterotopic ossification (the pathological mineralization of soft tissues). Wounds fail despite the use and application of novel wound-specific treatment modalities. An understanding of the molecular environment of acute wounds throughout the debridement process can provide valuable insight into the mechanisms associated with the eventual wound outcome.

Currently, we are examining wound biopsies and wound effluent, and exploring the use of vibrational spectroscopy to answer three clinical questions.

1) Are there bacteria present in the wound (i.e. infection)?
2) Is the wound developing heterotopic ossification?
3) Will the wound heal normally?

We have examined tissue sections of wound biopsies from patients with wounds that exhibited normal healing or impaired healing. In addition, we have explored ex vivo tissue biopsies and wound effluent.

7895-25, Session 5
Monitoring the morphochemistry of skin by multimodal imaging
N. Vogler, I. Latka, C. Krafft, Institut für Photonische Technologien e.V. (Germany); K. Svanberg, N. Bendsoe, Lund Univ. Hospital (Sweden); B. Dietzke, Friedrich-Schiller-Univ. Jena (Germany); J. Popp, Institut für Photonische Technologien e.V. (Germany)

Multimodal nonlinear imaging constitutes a contemporary approach to investigate the morphochemistry of complex samples in a noninvasively and label free. Here we discuss our recent success in jointly using various nonlinear microspectroscopic approaches such as CARS, two-photon fluorescence and second-harmonin generation to study the chemical composition of skin in healthy and diseased tissue. In particular we will show how multimodal nonlinear imaging in combination with linear microspectroscopy, i.e. single-photon fluorescence or Raman scattering can be employed to study the structural and chemical development of disease formation as well as to monitor important aspects of the treatment process.

Dynamic light scattering yields information about the index of refraction changes within the sample and is ideally suited to highlight morphological features. Raman microscopy on the other hand, records a vibrational spectrum at each sample position. Thereby, chemical contrast is obtained, i.e. a chemical image of the sample can be generated. One of the fundamental disadvantages of Raman scattering, its very low scattering cross-section and consequently long image acquisition times, can be overcome by the complementary use of Raman and CARS microscopy. CARS presents a coherent nonlinear variant of Raman microscopy and is used for fast monitoring of large sample areas. Finally, second-harmonic generation selectively highlights the presence of ordered structures, i.e. region of the sample with no inversion symmetry.

In combining the aforementioned microspectroscopic technique we obtain a case study on the morphochemistry of healthy and diseased human skin and present approaches to follow the effect of treatment on a molecular level.

7895-26, Session 5
Objective methods for achieve an early prediction of the effectiveness of regional block anesthesia using thermography and hyperspectral imaging
J. H. Klaessens, M. Landman, R. de Roode, H. J. Noordmans, Univ. Medical Ctr. Utrecht (Netherlands); R. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands)

An objective method to measure the effectiveness of regional anesthesia can contribute to time saving and unintended pain inflicted to the patient. A prospective observational study was performed on 20 patients during a local anesthesia before undergoing hand surgery. Two non-invasive techniques: thermal and oxygenation imaging were applied to observe the region affected by the peripheral block and the results were compared to the standard cold sensation test.

The supraclavicular block was placed under ultrasound guidance around the brachial plexus by injecting 20 cc Ropivacaine. The sedation causes a relaxation of the muscles around the blood vessels resulting in dilatation and hence an increase of blood perfusion, skin temperature and skin oxygenation in the lower arm and hand.

Temperatures were acquired with an IR thermal camera (FLIR ThermoCam SC640). The data were recorded and analyzed with the ThermoCamTM Researcher and Matlab software. Narrow band spectral images were acquired at selected wavelengths with a CCD camera either combined with a Liquid Crystal Tunable Filter (420-730 nm) or a tunable hyper-wavelength LED light source (450-880nm). Concentration changes of oxygenated and deoxygenated hemoglobin in the dermis of the skin were calculated using the modified Lambert Beer equation.

Both imaging methods showed distinct oxygenation and temperature differences at the surface of the skin of the hand with a good correlation to the anesthetized areas. The temperature response was within 5 minutes compared to the standard of 30 minutes. Both non-contact methods show to be more objective and have an earlier prediction for the effectiveness of the anesthetic block.

7895-27, Session 5
Raman scattering by light with orbital angular momentum
G. Milione, The City College of New York (United States)

We present the experimental investigation of Raman scattering in a uniaxial quartz crystal by light possessing orbital angular momentum. The interaction of light’s spin angular momentum in quartz associated with polarization reveals itself through the well-established phenomena of circular dichroism and optical activity. More recently, it has been shown theoretically that there is no interaction between light’s orbital angular momentum associated with the helical phase of optical vortices and molecular chirality, and the experimental investigation of beam’s with both spin and orbital angular momentum revealed no difference in circular dichroism in an enantiomeric solution. In this work, we investigate the interaction of quartz molecules in a uniaxial birefringent quartz crystal with the spin and orbital angular momentum of light through Raman spectroscopy. The scattered radiation of light propagating along the crystal optic axis with varying combinations of spin and orbital angular momentum is measured. The relative intensity differences in the Raman spectra corresponding to the normal vibration along the optic axis are studied. We discuss the possible relationship between the observed scattering intensities and Raman optical activity.
Inactivation of encephalomyocarditis virus and herpes simplex virus by using a visible femtosecond laser

S. D. Tsen, Washington Univ. in St. Louis (United States); K. Tsen, Arizona State Univ. (United States)

Recently, a variety of viral systems, including M13 bacteriophage, tobacco mosaic virus (TMV), human papillomavirus (HPV) and human immunodeficiency virus (HIV) have been shown to be inactivated by the irradiation of a near-infrared subpicosecond fiber laser [1, 2, 3]. These experimental results indicated that the inactivation of viruses by an ultrashort pulsed laser might involve disruption of their protein coat through laser-induced excitation of large-amplitude acoustic vibrations. In this work, we report experimental results on the inactivation of both encephalomyocarditis virus (EMCV) and herpes simplex virus (HSV) by using a visible femtosecond laser derived from the second harmonic generation of a cw mode-locked Ti-sapphire laser system. The inactivation of these viral particles has been demonstrated to depend on the laser exposure time as well as laser power density. Possible mechanisms for the inactivation will be discussed.

References:
Assessment of basic instrumental performance of time-domain optical brain imagers

H. Wabnitz, Physikalisch-Technische Bundesanstalt (Germany); A. Pifferi, A. Torricelli, Politecnico di Milano (Italy); D. R. Taubert, M. Mazurenka, O. Steinkellner, A. Jelzow, Physikalisch-Technische Bundesanstalt (Germany); A. Farina, I. Bargigia, D. Contini, M. Caffini, L. Zucchelli, L. Spinelli, Politecnico di Milano (Italy); P. L. Sawosz, A. Liebert, Institute of Biocybernetics and Biomedical Engineering (Poland); R. Macdonald, Physikalisch-Technische Bundesanstalt (Germany); R. Cubeddu, Politecnico di Milano (Italy)

To facilitate the development of novel approaches and technologies for time-domain optical brain imaging within in the European project “neURON”, the performance of various instruments has to be assessed and compared. This type of instruments relies on picosecond lasers with high repetition rates, fast detectors and time-correlated single photon counting. As a first step of the assessment, a number of basic tests are performed, including the recording of source parameters, of the temporal instrument response function and of the differential nonlinearity of the timing electronics. Specific figures are recorded to assess the stability of the system, crucial to functional imaging measurements. An additional test has been devised to measure the responsibility of the detection system, i.e. the overall efficiency to collect and detect light emerging from tissue. Dedicated solid slab phantoms have been developed and quantitatively spectrally characterized to provide sources of known radiance with nearly Lambertian angular characteristics. Radiance is obtained as a product of the phantom-specific, wavelength-dependent transmittance factor - on the order of 10^-20/(W m^-2 sr) - and the input power of the instrument. The responsibility is given by the ratio of photon count rate detected and input radiance. Exemplary results obtained applying the “basic performance” protocol to a number of instruments will be shown. By simulating typical measurements based on this data, it is possible to prospectively study the impact of differences between instruments on their performance in various applications of brain imaging. This approach greatly supports the design and optimization of time-domain optical brain imagers on a quantitative basis.

Correlations between time-domain NIRS and systemic physiological signals studied for a cognitive task

A. Jelzow, Physikalisch-Technische Bundesanstalt (Germany); I. Tachtsidis, Univ. College London (United Kingdom); E. Kirilina, M. Niessing, Freie Univ. of Berlin (Germany); R. Bruehl, H. Wabnitz, Physikalisch-Technische Bundesanstalt (Germany); A. Heine, Freie Univ. of Berlin (Germany); B. Ittermann, R. Macdonald, Physikalisch-Technische Bundesanstalt (Germany)

Near-infrared spectroscopy (NIRS) is widely applied in functional brain activation studies. It is known that some functional experiments can cause significant systemic changes (e.g. heart rate, blood pressure), which can influence the haemodynamic NIRS signal making its interpretation challenging. To investigate this we employed time-domain NIRS and multimodal systemic monitoring. Fifteen healthy subjects performed a modified continuous word performance task of 34 seconds followed by a 30 seconds rest period. The changes in oxy- and deoxymoglobin were measured with time-domain NIRS on the frontal lobe (bilateral Brodmann Area 10, two sources and four detectors). The analysis based on moments of time-of-flight distributions of photons yields superficial and depth-selective signals. In addition, a set of systemic parameters, i.e. mean blood pressure, electrocardiogram (ECG), blood volume pulse at the ear, respiration, galvanic skin response, scalp blood flow (flux), and scalp red blood cells (RBC) concentration changes, were recorded simultaneously. Cerebral oxygenation changes were localized by subsequent fMRI runs using the same paradigm. We analyzed time series, individual block averages and grand averages of systemic and optical data. Temporal behavior and mutual correlations were investigated. Mean blood pressure and heart rate show significant increases during the activation period. Relative flux and RBC concentration changes showed a heterogeneous response. The correlation of optical and systemic data was found to differ for depth-sensitive and superficial NIRS signals.

Cerebral hemodynamics in acute ischemic stroke patients probed with optical methods

R. C. Mesquita, M. N. Kim, Univ. of Pennsylvania (United States); C. G. Favilla, The Univ. of Pennsylvania Health System (United States); E. M. Buckley, Univ. of Pennsylvania (United States); J. H. Greenberg, J. A. Detre, S. E. Kasner, The Univ. of Pennsylvania Health System (United States); A. G. Yodh, Univ. of Pennsylvania (United States)

Diffuse Correlation Spectroscopy (DCS) and Diffuse Optical Spectroscopy (DOS) are emerging noninvasive diagnostics that have enormous potential for bedside monitoring of brain function in the clinic. With the ability to measure both perfusion and oxygenation, they are particularly useful to monitor the brain state in situations wherein hemodynamics are disrupted, such as in acute ischemic stroke (AIS) patients. In this population, medical interventions are intended to maximize blood perfusion in the affected region in order to salvage as much of the ischemic penumbra as possible. Examples of such interventions are head-of-bed (HOB) positioning - where the angle of the patient’s head-of-bed is manipulated - and induced hypertension - which allow for controlled blood flow change based on pressure changes. In this study we employed DCS and DOS to monitor blood flow and oxygenation in the frontal lobe in 15 AIS patients with unilateral middle cerebral arterial cortical infarction within 72 hours of onset. Measurements were made simultaneously with Transcranial Doppler (TCD) ultrasound, and each contralesional hemisphere served as a control. HOB positioning at 15o resulted in a 6% decrease in CBF ipsilesionally and at 30o resulted in 12% decrease in CBF ipsilesionally, relative to flat HOB. Similarly, a 30o HOB angle resulted in 8% decrease in blood velocity relative to flat HOB in the ipsilesional hemisphere. Although changes in the contralesional hemisphere were found, they were significantly smaller. In general, our results demonstrate the feasibility of both diffuse optical techniques as a potential bedside monitoring tool in a brain diseased population.
Cerebrovascular reactivity (CVR) reflects the compensatory dilatory capacity of cerebral vasculature to a dilatory stimulus and is an important indicator of brain vascular reserve. fMRI has been proven to be an effective imaging technique to obtain the CVR map when the subjects perform CO2 inhalation or the breathing holding task (BHT). However, the traditional data analysis inaccurately models the BOLD using a boxcar function with fixed time delay. We propose a novel way to process the fMRI data obtained during a blocked BHT by using the simultaneously collected near infrared spectroscopy (NIRS) data as regressor. In this concurrent NIRs and fMRI study, 6 healthy subjects performed a blocked BHT (5 breathholds with 20s durations intermitted by 40s of regular breathing). A NIRS probe of two sources and two detectors separated by 3 cm was placed on the right side of prefrontal area of the subjects. The time course of changes in oxy-hemoglobin ([δHbO]) was calculated from NIRs data and shifted in time by various amounts, and resampled to the fMRI acquisition rate. Each shifted time course was used as regressor in FEAT (the analysis tool in FSL). The resulting z-statistic maps were concatenated in time and the maximal value was taken along the time for all the voxels to generate a 3-D CVR map. The new method produces more accurate and thorough CVR maps; moreover, it also enables us to produce a comparable baseline cerebral vascular map if applied to resting state data.

7896-05, Session 1
Quantitative voxel-wise comparison of high-density diffuse optical tomography and fMRI mapping of visual cortex
A. T. Eggebrecht, B. R. White, S. L. Ferradal, A. Z. Snyder, J. P. Culver, Washington Univ. in St. Louis (United States)
High-density diffuse optical tomography (HD-DOT) is an emerging technology for performing non-invasive functional brain mapping at the bedside and in relatively naturalistic environments with high temporal resolution and moderately good spatial resolution. Recent studies have shown that HD-DOT can generate detailed activation maps in cortical regions such as visual cortex and motor cortex. With these promising results, HD-DOT now requires a compelling validation through a voxel-wise comparison with maps generated via functional magnetic resonance imaging (fMRI), the current gold standard of functional neuroimaging. While several studies have compared near infrared spectroscopy/NIRS with fMRI, the focus has been on evaluation of the time courses in the NIRS sensor space and not on image quality. In this study, subject-specific anatomical MRIs are used in subject-specific DOT image reconstructions allowing for direct spatial co-localization of DOT activations with fMRI activations in voxel space. Activation maps were generated with both DOT and fMRI in response to visual stimuli designed to map out the visual cortex. For both modalities strong CNR was obtained throughout the visual field of view and activation patterns moved as expected along the cortical gyri in response to dynamic visual stimuli. Quantitative comparisons of the images in voxels space are used to evaluate the differences in localization between the methods and the depth sensitivity limits of DOT. The implications of these results on the potential of HD-DOT as a neuroimaging tool will be discussed.

7896-06, Session 1
Adaptive cancellation of spontaneous fluctuations in combination with depth compensation algorithm enhances real-time brain imaging in diffuse optical tomography
F. Tian, H. Niu, B. Khan, G. Alexandrakis, K. Bebbehani, H. Liu, The Univ. of Texas at Arlington (United States)
Functional brain imaging with diffuse optical tomography (DOT) is limited by the high measurement sensitivity to the superficial tissues (i.e., the scalp and skull) and severely decreased sensitivity to the deep brain. Significant interference in functional DOT results from spontaneous fluctuations that are embedded in both the superficial tissues and brain, such as arterial pulsation (−0.1–1.2 Hz) and vasomotion (−0.1–1.2 Hz). In this study, first we investigate coherence and phase shift of the spontaneous fluctuations in resting state, within the superficial tissues and at various depths of the brain, respectively. We demonstrate that the spontaneous fluctuations originating from arterial pulsations are spatially global and temporally coherent, while the fluctuations originating from vasomotion tend to lose coherence with increased depth. Second, adaptive cancellation of the spontaneous fluctuations with a frequency-specific strategy is utilized and validated in both resting and activation (evoked by a finger-tapping task) states. Third, improved depth localization of motor activation in reconstructed rDOT images is achieved by combining adaptive cancellation with a depth compensation algorithm that we recently developed.

7896-07, Session 2
Diet-induced alterations in brain microvasculature: a non-invasive, near-infrared spectroscopy in rats
B. Hallacoglu, A. Sassaroli, I. H. Rosenberg, S. Fantini, A. Troen, Tufts Univ. (United States)
Abstract. Brain microvascular alterations are commonly associated with Alzheimer’s disease and other dementia. However, the extent to which microvascular abnormalities cause or contribute to cognitive impairment is unclear. Dietary vascular risk factors, such as deficiencies of vitamins B9 (folate), B12, and B6 are potentially modifiable predictors of cognitive impairment in humans. We have previously reported that a diet deficient in folate, vitamins B12 and B6 caused a 30% decrease in brain microvasculature volume in mice, as well as an impaired performance on tests of learning and memory. Here, we present a folate deficiency study in rats, based on non-invasive near-infrared spectroscopy (NIRS) of the cerebral cortex. We have found that absolute brain hemoglobin concentration ([Hb]) and oxygen saturation (StO2) were significantly lower in folate deficient rats (n=5) with respect to control rats (n=6) (for [Hb]: 73±10 μM vs. 95±14 μM; for StO2: 55%±7% vs. 66% ±4%). These results implicate microvascular rarefaction and diminished oxygen delivery as a mechanism of cognitive impairment in folate deficient rats. We also plan to report the results of an ongoing NIRS study on B12 deficient rats (n=6) and B12 replete rats (n=6). These studies establish initial steps towards improving our understanding of the link between diet, nutritional status, cerebral microvasculature volume, and cognitive function impairment, with the ultimate goal of studying vascular deficiency effects in humans.

7896-08, Session 2
Diffuse optical signals in response to peripheral nerve stimulation reflect skeletal muscle kinematics
M. K. Erb, Boston Univ. (United States); D. K. Chen, A. Sassaroli, S. Fantini, Tufts Univ. (United States); P. R. Bergherson, Boston Univ. (United States)
We have previously reported an optical response in human subjects following electrical stimulation of peripheral nerves. This optical signal has peak amplitude on the order of 0.1% which occurs at approximately 100 ms post stimulus. In the present study, an animal model has been created to directly investigate the contribution of myogenic components to the signal. In addition, further experiments have been performed in human subjects to investigate the signal’s neuroanatomical specificity, sensitivity to muscle motion, and spatial and spectral features. In the Sprague-Dawley rat, non-invasive optical responses are robust during stimulation of the exposed sciatic nerve. They can be abolished both with the delivery of a neuromuscular blocking agent and with surgical
denervation of muscles in the lower limb. In human studies, the signal is elicited on stimulation of mixed nerves (consisting of both sensory and motor fibers), both within and outside the tissue volume probed by the spectrometer. Stimulation of sensory nerves, however, does not elicit an optical response. Spatially resolved measurements and broadband spectral measurements revealed that the signal is characteristic of hemoglobin and probably due to blood vessel displacement. Additionally, the signal is unassociated with capillary bed hemoglobin, and unlikely due to optical coupling changes between optical fibers and skin, or to vascular dilation or constriction. These results suggest the observed optical signal derives from stimulus-induced motion associated with muscle contraction (either by direct muscle stimulation or as an indirect effect of nerve stimulation). Because it likely contains myological information of clinical value, current research is focused on elucidating which biomechanical parameters can be extracted from this signal.

7896-09, Session 2

Time-domain near-infrared spectroscopy monitoring of brain pathophysiology after injury, stroke, and subarachnoid hemorrhage

N. V. Iftitma, Physical Sciences Inc. (United States); J. J. Selb, E. Rosenthal, Massachusetts General Hospital (United States); M. Mujat, R. D. Ferguson, D. X. Hammer, Physical Sciences Inc. (United States)

The knowledge of brain pathology after traumatic head injury, stroke, or subarachnoid hemorrhage (SAH) is necessary for adequate and patient-oriented treatment. While the primary insult, which represents the direct damage, cannot be therapeutically influenced, the secondary damage can be prevented by treatment. Signs of danger for secondary damage include changes in cerebral blood flow (hypo- and hyperperfusion), impairment of cerebrovascular autoregulation, cerebral metabolic dysfunction, and inadequate cerebral oxygenation. Near-infrared spectroscopy seems to be a suitable technology for monitoring such pathologies. We present the development and preliminary testing of a TD NIR system on TBI, stroke, and SAH patients. Preliminary data show the TD NIRS findings correlate well with CT findings and patient health status monitored with NICU sensors. The development of the TD NIRS instrument and its preliminary NICU testing will be discussed.

7896-10, Session 2

Cerebral effects of blood transfusions in neonates with congenital heart defects

E. M. Buckey, Univ. of Pennsylvania (United States); D. Goff, The Children's Hospital of Philadelphia (United States); G. Hedstrom, Children's Hospital of Philadelphia (United States); D. Hance, T. Durduran, M. N. Kim, R. Mesquita, Univ. of Pennsylvania (United States); M. Putt, The Univ. of Pennsylvania Health System (United States); A. G. Yodh, Univ. of Pennsylvania (United States); D. J. Licht, Children's Hospital of Philadelphia (United States)

Approximately 6-8 in 1,000 infants born each year are diagnosed with congenital heart defects (CHD), a third of whom require major surgical repair in the first month of life. In the 12 hours immediately following cardiac surgery, we have monitored cerebral blood flow (CBF) and blood oxygenation changes in 23 CHD neonates using diffuse optical spectroscopy. During this time, we have captured 16 blood transfusions of varying duration and amount. The median (range) duration of transfusion was 24.1 (34.5) minutes and median amount of transfusion was 25.3 (10.7) mL. A hybrid diffuse correlation and diffuse optical spectroscopy (DOS/DCS) device, constructed in our lab, acquired changes in oxy-, deoxy- and total hemoglobin concentrations (ΔHbO2, ΔHb, and ΔTHC) and relative changes in CBF (rCBF) after transfusion. Changes were computed from a 5-minute mean of each parameter immediately before and after transfusion. ΔTHC showed a significant median (range) increase of 9.5 (10.9) uM after transfusion (p=0.002, Wilcoxon signed rank test). Transfusions had mixed effects on rCBF, leading to a non-significant (p=0.21) population averaged change in CBF of 4.5 (24.5) %. Additionally, after dividing results into palliative and corrective CHDs (N=9 and 7, respectively), we observe a significant difference in ΔHbO2 response to transfusion. Patients with corrected CHD show a significantly greater increase in ΔHbO2 than those with a palliative repair of their CHD. Currently we are investigating a piglet model of transfusion in order to have normative data to compare our CHD population results.

7896-11, Session 3

A non-contact time-domain scanning brain imaging system: results of proof of principle tests

M. Mazurenka, A. Jelzow, H. Wabnitz, Physikalisch-Technische Bundesanstalt (Germany); D. Contini, L. Spinelli, A. Pifferi, R. Cubeddu, A. Dalla Mora, A. Tosi, Politecnico di Milano (Italy); F. Zappa, Micro Photon Devices S.r.l. (Italy) and Politecnico di Milano (Italy); R. Macdonald, Physikalisch-Technische Bundesanstalt (Germany)

We report on the development of a scanning non-contact optical brain imager implementing null or small source-detector distance measurements without using optical fibers. The scanning approach potentially allows one to image an area of the head of several cm² with small and adjustable scanning steps, i.e. high density of mapping points. To test the feasibility of the proposed method, an experimental setup for single-point measurements was built and tested. The optical system included a set of image transfer lenses as well as a polarizer, an analyzer, and a polarization splitting cube. The source and detection spots were adjusted to be 1 mm apart. By polarization-selective detection, light reflected directly from optical components as well as from the sample surface was efficiently suppressed. To retrieve signals from deep within tissues, the detection of late photons was achieved by employing a state-of-art fast-gated single-photon avalanche diode (SPAD) that allows to cut off the large amount of early photons. The experimental setup was tested on a liquid phantom using a small black PVC cylinder as a lesion which was translated in X and Z directions to estimate depth sensitivity and lateral spatial resolution of the instrument. The results were compared with simulations of light propagation in the turbid medium and with similar previous experiments performed with a fiber-based system with the same detector. The non-contact approach is shown to have similar depth sensitivity and spatial resolution, and is very promising for the realization of a high density topological NIRS brain imaging system.

7896-12, Session 3

Repeatability of end-expiratory breath hold responses measured with near-infrared spectroscopy

J. Virtanen, Aalto Univ. School of Science and Technology (Finland); T. Noponen, Univ.of Turku (Finland); R. Ilmoniemi, Aalto Univ. School of Science and Technology (Finland)

Near-infrared spectroscopy (NIRS) can be used to assess the cerebrovascular response to CO2 during breath hold. We measured 8 healthy subjects during voluntary end-expiratory breath hold to study inter- and intraindividual variability of deoxy- and oxyhemoglobin (HHb and HbO2, respectively) responses for the scalp and cerebral cortex. 143 breath holds of 27.5 ± 5.0 s (mean duration ± standard deviation (STD)) were analysed. Cortical [HbO2] started to increase 7.5 ± 2.1 s (mean ± STD between subjects) after beginning of breath hold. This increase was in some subjects accompanied by a decrease in [HHb]. Breath hold termination
was often associated with a transient increase in [HbO2] before both [HbR] and [HbO2] returned to baseline levels. [HbO2] started to decrease 11.0 ± 2.4 s after breath hold termination, with a return to baseline occurring 22.5 ± 4.3 s after the termination. In the scalp, [HbR] and [HbO2] changes varied more between subjects.

The mean change in cortical [HbO2] during the breath hold ranged between individuals from 7.6 to 20.9 µM, with intraindividual STD ranging from 4.1 to 8.7 µM. The slope of cortical [HbO2] during the breath hold was found to have better measurement repeatability, with the mean slope ranging from 0.14 to 1.40 µM/s and intraindividual STD from 0.19 to 0.81 µM/s. The mean slope was also a good indicator of an individual’s CO2 tolerance, with Pearson’s linear correlation coefficient between the mean slope and breath hold duration being 0.71. Thus, the cortical [HbO2] slope can be used for quantitative assessment of cerebrovascular response to CO2.

7896-13, Session 3

Monte Carlo based modeling of indocyanine green bolus tracking in the adult human head

J. T. Elliott, M. Diop, K. M. Tichauer, Lawson Health Research Institute (Canada); T. Lee, Robarts Research Institute (Canada); K. St. Lawrence, Lawson Health Research Institute (Canada)

The use of near-infrared spectroscopy (NIRS) is increasingly being investigated in critical care settings to measure cerebral hemodynamics, because of its potential for guiding therapy during the recovery period following brain injury. Cerebral blood flow (CBF) can be quantified by NIRS using indocyanine green (ICG) as an intravascular tracer. However, extracting accurate measurements from complex tissue geometries, such as the human head, is challenging and has hindered the clinical applications of NIRS.

With the development of fast Monte Carlo simulations that can model arbitrary geometries from MRI/CT imaging data, it is now possible to investigate signal contamination from extracerebral layers or other structural peculiarities, such as hematomas, which can confound CBF measurements. Here, we present a theoretical model that utilizes current graphics processing unit based Monte Carlo simulations to simulate the flow of ICG through extra- and intra-cerebral tissues defined by a segmented MRI image of the head. To produce time-dependent changes in tissue absorption coefficients, arterial ICG input functions were convolved with tissue-specific impulse residue functions, which contain information about blood flow, blood volume, and mean transit time. Each time point required on average 11.8 minutes to simulate 200 million photons.

Using the developed model, we assessed the ability of NIRS to recover the pre-defined CBF values in clinically relevant scenarios. For example, the presence of a 10-mm diameter subdural hematoma, centered in the depth-resolved NIRS field-of-view, caused a 13.53%-reduction in the measured CBF. The sensitivity of NIRS to a variety of different ischemic-related pathologies was also investigated.

7896-14, Session 3

Continuous monitoring of absolute cerebral blood flow by combining diffuse correlation spectroscopy with time-resolved near-infrared technology

M. Diop, Lawson Health Research Institute (Canada); T. Lee, Robarts Research Institute (Canada); K. St. Lawrence, Lawson Health Research Institute (Canada)

Continuous monitoring of cerebral blood flow (CBF) at the bedside has the potential to improve neurointensive care management of patients with brain injury, since cerebral ischemia during the recovery period is a major cause of secondary brain injury. Diffuse correlation spectroscopy (DCS) provides the ability to monitor relative flow changes; however, this technique cannot quantify blood flow, which makes it difficult to determine if CBF has fallen below ischemic thresholds. Our group has developed a time-resolved near-infrared (TR-NIR) method for measuring CBF using the indocyanine green (ICG) as a blood flow tracer. To monitor changes in absolute CBF, we propose to use single time-point CBF measurements acquired by TR-NIR to calibrate the DCS signal.

The TR-NIR technique used time-correlated single photon counting technology to record temporal point spread functions, from which can be determined the concentration of ICG in the brain following an intravenous bolus injection of ICG. The DCS system consisted of a long coherence laser (785 nm), an avalanche photodiode detector, and an autocorrelator board. Experiments were conducted using a newborn piglet model, and CBF was altered by changing the arterial CO2 pressure from normocapnia to hypercapnia (40 to 60 mm Hg). Using TR-NIR, CBF in one piglet increased from 36 to 56 ml/min/100g or 55%, which is in excellent agreement with the relative flow change measured by DCS (53%). These preliminary data demonstrate the potential of this hybrid approach for monitoring CBF in critical-care patients.

7896-15, Session 3

Phasor representation of oxy- and deoxy-hemoglobin concentrations at rest and during brain activation

F. Zheng, M. Pierro, A. Sassaroli, S. Fantini, Tufts Univ. (United States)

We introduce a novel phasor representation of cerebral hemodynamic oscillations at a specific frequency (or relatively narrow frequency band) as measured by near-infrared spectroscopy (NIRS). Specifically, we have used the temporal cross-correlation function between oxy-hemoglobin ([HbO]) and deoxy-hemoglobin ([Hb]) concentrations to define a cross-correlation phasor ([R[Hb],[HbO]]) whose amplitude represents the level of correlation between [Hb] and [HbO], and whose phase represents the delay between [Hb] and [HbO] oscillations. We have initially focused our attention on spontaneous low frequency oscillations, typically centered at 0.08-0.10 Hz in the brain of human subjects, and used phasor notation to investigate spatio-temporal relationships of associated cerebral [Hb] and [HbO] oscillations. In our initial measurements, we have found that the phasor [R[Hb],[HbO]] features different phase angles during rest and brain activation conditions, indicating a change in the relative phase of [Hb] and [HbO] low frequency oscillations. The phasor representation of [Hb] and [HbO] oscillations lends itself to physiological interpretations by considering that different hemodynamic processes (say, changes in blood volume, flow velocity, and oxygen consumption), which may affect one or more vascular compartments, contribute to the measured [Hb] and [HbO] phasors. Such contributions may be represented by individual phasor components that result in the measured [Hb] and [HbO] phasors, providing a novel analytical tool to investigate hemodynamics measured with NIRS. We apply this novel analytical tool to the analysis of NIRS data collected on the brain of human subjects during rest and activation conditions, and explore its potential for the study of functional connectivity networks.

7896-16, Session 4

A framework for mapping prior information into NIR spectral tomography

B. W. Pogue, Dartmouth College (United States)

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7896-17, Session 4

Application of spectral derivative data in near-infrared spectroscopic tomography

H. Dehghani, The Univ. of Birmingham (United Kingdom); F. Leblond, B. W. Pogue, Dartmouth College (United States); F. Chauchard, Indatech (France)

Multi-spectral Near Infrared (NIR) tomographic imaging has the potential to provide qualitative and quantitative information about tissue specific function, as well as the subcellular structures scattering light, based upon NIR transmission measurements. The use of the spectral derivative method in Near Infrared optical spectroscopy will be presented, whereby instead of using discrete measurements around several wavelengths, the difference between nearest neighboring spectral measurements are utilized. The proposed technique is shown to be insensitive to the unknown tissue and fibre contact coupling coefficients providing substantially increased accuracy as compared to more conventional techniques. The self-calibrating nature of the spectral derivative technique is shown to increase its robustness in clinical applications, as is demonstrated based on simulated results as well as experimental data. This concept is then extended to spectral derivative image reconstruction algorithms. The results indicate a dramatic improvement in image reconstruction and the elimination of image artifacts often associated with unknown measurement errors such as coupling coefficient and external boundary variations. The underlying theory as to the self-calibrating nature of this technique is presented, together with the algorithms adapted for bulk parameter recovery as well as full tomographic image reconstruction.

7896-18, Session 4

Implementation of the unstructured finite volume approximation to the simplified spherical harmonics equations for modeling light propagation in tissue

L. D. Montejo, H. Kim, A. H. Hielscher, Columbia Univ. (United States)

In this work we implement the simplified spherical harmonics (SPN) approximation to the radiative transfer equation (RTE) for modeling light propagation in biological tissue. Under non-diffuse conditions the SPN approximation (N=1) is known to approximate the RTE more accurately than the diffusion equation (SP1). The goal of this work is to develop an algorithm to solve the light propagation problem using the SPN equations in heterogeneous tissue at minimal computational cost. We formulate the finite volume approximation to the SPN equations with partly reflective boundaries on unstructured grids in three-dimensions, and solve the resulting system of linear equations using the generalized minimal residual (GMRES) method. The SPN equations are solved for N = 1, 3, and 5. The accuracy of the algorithm is verified by comparing solutions from numerical simulations to results from the more accurate, but computationally demanding, light propagation model based on the RTE. The heterogeneous numerical phantoms we employ to verify our algorithm include a disk, a rectangle, a cylinder, and an arbitrarily shaped three-dimensional object. The solution to the boundary source light propagation problem on a circular phantom, with embedded inhomogeneities, discretized by triangular elements (4886 elements, 2572 nodes) is computed in 1.56s, 2.09s, and 3.68s, using the SP1, SP3, and SP5 approximations, respectively.

7896-19, Session 4

Fluorescence-enhanced optical tomography using phase information

Y. Lu, E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

Fluorescence molecular imaging or fluorescence enhanced optical tomography could play an important role in preclinical research and clinical diagnostics. Improvements in spatial resolution and quantification have been investigated in recent years using time-, frequency-, and continuous wave (CW)-resolved techniques. However, because of the added contrast due to fluorescence lifetime, time- and frequency-resolved fluorescence tomography is less impacted by endogenous optical properties than the counterpart in CW mode potentially. In addition, improved reconstruction should be possible with time-dependent measurements when compared with continuous wave (CW) measurements because of the added dimension of time. However, until recently, the information content of the data type has not been thoroughly investigated. In this paper, phase-only fluorescence enhanced optical tomography is demonstrated and evaluated. The approach involves finite element methods representation in which a simple linear relationship between the unknown fluorophore variables and the boundary measurable information is established via the phase information. We use the full information of both amplitude and phase as well as phase-only information obtained from time- and frequency-domain measurements to reconstruct fluorophore distribution. Using synthetic data, we compared amplitude/phase and phase-only-based reconstructions to show that the time cost in phase-only-based reconstruction is remarkably reduced. The reconstructed results show that the tomography with high frequency phase information can significantly improve the reconstruction quality to spatially resolve multiple-fluorophores at increasing depths. The work demonstrates improvements for not only enhanced optical tomography conducted with frequency domain, but also with time-domain measurements.

7896-80, Poster Session

Time-gated near-infrared spectroscopic imaging of brain activation: a simulation proof of concept

G. Diaz-Ayli, F. Nouizi, Univ. de Strasbourg (France); W. Uhring, B. Dubois, Institut d’Électronique du Solide et des Systèmes (France); P. Poulet, Univ. de Strasbourg (France)

3 companies, Photonis, Montena and Telmat, and 2 academic laboratories, University Strasbourg/CNRS, merged their skills to build a spectroscopic imaging device, without any scan nor contact, to study brain activation in normal humans and patients 1. The entire instrument will be assembled in a unique setup. Light emitted by 4 near infrared laser diodes, working sequentially in a picosecond regime, is injected in a four-furcated optical fiber ended with a frontal light distributor to obtain a uniform illumination spot. Back scattered photons are detected by an intensified CCD camera. The photocathode of the micro-channel-plate (MCP) intensifier is powered with electrical pulses of some hundred of picoseconds. The instrument is controlled by an FPGA based module which generates the pulse sequence for laser diodes and, after a programmable delay, for the MCP photocathode, and the trigger of the CCD camera.

A time resolved 3D simulation study, using the Finite Element Method, was performed in order to evaluate the proposed method for brain activation imaging. Results will be presented with special attention on the sensitivity and accuracy for detection of optical absorption changes. The sensitivity is expressed through the contrast to noise ratio and the contrast to physiological clutter ratio as a function of the intensity, depth and volume of the absorption changes. Expected instrumentation performances: light pulse energy, time gate width and detector sensitivity are used and simulation data were calibrated thanks to time-resolved experiments.

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**7896-81, Poster Session**

**Multi-wavelength time-resolved diffuse reflectance measurement carried out on the head of an adult during injection of indocyanine green**

A. Gerega, D. Milej, M. Kacprzak, Institute of Biocybernetics and Biomedical Engineering (Poland); W. Weigl, Medical Univ. of Warsaw (Poland); A. Liebert, Institute of Biocybernetics and Biomedical Engineering (Poland)

Near infrared spectroscopy (NIRS) is a method which can be used to assess deficits of perfusion or oxygenation of the human brain. For assessment of brain perfusion, the NIRS technique can be utilized in combination with intravenously injection of the Indocyanine Green (ICG) revealing high absorption in near-infrared spectral region. Multimwavelength time-resolved laboratory setup based on broadband supercontinuum radiation source, sensitive multimwavelength photodetection and TCSPC electronics was constructed. The laser light was delivered to the surface of the head with the use of an optical fiber and the diffusely reflected photons were transmitted to the detection system with the use of fiber bundle. In this paper we report results of an in-vivo experiment carried out on the head of an adult healthy volunteer. The instrument was applied during intravenous injection of ICG and the distributions of times of flight of photons (DTOFs) were successfully acquired at multiple wavelengths showing inflow and washout of the dye. The analysis of DTOFs have shown that the time-resolved signals detected at longer wavelengths contain large contribution of remitted fluorescence photons. The observed phenomenon can influence the optical signal detected by NIR diffuse reflectance spectroscopy carried out at single laser source wavelength and broad spectrum detection. The results show that the filtration of the fluorescence light may be essential in analysis of inflow and washout of ICG using time-resolved reflectometry.

**7896-82, Poster Session**

**Algorithmic depth compensation improves transverse resolution and quantification in functional diffuse optical tomography**

F. Tian, H. Niu, S. Khadka, Z. Lin, H. Liu, The Univ. of Texas at Arlington (United States)

Accurate depth localization of a regional activation has been a major challenge in functional brain imaging by diffuse optical tomography (DOT). The photon density drops rapidly with increased depth in tissue, for which conventional DOT reconstruction yields a significant error in depth localization. Recently we have developed a depth compensation algorithm to improve the accuracy of depth localization in DOT. In this paper, we first present a depth-compensation-based approach to improve the quantification of absorption perturbation in DOT by defining a spatial prior. Simulative experiments show a recovery rate of 50-60% of actual absorption perturbation can be achieved by using this approach. Next, we present comprehensive analysis on the spatial resolution of three-dimensional (3D) DOT without and with depth compensation. It reveals that the depth compensation algorithm improves the resolution of DOT in transverse direction (parallel to the measurement surface), which is confirmed in tissue-like phantom experiments. At last, we will further discuss how to improve the spatial resolution by combining the depth compensation algorithm with high-density measurement.

**7896-83, Poster Session**

**Quantitative evaluation of systematic imaging error due to uncertainty in tissue background optical properties in high-density diffuse optical tomography of the human brain**

Y. Zhan, The Univ. of Birmingham (United Kingdom); A. T. Eggebrecth, Washington Univ. in St. Louis (United States); H. Dehghani, The Univ. of Birmingham (United Kingdom); J. P. Culver, Washington Univ. in St. Louis (United States)

Diffuse optical tomography (DOT) of human brain is an emerging neuroimaging methodology that allows noninvasive imaging of human brain activity by taking NIR optical measurements using sources and detectors placed on the human scalp. The image reconstruction accuracy depends on multiple factors, such as system design, data collection and calibration, and realistic physical modeling of the human head which has proven to be an important aspect for model based image reconstruction. In magnetic resonance imaging (MRI)-guided DOT where both T1 and T2-weighted head images are available, a three-dimensional (3D) anatomical head model consisting of up to five segmented tissue types, i.e. scalp, skull, cerebrospinal fluid (CSF), white matter and grey matter, can be specified as the underlying physical model used for DOT image reconstruction. With disregard to either misclassification between different tissues, or the assumption that perfect tissue segmentation is possible, uncertainty in the static background optical properties becomes the dominant cause of systematic error in the predicted optical measurement sensitivity and the reconstructed image. In this work we present a quantitative evaluation of measurement sensitivity, image resolution and localization error dependence due to such uncertainty in the static background properties of the various tissue types. Results will be presented using a 3D finite element model (FEM) of an adult head generated from MRI-based tissue segmentation, and up to 4th Nearest Neighbor (NN) continuous-wave measurements in high-density diffuse optical tomography (HD-DOT) setup, demonstrating the effect of such factors on image reconstruction.

**7896-84, Poster Session**

**Influence of non-cortical contributions and probe pressure on measurements of cerebral blood flow using diffuse correlation spectroscopy**

M. N. Kim, R. Mesquita, C. G. Favilla, J. H. Greenberg, J. A. Detre, A. G. Yodh, Univ. of Pennsylvania (United States)

This study aimed to investigate the contribution of non-cortical flow on DCS-CBF measurements of the human brain. Because flow components mostly due to superficial scalp flow are present in the DCS signal (although minimized at larger source-detector separations), we analyzed the influence of pressure on the probe face to account for these contributions. Increasing the pressure on the probe would most likely push away scalp blood flow from the detection area underneath, and allow us to quantify how much effect scalp flow has on the signal when probing deeper tissue.

We used four source-detector separations (0.5, 1.0, 2.5, and 3.0 cm) arranged on the same probe. Baseline data was taken with no pressure on the probe, the subject sitting upright and probe adhered to the forehead with an adhesive pad. Then a head frame was positioned over the probe and tightened. The 0.5 cm signal blood flow index (BFI) dropped -90.9%, while the 1.0 cm BFI dropped -57.2%. In contrast, the larger separations were not significantly affected by pressure: 2.5 cm BFI fell only -1.3%, and the 3.0 cm BFI increased 1.0%.

These results from a test subject suggest that, although DCS signals from large source-detector separations have a non-cortical component, this contribution is negligible and can be further discounted by applying pressure on the optodes. Future work with additional subjects will look...
at relative blood flow during functional interventions and better quantify changes in BFI with units of pressure.

7896-85, Poster Session

Cerebral blood flow monitoring with diffuse correlation spectroscopy to assess autoregulation after fluid percussion brain injury in a piglet model

J. Liang, Univ. of Pennsylvania (United States) and Xi’an Jiaotong Univ. (China); W. Kiessling, The Univ. of Pennsylvania Health System (United States); M. N. Kim, R. C. Mesquita, A. G. Yodh, Univ. of Pennsylvania (United States); W. M. Armstead, The Univ. of Pennsylvania Health System (United States)

Fluid percussion brain injury (FPI) in newborn piglets is a standard model used to simulate conditions following traumatic brain injury (TBI) in infants. We employed diffuse correlation spectroscopy (DCS) to monitor cerebral blood flow (CBF) changes continuously before, during, and up to 90 minutes following injury in both male and female piglets. The role of phenylephrine to alleviate brain damage was evaluated when administered pre-injury versus post-injury, and both were compared to untreated responses to FPI. The correlation coefficient between CBF and MAP time-series was calculated to estimate an “autoregulation index”, which can be used to assess cerebral autoregulation. A high autoregulation index is a sign of impaired cerebral autoregulation.

Twenty-two 2 to 5day-old male (n=12) and female (n=10) healthy piglets were used for the study. CBF and MAP decreased markedly during hypotension and fluid percussion brain injury in males, but of less magnitude in females. The use of phenylephrine caused less of a decrease in CBF and MAP, reduced autoregulation index in females. However, phenylephrine caused the opposite effect in males.

In summary, our data suggest that hemodynamic responses to FPI are significantly dependent on gender. Elevation of MAP with phenylephrine prevented impairment of cerebral autoregulation during hypotension after FPI in females only, indicating the potential role for gender-dependent mechanisms in cerebral autoregulation in pediatric TBI. More piglets and addition of concurrent measurements of oxygen- and deoxy-hemoglobin concentrations in the future will permit further exploration of this mechanism.

7896-86, Poster Session

CCD-camera-based diffuse optical tomography to study ischemic stroke in preclinical rat models

Z. Lin, H. Niu, L. Li, H. Liu, Y. Liu, J. Su, The Univ. of Texas at Arlington (United States); M. Ren, S. Yang, Univ. of North Texas Health Science Ctr. at Fort Worth (United States)

Stoke, due to ischemia or hemorrhage, is the neurological deficit of cerebrovasculature and is the third leading cause of death in the United States. More than 80 percent of stroke patients are ischemic stroke due to blockage of artery in the brain by thrombosis or arterial embolism. Hence, development of an imaging technique to image and monitor the cerebral ischemia and effect of anti-stroke therapy is more than necessary. Near infrared (NIR) optical tomographic technique has a great potential to be utilized as a non-invasive image tool to image the embedded abnormal tissue, such as a dysfunctional area caused by ischemia, due to its low cost and portability. Moreover, NIR tomographic techniques have been successively demonstrated in the studies of cerebro-vascular hemodynamics and brain injury. As compared to a fiber-based diffuse optical tomographic system, a CCD-camera-based system is more suitable for pre-clinical animal studies due to its simpler setup and lower cost. In this study, we have utilized the CCD-camera-based technique to image the embedded inclusions based on tissue-phantom experimental data. Then, we are able to obtain good reconstructed images by two recently developed algorithms: (1) depth compensation algorithm (DCA) and (2) globally convergent reconstruction (GCR). In this study, we will demonstrate the volumetric tomographic reconstructed results taken from both tissue-phantom and animal studies; the latter has a great potential to determine and monitor the effect of anti-stroke therapies.

7896-87, Poster Session

A study of solving diffusion equation using wavelet finite element method

F. Yang, F. Gao, Tianjin Univ. (China)

The use of optical radiation is important in many fields, such as neonatal brain and breast imaging. For deriving photon density inside an object and photon flux at its boundary, a wavelet finite element method (WFEM) is proposed herein to solve the two-dimensional steady-state diffusion equation which is well known as the P1 approximation to the radiative transfer equation. Different from the typical finite element method (FEM), the scaling function of Daubechies wavelets are adopted as basis functions providing flexibility to represent the unknowns at different levels of resolution. Due to lack of explicit Daubechies scaling function expression, the calculation of the connection coefficients for stiffness matrices is presented for reference as well. In this article a high degree of agreement with a Monte Carlo method is demonstrated.

7896-88, Poster Session

Development and evaluation of a diffusion model for time-domain near-infrared fluorescence imaging using a finite element method

Q. Zhu, The Univ. of Birmingham (United Kingdom); F. Leblond, F. El-Ghussein, B. W. Pogue, Dartmouth College (United States); H. Dehghani, The Univ. of Birmingham (United Kingdom) and Dartmouth College (United States)

NIRFAST is an open source software package designed for modeling near-infrared light transport in biological tissue based on solutions to the diffusion equation obtained with a finite element method (FEM). The current version of NIRFAST allows simulations of continuous-wave and frequency-domain fluorescence signals. In this work we introduce a generalization of the approach allowing time-domain excitation and fluorescence data to be generated. This new functionality allows simulation of temporal point-spread functions (TPSF) for heterogeneous scattering and absorbing media of arbitrary geometry. In the first part of this paper we present the approach used to develop a computationally efficient approach for solving the time-dependent diffusion equation for excitation and fluorescence data. In the second part, a detailed evaluation of the method is presented comparing FEM numerical simulations with analytical solutions and Monte Carlo simulations. Simulated TPSFs are also compared with experimental data obtained with a small-animal time-domain fluorescence tomography system. The potential of time-dependent diffusion-based light transport modeling is assessed by comparing the results with excitation and fluorescence TPSFs acquired with the tomography system on a tissue-simulating phantom with a thickness varying from 15 mm to 50 mm. Comparison of TPSF datatypes such as total light fluence, mean time of photon arrival and variance of the time-dependent curve shows that the model is reliable and warrants use for future time-domain applications where diffusion modelling can be used.
These findings suggest that on the cylindrical applicator interface there is a greater rate of photon fluence decay along the azimuth direction but a longitudinally reduced (more than 1000x on 5 GPGPU processors) in comparison to the planar semi-infinite geometry having the same source-detector distance. The comparisons between the different time-dependent solutions were performed on selected parameters sets for a two- and a three-layer medium. The results and the performances of the forward solvers are presented. Monte Carlo procedures and solutions of the diffusion equation for the time domain. For Monte Carlo we included four independent codes. For the solutions of the diffusion equation, we present: semi-analytical approaches based on the Green's function method, solutions obtained with the Finite Element Method, and solutions obtained with the discontinuous Galerking model. Finally, fast simulations of temporal point spread functions from the moments of the distribution are also presented. The comparisons between the different time-dependent solutions were performed on selected parameters sets for a two- and a three-layer medium. The results and the performances of the forward solvers are discussed. The intent of this presentation is to make an overview of some typical approaches frequently used in photon migration studies by several research groups working in tissue spectroscopy and diffuse optical tomography. The results obtained show that the same information on photon migration can be obtained following completely independent and different approaches.
7896-120, Poster Session

**Tomographic optical imaging in Gaussians**

H. Gao, Univ. of California, Los Angeles (United States); H. Zhao, Univ. of California, Irvine (United States); W. Cong, G. Wang, Virginia Polytechnic Institute and State Univ. (United States)

Due to the diffusive nature of light in tissues, the inverse problem is severely ill-posed. As a result, the accurate reconstruction of the optical property or the fluorescence/bioluminescence source distribution in voxels is highly challenging. However, one can simplify the representation and reconstruction using characteristic geometry of the underlying parameters.

For example, it is not only quantitatively accurate, but also physiologically reasonable to represent bioluminescent source as the summation of multiple Gaussians.

Mathematically, it was rigorously shown that the solution of (bioluminescence tomography) BLT is non-unique without the incorporation of effective a priori knowledge on the source distribution; in particular the uniqueness can be established when source is assumed to be composed of solid balls with the known intensities. On the other hand, the anisotropic Gaussian representation is an effective mathematical approximation in the sense that it parameterizes the major quantitative information of interest, such as the intensity, the center and the size of the source. Physiologically, the Gaussian shape is indeed a natural choice. According to http://www.humpath.com/tumorigenesis, tumorigenesis is a collection of complex genetic diseases characterized by multiple defects in the homeostatic mechanisms that regulate cell growth, proliferation and differentiation.

With this shape prior, the reconstruction is reduced to that with a few parameters. Therefore, the method is robust to the measurement noise and the mismatch in optical background.

Various simulations are performed to demonstrate its superiority over the conventional voxelbased method and in vivo experimental data are utilized to validate the propose algorithm.

7896-121, Poster Session

**Indentifying constituent spectra sources in multispectral images to quantify and locate cervical neoplasia**

K. Baker, S. B. Bambot, Guided Therapeutics, Inc. (United States)

No abstract available

7896-39, Poster Session

**Optical properties in highly scattering medium using an approach by interference and heterodyne technology**

C. Chou, Y. Lan, Chang Gung Univ. (Taiwan)

We present and develop an approach using optical interference and heterodyne technology to investigate the light migration in highly scattering media. The theoretical model is based on diffusion approximation in steady-state frequency domain. The model incorporates pair-photon dipole source in order to satisfy the emulsion boundary condition and is suitable for either refractive index matched or mismatched surface. The experimental results showed that the diffusion theory applies in this study. Under the appropriate boundary and interference condition, the study accurately estimates the optical parameters of the medium.

7896-93, Poster Session

**Experimental estimation of the sensitivity profile of time-resolved reflectance measurement: a phantom study**

P. L. Sawosz, M. Kacprzak, Institute of Biocybernetics and Biomedical Engineering (Poland); W. Weigl, Medical Univ. of Warsaw (Poland); N. S. Zolek, S. Wojtkiewicz, R. Maniewski, A. Liebert, Institute of Biocybernetics and Biomedical Engineering (Poland)

In near infrared spectroscopy the light penetration depth and spatial distribution of visiting probability of photons should be considered for proper interpretation of the acquired signals. The sensitivity of the measurement to the changes of absorption can be calculated with the use of numerical methods like Monte Carlo simulations or diffusion theory. In this study, we proposed a method for experimental estimation of distribution of light penetration probability in an inhomogeneous, optically turbid tissue-phantom. Time-gated, intensified CCD camera was applied for imaging of physical phantom of a human head. A phantom was constructed with the use of a human skull which top was cut off. The skull was filled with optically turbid liquid (Intralipid and ink solution). The camera was positioned above the skull allowing for imaging of the phantom in the plane perpendicular to the direction of the incident light. Two fibers were attached at the edge of the skull in separation of 3 cm. The fibers delivered femtosecond light pulses from a Ti:Sapphire laser at 830 nm. The images were acquired for each fiber separately with recording of distribution of time of flight of photons (DTOF) for each voxel located on the surface of the phantom. Distribution of visiting probability of photons virtually travelling between source and detector was calculated by convolving the DTOFs recorded in each voxel for the two source positions. The experiments allowed to assess influence of inhomogeneities of the skull on the time-resolved distribution of light penetration probability.

7896-94, Poster Session

**Fast Monte Carlo fitting of two-layered tissue structures for short source-detector distances**

J. T. Elliott, K. M. Tichauer, M. Diop, K. St. Lawrence, Lawson Health Research Institute (Canada)

Scalable Monte Carlo algorithms have been successfully employed to recover optical properties of homogeneous tissue accurately and rapidly. However, this technique is inappropriate for two layered tissue structures, such as that found in prostate endoscopy applications. Here we demonstrate that a fast Monte Carlo algorithm can be used to iteratively fit and recover the optical properties of both tissue types in a rectal wall/prostate phantom for short source-detector distances.

A two-layer phantom was constructed from two different silicon materials, to model the rectal wall and prostate. The optical properties of each layer were first determined independently using a time-resolved near-infrared spectroscopy system. Once the layers were placed together, the phantom was imaged using the eXplore optix system. Fitting was performed in MATLAB by invoking the Monte Carlo eXtreme algorithm executable (Photon Migration Imaging Lab, Cambridge, MA) within a constrained fminsearch loop. Approximately 100 iterations per pixel were required to achieve convergence on average, which corresponded to 10 minutes of computational time per pixel on a single nVidia GTX480 GPU card.

The errors in recovered optical properties were 6.7 and 13.5% for absorption and scattering coefficients, respectively for the 5-mm thick top layer. Optical properties for the bottom layer were recovered with errors of 10.5 and 7.2% for absorption and scattering coefficients, respectively.

This work demonstrates the promise of highly accurate and ultrafast
Monte Carlo algorithms for applications within an iterative fitting routine - a development made only recently feasible due to advances in graphics processing unit technology.

7896-95, Poster Session

Multichannel photon counting DOT system based on digital lock-in detection technique
H. Zhao, Z. Wang, S. Hou, F. Gao, Tianjin Univ. (China)

Relying on deeper penetration of light in the tissue, DOT (Diffuse Optical Tomography) achieves organ-level tomography diagnosis, which can provide information on anatomical and physiological features. DOT has been widely used in imaging of breast, neonatal cerebral oxygen status and blood oxygen kinetics observed by its non-invasive, security and other advantages.

Continuous wave DOT image reconstruction algorithms need the measurement of the surface distribution of the output photon flow inspired by more than one driving source, which means that source coding is necessary. Most currently used source coding in DOT is time-division multiplexing technology, which utilizes the optical switch to switch light into optical fiber of different locations. In case of large amounts of the source location or using the multi-wavelength, the measurement time and the measurement interval between different locations within the same measurement period will therefore become too long to capture the dynamic changes in real-time.

In this paper, a frequency division multiplexing source coding technology is developed, which uses several light sources modulated by different frequency sine wave incident to the imaging chamber simultaneously. Signal corresponding to the various sources are obtained from the mixed output light using digital phase-locked detection technology in the detection end. A digital lock in detection circuit for photon counting measurement system is implemented in a FPGA development platform. A dual-channel DOT photon counting experimental system is preliminary established, including the two continuous lasers, photon counting detectors, digital lock in detection, control circuit, and computer programs to control and display. A series of experimental measurements is taken to validate the feasibility of the system.

This method greatly accelerates the DOT system measurement, and can also obtain the multiple measurements in different source-detector locations. The imaging results reflect the objective phenomenon more accurately.

7896-96, Poster Session

A low-cost multi-wavelength tomography system based on LED sources
A. Chen, A. Aguirre, U. S. Alqasemi, Q. Zhu, Univ. of Connecticut (United States)

Diffused optical tomography, time-domain, frequency-domain, and DC systems are used by many research groups to acquire data from biological tissues. Compared with the time-domain and frequency-domain systems, DC systems are cheaper and easier to construct. Laser diodes are typically used as sources in DC systems. In this talk, we present a multi-wavelength DC system using Light Emitting Diode (LED) sources of four wavelengths in the near infrared range of 740nm to 830nm. The LEDs in this wavelength range are commercially available with power ranging from 10 mW to 30 mW, which is adequate to probe deeply seated lesions up to several centimeters. The cost of the LED is a fraction of the laser diode. In our system, 8 groups of LEDs of 740nm, 780nm, 810nm, and 830nm were deployed on a hand-held probe and 14 Photomultiplier Tube (PMT) detectors were fiber coupled to the probe. A co-registered ultrasound array was located in the middle of the probe to provide the lesion structure information. The LEDs were modulated at a lower kHz value to avoid the DC noise and the detected signals were directly sampled by parallel A/D converters. Two laser diodes with frequencies modulated at 140 MHz were used with 14 detectors to provide the background optical properties which were not readily available from the DC system. Experiments were conducted to evaluate the performance of the LED based DC system and the laser diode based frequency domain system and the results were comparable.

7896-97, Poster Session

Target detection and characterization using a hybrid handheld diffusive optical tomography and photo-acoustic tomography system
P. D. Kumavor, A. Aguirre, C. Xu, J. Gamelin, Y. Ardeshipur, B. Tavakoli, S. Zanganeh, U. S. Alqasemi, Q. Zhu, Univ. of Connecticut (United States)

DOT guided by co-registered ultrasound, MRI, and x-ray has demonstrated a great clinical potential to overcome lesion location uncertainty and to improve light quantification accuracy. However, due to the different contrast mechanisms, some lesions may not be detectable by a non-optical modality but yet have high optical contrast. We present a photoacoustic tomography (PAT)-guided diffusive optical tomography (DOT) approach for detection and characterization of deeply seated targets embedded in a turbid medium. In PAT, the absorbed energy distribution is reconstructed at ultrasound resolution.

For co-registration, the light illumination used for PAT was coupled into a pair of optical fibers and housed by a probe which also included the optical fibers for DOT and ultrasound transducer. In reflection geometry PAT, the front-face of the ultrasound transducer generates ultrasound waves upon light illumination. These secondary echo signals propagate back into the tissue and produce pulse-echo artifacts that interfere with the photoacoustic images. To circumvent this, a rectangular cuboid of acrylonitrile butadience styrene (ABS) material with the top and bottom covered with a polyethylene membrane was constructed. This cuboid of 3mm thickness was filled with intralipid (200 cm-1) and placed over the front face of the transducer to reduce the light absorption. This method reduced the artifact signal by a factor of two but showed no significant decrease of ultrasound signals from the target. Measurements of the absorption coefficient of polyvinyl plastisol phantoms using this approach yielded values that were 70% accurate.

7896-98, Poster Session

Simultaneous bilateral breast imaging using a novel handheld optical device
J. Gonzalez, J. DeCerce, S. Martinez, S. Erickson, A. Godavarty, Florida International Univ. (United States)

Hand-held optical imaging is an emerging approach in the clinical translation of the technology towards breast imaging. Various hand-held optical devices developed to date focus on spectroscopic imaging of small tissue region. In the Optical Imaging Laboratory, a novel hand-held optical device has been developed that is capable of performing simultaneous bilateral imaging of large tissue surfaces. The hand-held optical device consists of a unique probe head that can flex to the tissue curvature and also allow rapid data acquisitions via simultaneous illumination and detection. Fluorescence-enhanced optical imaging studies are performed on large (~1000 ml) cubical tissue phantoms to assess the capability of the device in simultaneous bilateral imaging. Two cubical phantoms (one with and one without a fluorescence target) are simultaneously imaged using the hand-held probe to detect the presence or absence of targets in real-time. Preliminary results demonstrate that this novel device can help in 2D target detection(s) when comparing a no-target and target-based phantoms simultaneously. Currently, in-vivo bilateral breast imaging studies on healthy female volunteers (> 21 years of age) is carried out, where a simulated target is embedded under the flap of one breast tissue. The study has greater potential towards clinical breast cancer screening, where simultaneous bilateral imaging can detect potential tumors in real-time.
Clustered targets imaged by multizone optical tomography reconstruction method

Y. Xu, C. Xu, Q. Zhu, Univ. of Connecticut (United States)

Clustered small breast lesions may be present in the neighboring areas which are difficult to resolve and quantify accurately in diffuse optical tomography. In addition, advanced breast cancers are often accompanied by clustered satellite lesions in the neighboring areas, which are also difficult to resolve and quantify. To improve the light quantification of clustered lesions, a multi-zone reconstruction algorithm guided by co-registered ultrasound was investigated using simulations and phantoms. The performance of the algorithm was demonstrated using clinical examples. This method separated one larger region of interest (ROI) into several ROIs based on the location information provided by ultrasound images. The performance of the multi-zone reconstruction method was compared with single ROI algorithm under different imaging conditions, for example, two targets of same or different size with same or different contrast located at the same depth or different depth. In simulation, Finite-element method was used to generate the forward data with two targets embedded in the medium. In experiments, phantom targets were submerged in the Intralipid background. With the multi-zone reconstruction method, two targets can be distinguished from each other when their center-to-center separation was larger than 1.5 cm and the depth was shallower than 2.5 cm. In contrast, these targets cannot be separated using the single ROI algorithm unless their separation was larger than 2.5 cm and the depth was shallower than 2.0 cm. The accuracy of reconstructed absorption coefficients could reach 81% using the multi-zone method while the accuracy for one ROI algorithm was about 50%.

Improving light quantification of large breast lesions imaged by diffuse optical tomography

B. Tavakoli, Q. Zhu, Univ. of Connecticut (United States)

Diffuse Optical tomography (DOT) is a non-invasive functional imaging modality that has a great potential for distinguishing benign from malignant lesions. To overcome the limitation of DOT’s low spatial resolution and inaccurate target quantification, we have used co-registered ultrasound (US) to guide DOT. However, because of the exponential decay of the photon density wave, the accuracy of quantifying deeper portion of the large lesions in reflection geometry is much lower than that of the top portion. In this study, we introduce a modified depth correction method that incorporates the target depth information provided by co-registered US. The correction method properly regulates the weight matrix before image reconstruction. The maximum singular values, MSVs, of the weight matrix are calculated for all depth layers of the lesion and background, respectively. Then the weight matrix is scaled using inversely arranged MSVs in the order from bottom to top layer. After inversion, the weight matrix is rescaled back to obtain the absorption distribution. Simulations and phantom experiments of homogenous and inhomogenous large lesions were performed. It was found that by implementing depth correction, lesions were more accurately characterized. The ratio of the maximum absorption coefficient of the top-half to bottom-half of the lesion was calculated to quantify the improvement. After depth correction, the ratio improved from 4.2 to 1.01 for homogenous targets. The ratio was improved about 2.5 times for targets of top-half less absorptive than that of the bottom-half. Clinical examples demonstrated a consistent improvement of reconstructed absorption distribution and accurate characterization of large lesions.

A time-domain fluorescence diffusion optical tomography system for breast tumor diagnosis

W. Zhang, F. Gao, X. Wang, J. Li, F. Yang, Z. Zhou, L. Zhang, H. Zhao, Tianjin Univ. (China)

A prototype time-domain fluorescence diffusion optical tomography (FDOT) system using near-infrared light is presented. The system employs two pulsed light sources, 32 source fibers and 32 detection channels, working separately for acquiring the temporal distribution of the photon flux on the tissue surface. The light sources are provided by low power picosecond diode lasers at wavelengths of 785 nm and 830 nm, and a 32×1-fiber-optic-switch sequentially directs light sources to the object surface through 32 source fibers. The light signals re-emitted from the object are collected by 32 detection fibers connected to eight 4×1-fiber-optic-switches and then routed to 4 time-resolved measuring chains, each of which consist of a collimator, a filter wheel, a photomultiplier tube (PMT) and a time-correlated single photon counting (TCSPC) module. The performance and efficiency of the designed system are assessed by reconstructing the fluorescent- and optical-images of a solid phantom.

Study on diffuser-aided diffuse optical tomography for breast imaging based on three-dimensional Monte Carlo modeling

C. Chuang, National Taiwan Univ. (Taiwan); C. Sun, National Yang-Ming Univ. (Taiwan); C. Lee, C. Chen, National Taiwan Univ. (Taiwan); C. Wang, Y. Hsieh, National Chiao Tung Univ. (Taiwan)

For human breast imaging, DOT reveals pathological tumor contrast directly in vascularity, hemoglobin concentration and tissue scattering property. Usually, the optical properties of normal breast tissue (as background) are measured from the contralateral of detected breast generates concomitant prediction errors of image reconstruction. Thus, we propose the diffuser-aided diffuse optical tomography (DADOT) as a new approach for breast tumor imaging with modified Beer-Lambert’s law calculation to overcome this problem in this study. DADOT was introduced by means of Monte Carlo simulation with the following purposes: 1) to evaluate its feasibility in detecting breast tumor from three dimensional Monte Carlo simulation, 2) to demonstrate its capability of diffuse background reduction by use of diffuser, and 3) to illustrate its improved performance over the conventional DOT approach for breast image. The simulation results demonstrate the time-resolved Monte Carlo method can be capable of performing source-detector separation analysis. The dynamics of photon migration with various source-detector separations are analyzed for characterization of breast tissue and estimation of optode arrangement. The source-detector separations should be less than 4 cm for breast imaging in DOT system. Meanwhile, the feasibility of DADOT was manifested in this study. In the results, the DADOT approach can provide better imaging contrast than conventional DOT measurement. The DADOT approach possesses great potential to detect the breast tumor in early stage and chemotherapy monitoring.

Resolution in diffuse optical tomography of the human breast near the chest wall

H. Y. Ban, D. R. Busch, Univ. of Pennsylvania (United States); S. D. Konecky, Univ.of California, Irvine (United States); M. Machida,
Trimensional spectra: high discrimination of benign and malignant prostate tissue

V. Maslamlani, M. Al Salhi, King Saud Univ. (Saudi Arabia); V. Trinka, Thendrel Inc. (United States); D. Rabah, King Saud Univ. (Saudi Arabia); M. R. Turki, King Saud Medical Complex (Saudi Arabia)

Cancer of prostate occurs more frequently for older men and for many of them the malignancy is not aggressive. So a non-invasive technique of spectral scaling of virulence of malignancy is our ultimate goal. The first step is spectral discrimination of benign from malignant tumor. In this paper, we have done fluorescence emission spectrum (FES), Stokes' shift Spectrum (SSS) and reflectance spectrum (RLS) of excised benign and malignant tumor tissues (N = 15 each).

The tissues were minced, washed five times and dried before loading into the quartz cuvette for spectral analysis. The FES was done with excitation at 325nm only; SSS with Δλ = 70, and Δλ = 0, the latter being equivalent to reflectance spectra.

Of the three modes of spectra, SSS with Δλ = 70nm showed the best discrimination. There were four important bands, one at 280nm (due to tryptophan); 320nm (due to elastin & tryptophan); 355 and 385 (due to flavin). From the relative intensities of these bands, three ratios were evaluated. Similarly another two ratios were obtained from reflectance spectra and two more from FES. Thus, there are 7 ratio parameters which represent the relative concentration of tryptophan, elastin, NADH and flavin. A statistical analysis showed that benign and malignant tissues could be classified with accuracy greater than 90%.

This report is only for in vitro analysis; but employing optical fiber, this can be extended to in vivo analysis too, so that benign tumor could be distinguished without surgery.

Improving the spatial resolution of fluorescence diffuse optical tomography using nonlinear fluorophores

C. T. Xu, P. Svenmarker, H. Liu, S. Andersson-Engels, Lund Univ. (Sweden)

Fluorescence diffuse optical tomography (FDOT) is a relatively inexpensive 3-D imaging modality that can be used to non-invasively detect and quantify the concentration of a fluorophore within scattering material, such as tissues. Due to the diffuse nature of light, the low spatial resolution of the reconstructed fluorophore distributions has been one major drawback of FDOT. In addition, FDOT is generally an ill-posed problem, i.e., it is extremely sensitive to perturbations of the input data. In this work, we quantify and demonstrate, using simulated and experimental data, means of increasing the spatial resolution using nonlinear fluorophores, e.g., upconverting nanoparticles.

The increased spatial resolution can be understood by considering and comparing the excitation fields of a linear and a nonlinear fluorophore. It is obvious that for a multi-photon process, the effective excitation field will be much more sharply defined than for a linear process. Thus by scanning the excitation field, it is possible to obtain a much higher spatial sensitivity, i.e., fluorescence will only be strong in the vicinity of the source position followed by a quick decay as a function of the source-to-fluorophore distance. The quantification of the increased spatial resolution in the reconstructions was performed both through simulations and experiments, by reconstructing two closely situated fluorophores.

Our preliminary studies have shown that the resolution enhancement while using a quadratic fluorophore as compared with a linear fluorophore, for typical optical and geometrical properties of small animals, can be up to 40%.

Toward robust high-resolution fluorescence tomography: A hybrid row-action edge preserving regularization

A. Behrooz, H. Zhou, A. A. Eftekhar, A. Adibi, Georgia Institute of Technology (United States)

Fluorescence molecular tomography (FMT) is an in vivo non-invasive optical imaging modality that aims at localization and quantification of fluorescent-tagged inclusions deep in biological tissue. Depth-resolved reconstruction of fluorescence distribution in tissue is highly ill-conditioned as depth information should be extracted from limited number of surface measurements. Conventionally, inverse solvers resort to regularization algorithms that penalize the Euclidean norm of the solution to overcome ill-posedness. While this class of regularization algorithms offer good accuracy, they possess smoothing effects that result in continuous distributions which lack certain features of the actual fluorescence distribution, e.g. sharp transitions or edges, and hence limit the resolution offered by FMT.

In this work we propose an algorithm that penalizes the total variation (TV) norm of the solution to preserve sharp transitions and high-frequency components in the reconstructed fluorescence map while overcoming ill-posedness. The hybrid algorithm is composed of two levels: 1) An Algebraic Reconstruction Technique (ART), performed on FMT data for fast recovery of a smooth solution that serves as an initial guess for the iterative TV regularization, 2) A time marching TV regularization algorithm, inspired by the Rudin-Osher-Fatemi TV image restoration, performed on the initial guess to further enhance the resolution and accuracy of the reconstruction.

The performance of the proposed method in resolving fluorescent tubes inserted in a liquid tissue phantom imaged by a non-contact CW trans-illumination FMT system is studied and compared to conventional regularization schemes. It is observed that the proposed method performs better in resolving fluorescence inclusions at higher depths.

Early detection of breast cancer: 3D modeling, simulation and in-vitro studies to characterize the in-vivo performance of synthesized novel NIR-f estrogen conjugate dye

S. Bhattacharjee, I. Jose, Birla Institute of Technology and Science (India)

Estrogen induced proliferation of mutant cells is widely understood to
be the one of major risk determining factor in the development of breast cancer. Hence determination of the Estrogen Receptor[ER] status is of paramount importance if cancer pathogenesis is to be detected and rectified at an early stage. Near Infrared Fluorescence [NIRf] Molecular Optical Imaging is emerging as a powerful tool to monitor bio-molecular changes in living subjects. We have successfully carried out the synthesis and characterization of a novel target-specific NIRf dye conjugate aimed at measuring Estrogen Receptor[ER] status. The conjugate was synthesized by ester formation between 17-β estradiol and a hydrophilic derivative of Indocyanine Green (ICG) cyanine dye, bis-1,1-(4-sulfobutyl) indotricarbocyanine-5-carboxylic acid, sodium salt. In-vitro studies regarding specific binding and endocytosis of the dye performed on ER+ve [MCF-7] and ER−ve [MDA-MB-231] adenocarcinoma breast cancer cell lines clearly indicated nuclear localization of the dye for MCF-7 as compared to plasma level staining for MDA-MB-231. Furthermore, MCF-7 cells showed ~4.5-fold increase in fluorescence signal intensity compared to MDA-MB-231. A 3-D mesh model mimicking the human breast placed in a parallel-plate DOT Scanner is created to examine the in-vivo efficacy of the dye before proceeding with clinical trials. Photon migration and fluorescence flux intensity is modeled using the finite element method with the coefficients (quantum yield, molar extinction co-efficient etc.) pertaining to the dye as obtained from photo-physical and in-vitro studies. We conclude by stating that this lipophilic dye can be potentially used as a target specific exogenous contrast agent in molecular optical imaging for early detection of breast cancer.

7896-108, Poster Session

Reconstruction and quantification of fluorescent parameters imaging using fluorescence diffuse optical tomography

J. Li, F. Gao, X. Yi, L. Wu, L. Zhang, H. Zhao, Tianjin Univ. (China)

Near-infrared fluorescence diffuse optical tomography has proven to be an efficient tool for visualizing the bio-distributions of fluorescent markers in tissue. We present a two-dimensional image reconstruction method for time-domain fluorescence diffuse optical tomography on a turbid medium of circular domain. The methodology is based on a linear generalized pulse spectrum technique that employs the analytical solution to the Laplace-transformed time-domain photon-diffusion equation to construct a Born normalized inverse model, which enable that the absolute three-dimensional problem is approximately equivalent to the relative two-dimensional problem. A pair of real domain transform-factors is introduced to simultaneously reconstruct the fluorescent yield and lifetime images and the resultant linear inverses are solved using an algebraic reconstruction technique. A set-up, based on a multi-channel time-correlated single-photon-counting system, to localize fluorescent target in diffusing phantom was developed. The cylinder phantom embeds a fluorescent target made from 1%-Intralipid solution and fluorescent agent. We studied the dependence of time-resolved data on the number of light source points of excitation light and the number of detection points of fluorescence emission. The results show that the approach retrieves the position, shape and quantification of the target with a reasonable accuracy.

7896-109, Poster Session

The effect of time-gating on instrument photon density sensitivity functions for small animal fluorescence tomography

N. Valim, J. L. Brock, M. J. Niedre, Northeastern Univ. (United States)

Despite significant advances in fluorescence mediated tomography, low imaging resolution is still a problem due to the high degree of light scatter in biological tissue. It has been shown previously that time-gated detection of early-arriving photons improves imaging resolution since they undergo significantly less scatter than continuous wave (CW) photons. Although this principle is well understood, significant details with respect to the practical implementation remain unclear, for example, i) the relative reduction in instrument photon density sensitivity function (PDSF) volume obtainable by measuring early photons, ii) the dependence of this improvement on the detection time gate, and iii) the dependence on tissue optical properties and pathlengths.

In this work, we measured PDSFs of our time gated system using a point perturbation approach. The output of a picosecond pulsed laser was transmitted through liquid optical phantoms and the emitted light was detected with a photomultiplier tube operating in photon counting mode. Instrument PDSFs were determined by analyzing the effect of the position of a small absorbing or fluorescent object on detected light at different time gates. Our results indicated that the optimal early time gate with reduced photon scattering and large signal to noise ratio centered on the 5% rise portion of the peak intensity. This yielded a ~6-9-fold reduction in the volume of the PDSF versus quasi-CW photons. This improvement was surprisingly consistent over a range of optical properties and pathlengths relevant for optical tomography with near-infrared light in biological tissue. Results were validated using theoretical models of photon propagation.

7896-110, Poster Session

Sparse reconstruction for fluorescence diffuse optical tomography

A. Jin, B. Yazici, Rensselaer Polytechnic Institute (United States)

Fluorescence diffuse optical tomography (FDOT) is an emerging molecular imaging modality that uses near infrared (NIR) light to excite the fluorophore injected into the tissue, and reconstruct the fluorophore concentration from measurements of scattered light. The inverse problem of FDOT is often highly ill-posed, since the number of measurements is usually much smaller than the number of unknowns. In FDOT, the fluorophore concentration is typically targeted to accumulate in a small region of interest, e.g., tumor, thus it is often very sparse in the imaging domain. Recently, the emerging field of compressive sensing (CS) has shown that sparse signals can be recovered from only a small number of measurements. When the sampling matrix is incoherent, i.e., the normalized inner product between every pair of columns in the sampling matrix is small, a sparse signal can be accurately reconstructed by L-0 or L-1 norm constraint optimization. In this work, we apply the CS theory for the reconstruction of sparse fluorophore concentration images. We design the FDOT forward matrix, which maps fluorophore concentration to measurements, to be incoherent as required by the CS theory. Specifically, we design two linear filters, one acting on the source field and another acting on the detected field, to achieve incoherency of the resulting forward matrix. We refer to these two filters acting on the source and detected fields, as optical and digital masks, respectively. Finally, we use L-1 norm constraint optimization to solve for the fluorophore yield which is proportional to the fluorophore concentration. We show the advantage of the method by both visual results and quantitative measurements in 3D numerical simulations.

7896-111, Poster Session

Near-infrared spectroscopic system and fast inverse Monte Carlo algorithm for endoscopic measurement of tubular vessel

X. Zhou, H. Zhao, Z. Wang, F. Gao, Tianjin Univ. (China)

A near infrared diffuse reflectance spectroscopic system and an inverse algorithm for deriving optical properties are developed. The system works on frequency domain (FD) and a probe was specially designed for endoscopic detection. Measurements for evaluating the accuracy of the system indicate that the deviation in measuring the AC amplitude and phase lag is less than 3.7% and 6.7%, respectively. To eliminate the influence of the initial guess of optical properties on the reconstruction accuracy, an inverse Monte Carlo simulation algorithm with cluster
Diffuse optical spectroscopy monitoring of cytochrome c oxidase redox state during respiratory challenges in a sublethal rabbit cyanide model

J. Lee, J. G. Kim, S. B. Mahon, D. S. Mukai, B. J. Tromberg, M. Brenner, Beckman Laser Institute and Medical Clinic (United States)

Cyanide (CN) poisoning induces lethal histotoxic anoxia and stops aerobic cell metabolism by disabling the function of cytochrome c oxidase (CcO), terminal oxidase of the mitochondrial respiratory chain. CN has a high binding affinity for active sites on cytochrome-c oxidase. When bound, the electron transport chain is arrested with the near infrared (NIR) optically active copper core in reduced form, preventing the donation of an electron. We have previously demonstrated that non-invasive diffuse optical spectroscopy (DOS) can be used to detect the physiologic events occurring during development of CN toxicity in an animal model. In this study, we extend the investigations to determine the feasibility of using DOS to detect CcO redox states by subjecting animals to respiratory challenges from 100% to 21% inspired oxygen levels during CN poisoning.

For measurements, New Zealand white rabbits (~4kg) were administered 10mg of NaCN in 60cc normal saline via the left femoral vein at a rate of 1cc/min. Respiratory challenges were applied before, during, and post cyanide infusion and changes in oxy- and deoxyhemoglobin concentrations and cytochrome c oxidase redox states throughout the experiment were monitored in the right hind leg muscle using DOS. Due the similarities of absorption spectra of hemoglobins and CcO redox states, non-invasive spectroscopic determination of CcO state has been difficult. However, with combined physiological perturbations of respiratory challenges and CN poisoning, DOS shows the feasibility of detecting the CcO redox state changes apart from hemoglobin concentration changes.

Inverse problem for biomedical applications: use of prior information on target and forward model parameters

F. Martelli, Univ. degli Studi di Firenze (Italy); S. Dei Bianco, Istituto di Fisica Applicata Nello Carrara (Italy); G. Zaccanti, Univ. degli Studi di Firenze (Italy)

We propose the use of a retrieval operator for biomedical applications in near-infrared spectroscopy. The proposed retrieval operator is based on the "Optimal Estimation" method. The main characteristic of this method relates to the possibility to include prior information both on target and on forward model parameters of the inversion procedure. The possibility of the retrieval operator to elaborate a-priori information can in principle be a benefit for the whole retrieval procedure. This means that a larger number of target parameters can be retrieved, or that a better accuracy can be achieved in retrieving the target parameters. What does it mean to know something about a parameter a-priori? In the sense here adopted it means to know something about a parameter before observing the data. For instance, the a-priori information can be summarized as an estimate of the parameter together with a variance range for the true value of the parameter. With the Bayesian inversion the probabilistic prior information is combined with the information contained in the observed data in order to update the prior information. The final goal of this procedure is to have an improved estimate of the target parameters. The procedure has been tested on time-resolved simulated experiments obtained with a Monte Carlo code. The results obtained show that an improved performance of the procedure is obtained when prior information on target and forward model parameters is available.

Reconstruction algorithm for diffuse optical tomography using x-ray CT anatomical information

M. A. Naser, M. S. Patterson, Juravinski Cancer Ctr. (Canada)

The diffuse optical tomography (DOT) problem is ill-posed and ill-conditioned. The only accessible data points are at the surface and their number is much less than the number of variables representing the absorption and scattering coefficients at all points inside the tissue. This precludes a unique solution to the problem. Therefore, the solution should include some prior information about the distribution of the optical properties. Using anatomical information from x-ray CT images, different regions inside the animal can be distinguished due to their differences in x-ray attenuation. By assuming that each identified region has the same optical properties, the number of variables will be significantly reduced and a unique solution can be obtained.

In the model reported here, the light fluence rate at the boundary is written as a Taylor series expansion around an initial guess corresponding to a homogenous object with the same optical properties in all tissues. The expansion is approximated by the first three terms including the first and the second order derivatives which are calculated by the direct method and give the change of light fluence rate at the boundary due to small change in the tissue optical properties. The first and second order derivatives are used in an iterative algorithm to reconstruct the tissue optical properties. For low noise, the first order approximation is sufficient to give good results with small relative error. For higher noise power, the second order term shows better performance and faster reconstruction.
7896-23, Session 5

Characterizing scattering property of random media from phase map of a thin slice: the scattering phase theorem and the intensity propagation equation approach

B. DeAngelo, G. Arzumanov, C. Matovu, P. Shanley, Fairfield Univ. (United States); Z. Xu, Wenzhou Medical College (China); M. Xu, Fairfield Univ. (United States)

Light scattering by tissue originates from light interaction with cellular and extracellular matrix structure. The power spectrum of the fluctuation in the three dimensional distribution of the refractive index in tissue or other random media determines its scattering property. The two extreme limits of multiple scattering and migration of light inside random media-diffusion of light after sufficient scattering events and transmission of light through a thin slice undergoing few scattering events—has been shown inherently connected earlier. The scattering coefficient and the reduced scattering coefficient of the bulk media is found to be proportional to the variance of the phase and the variance of the phase gradient, respectively, of the phase map of light passing through one thin slice of the medium. This is so called “scattering-phase theorem.” We report first a new derivation of the scattering phase theorem and provide, for the first time, the correct relation between the variance of phase gradient and the reduced scattering coefficient. The condition for the scattering-phase theorem to be valid is also illustrated.

We will also report the results of applying the scattering-phase theorem in investigation of the bulk scattering property from phase maps of thin slices of tissue phantoms and ex vivo tissue samples. The phase gradient map and phase map of the sample is measured using a differential phase interference (DIC) microscope (Axio 40cfl) using the intensity propagation equation approach. The scattering coefficient, the reduced scattering coefficient, and the anisotropy factor of the sample is then computed from the phase maps. The efficacy of the scattering-phase theorem and the intensity propagation equation approach is assessed by comparing the results to known scattering property of the bulk samples.

7896-24, Session 5

Reduction of artifacts in diffuse optical tomography breast image reconstruction

S. Pathak, R. Choe, H. Y. Ban, S. H. Chung, D. R. Busch, A. G. Yodh, Univ. of Pennsylvania (United States)

In diffuse optical tomographic (DOT) breast image reconstruction, results are often obfuscated by the presence of artifacts, which are regions of spuriously high optical parameters. They arise in different varieties of image reconstruction methods, be they direct or iterative, and are the effect of noise in the data as well as the framing of the reconstruction problem. Artifacts are especially problematic because they make statistical characterization of tumor tissue properties very difficult.

We present a study of artifacts and their growth in DOT breast image reconstruction based on iterative method in the parallel plate geometry. We use a series of simulated multispectral continuous wave (CW)/frequency domain (FD) data to study and characterize artifacts and their iterative growth. The simulation is based on our clinical breast imaging system that collects large CCD based CW/FD data in parallel plate transmission geometry. It is generally seen that whereas expected quantification stops improving after a number of iterations, artifacts continue to grow. We present an implementation of the early stopping regularization method to stop the iterative process when artifacts begin to overwhelm the results and propose this as an objective stopping criterion that could be used on both healthy and tumor breasts. We finally show the efficacy of such methods on silicone phantom data simulating breast placed in Intralipid/ink solution.

7896-25, Session 5

A multiscale sparse approach to reconstruct fluorescent objects of different sizes from FDOT measurements

L. Lecordier, L. Hervé, J. Dinten, Commissariat à l’Énergie Atomique (France); F. Peyrin, CREATIS-IRMN INSA (France)

Fluorescence Diffuse Optical Tomography (FDOT) consists in localizing fluorescent markers in biological tissues using near-infrared light. The use of targeting biomarkers provides a promising approach for cancer diagnosis. The 3-D fluorescence yield map is reconstructed from a set of optical measurements by solving an inverse problem. Unfortunately, FDOT is known to be an ill-posed problem, which implies noise sensitivity and non-uniqueness of solution.

Prior information about medium properties or fluorescent yield shape is needed to get satisfying solution. Tumors we are trying to detect may have several different sizes but they represent in most cases a small part of the entire volume of the medium. Sparse solutions - that is to say solutions with a minimized number of non-zero values - seem well adapted to the problem. The FOCal Underdetermine System Solver algorithm (FOCUSS) is a recursive algorithm developed for electroencephalography which converges to a sparse solution. We developed a regularized FOCUSS-Based algorithm for FDOT which has proven its efficiency on simulated data to reconstruct small size fluorescent objects from noisy data. Yet, the algorithm cannot provide satisfying solution if the fluorescent yield shape is not sparse enough.

We propose a multiscale approach by using a dictionary containing objects of different sizes. The algorithm first provides a sparse solution and determines if the solution is acceptable thanks to a sparsity criterion. If not, the algorithm provides less and less sparse solutions using the dictionary until a solution matches the sparsity criterion.

7896-26, Session 6

Preclinical and clinical validation of a novel oxygcnation imaging system

S. Gioux, Commissariat à l’Energie Atomique (France); A. Mazhar, Univ. of California, Irvine (United States); D. J. Cuccia, Modulated Imaging, Inc. (United States); A. Stockdale, R. Oketokoun, B. T. Lee, Y. Ashitate, Beth Israel Deaconess Medical Ctr. (United States); A. J. Dunkin, B. J. Tromberg, Univ. of California, Irvine (United States); J. V. Frangioni, Beth Israel Deaconess Medical Ctr. (United States)

Introduction: Two major disadvantages of currently available oxygenation probes are the need for contact with the skin and long measurement stabilization times. A novel oxygenation imaging device based on spatial frequency domain and spectral principles has been designed, validated pre-clinically on pigs, and validated clinically on humans. Importantly, this imaging system has been designed to operate under the rigorous conditions of an operating room.

Materials and Methods: Optical properties reconstruction and wavelength selection have been optimized to allow fast and reliable oxy- and deoxy-hemoglobin imaging under realistic conditions. In vivo pre-clinical validation against commercially available contact oxygenation probes was performed on pigs undergoing arterial and venous occlusions. Finally the device was used clinically to image skin flap oxygenation during a pilot study on women undergoing breast reconstruction after mastectomy.

Results: A novel illumination head containing a spatial light modulator (SLM) and a novel fiber-coupled high power light source were constructed. Pre-clinical experiments showed similar values between local probes and the oxygenation imaging system, with measurement times of the new system being < 500 msec. During pilot clinical studies, the imaging system was able to provide near real-time oxy-Hb, deoxy-Hb, and saturation measurements over large fields-of-view (> 300 cm2).
Conclusions: A novel optical-based oxygenation imaging system has the potential to replace contact probes during human surgery and to provide quantitative, wide-field measurements in near real-time.

7896-29, Session 6
Method for depth-resolved quantitation of optical properties in layered media using spatially modulated quantitative spectroscopy (SMoQS)
R. B. Saager, Beckman Laser Institute and Medical Clinic (United States); D. J. Cuccia, Modulated Imaging, Inc. (United States); A. J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

SMoQS, a spatial frequency domain (SFD) instrument, is capable of extracting ~1 nm resolved, quantitative optical properties from homogeneous tissue phantoms that span both the visible and near infrared regimes in reflectance geometry. Biological tissue such as skin, however, is highly structured and hence presents challenges to quantitative spectroscopic techniques which are based on homogeneous models. The average optical properties of skin in the visible regime are significantly greater than that within the near infrared. As a consequence, the SFD measurements within the visible regime typically represent optical properties within the top 1 mm of tissue, whereas the near infrared represents ensemble average of properties within several millimeters of tissue.

For this initial investigation, a series of two-layer tissue simulating phantoms of independently verified optical property were created, where the top layer thickness ranges 100-1000 microns and each layer contains unique absorbing agents. Measurements of these layered structures are analyzed in the context of a two layer model, where the detected concentrations of layer specific absorbers are allowed to vary as a function of wavelength specific optical properties (i.e. the change in the respective contribution of each layer as a function of the wavelength dependent volume of the layered medium probed). With only a priori assumptions of the chromophores present in the layers, SMoQS can 1) isolate superficial chromophores from those deeper in the media, 2) estimate layer thickness 3) resolve the layer specific concentration.

7896-28, Session 6
Novel approaches based on structured light for fast diffuse optical tomography
A. Bassi, N. Ducros, C. D’Andrea, G. Valentini, Politecnico di Milano (Italy); S. R. Arridge, Univ. College London (United Kingdom)

Diffuse optical tomography (DOT) and Fluorescence mediated tomography (FMT) are powerful in-vivo optical imaging techniques but they are affected by long acquisition and computational times. Recently, the use of structured light has been proposed in order to reduce the acquisition time and also the computational time of the inverse problem. Additionally, it has been proposed to compress the measured data set to reduce the reconstruction time.

First, we review the state of the art of DOT and FMT based on structured light and we show recent advancements in the field. We present our experimental approach, describing the instrument for structured illumination and wide field detection and we discuss the advantages to use a finite elements based approach.

Then, we introduce the use of spatial wavelets. Our method is based on the projection of a small number of wavelet patterns (Haar and Battle-Lemarie wavelets). The detected images are wavelet transformed and the information content is compressed to achieve fast 3D reconstruction. Experimental results are presented, showing fast reconstruction of complex absorbing/fluorescent objects in thick diffuse samples. Implications for fast small animal imaging are discussed.

7896-30, Session 7
Frequency domain diffuse optical tomography in parallel plate geometry
K. Lee, J. Ho, J. Dong, Nanyang Technological Univ. (Singapore)

Frequency domain diffuse optical tomography (FD-DOT) is a popular means of probing both absorption and scattering properties in deep tissue, but so far most of them were implemented using optical fibers with limited number of probes. In this paper, we show an instrumentation of FD-DOT that enables massive data acquisition in a fast manner in a parallel plate geometry. This type of instrumentation is especially suitable for recently developed analytic image reconstruction method, which is fast and significantly less expensive in terms of computational load. It makes use of the known mathematical form of the Fourier domain Green’s function in simple geometries such as the slab or cylindrical geometry.

We will present the results of a table-top phantom RF-DOT experiment and assess the quality of the images reconstructed by the analytic method in Fourier domain. In the experimental setup, we used a 70MHz-modulated laser source of 660 nm, along with a gain-modulated image intensifier attached to an electron-multiplying CCD camera. Using a homodyne method, we acquired phase-sensitive transmission images at multiple source positions on a 17×17 grid, both with and without a target inside the diffuse medium. The resulting images were sampled and fitted to a sinusoidal wave to obtain the amplitude and phase for each virtual detector position. The resolution, contrast, and level of crosstalk between absorption and scattering are assessed, and future possible uses of this algorithm in real-time clinical imaging will be discussed.
Feasibility of rapid near-infrared diffuse optical tomography by swept-spectral-encoded sequential light delivery

G. Xu, D. Piao, Oklahoma State Univ. (United States)

We investigate the feasibility of rapid near-infrared diffuse optical tomography (DOT) by spectrally-encoded sequential light delivery based on wavelength-swept light source. Approximately 10nm sweep-range of a 4mW wavelength-swept source centered at 840nm is fiber-coupled to and dispersed by a spectrometer that has a focal-length of 500mm and a grating of 1200 grooves/mm. The wavelength-swept light becomes a point-like beam that scans linearly across the exit window of the spectrometer. A “swept-spectral-encoding” of the light beam is thus formed that delivers sequential illumination, without switching by mechanical means, to linearly bundled fibers coupled to the exit window. The wavelength-sweep is synchronized with the CCD acquisition to obtain multiple data sets corresponding to illuminating the source channels sequentially. A data acquisition rate of over 1 frame/second is reached for a 20nm-diameter circular intra-lumenal applicator with 8 source and 8 detector channels placed in a liquid phantom of biological tissue. Compared with a previously reported “spread-spectral-encoding” configuration based on a broad-band light source, this “swept-spectral-encoding” configuration reduces the horizontal overlapping of the signals when the detection is made by a spectrometer-CCD pair. This configuration reduces CCD exposure time by improving the power coupled per channel, but induces channel transition time due to wavelength-sweeping. Thus the overall data acquisition rate may be equivalent to that of the “spread-spectral-encoding” configuration for the same source power level. One distinct advantage of this new configuration is that it can be readily extended to rapid fluorescence diffuse optical tomography (FDOT) by enabling sequential source-channel-encoded excitations of fluorophores.

Coherent detection of diffusely scattered light for extended depth optical coherence tomography

M. G. Giacomelli, A. P. Wax, Duke Univ. (United States)

Optical coherence tomography (OCT) systems image by coherently detecting photons that have undergone a single scattering event. While this approach ensures high resolution and SNR, it limits the depth of penetration in scattering media to approximately 13 mean free path lengths. However, although ballistic light is strongly scattered, light that has undergone multiple scattering events is attenuated much more slowly. The increased value of transport mean free path relative to the ballistic free path has been exploited in techniques such as Diffuse Optical Tomography (DOT) which has excellent penetration but poor resolution because photon paths are only approximately known from the system geometry.

As means of extending the useful range of OCT, we explore interferometric detection of diffuse low coherence light to image beyond the ballistic limit with good spatial resolution. A Mach-Zehnder time domain interferometer combined with lock-in detection, a novel structured illumination scheme and angle-resolved detection are used to perform time-gated B-scans through a strongly scattering media with greater than 130 dB SNR. The resulting scans demonstrate imaging extending beyond the range of ballistic light into the multiply scattered regime but retain good resolution using a combination of coherence gating and system geometry to infer the transit path of collected light. We characterize the resolution and maximum imaging depth using singly and multiply scattered light.

Toward single-fiber diffuse optical time-of-flight spectroscopy


In this work we investigate the possibility to perform photon time-of-flight measurements of scattering materials using a setup with a single optical fiber for both delivery and collection of light. Using a fast time-gated Single Photon Avalanche Diode (SPAD) in combination with standard Time Correlated Single Photon Counting (TCSPC) electronics and a picosecond laser, the time-of-flight distribution of photons re-entering an optical fiber immersed in a scattering medium may be recorded. The great dynamic range of the time-gated SPAD allows data to be recorded with sufficient dynamic range (spanning over more than nine orders of magnitude) to differentiate between responses of samples with different optical properties. While the single fiber approach requires more complicated instrumentation than conventional diffuse optical spectroscopy (DOS) techniques, it relieves the requirement of separating the source and detector by a well known distance, hence increasing the applicability of DOS for clinical applications, in particular interstitial in vivo situations.

We present results from preliminary measurements performed on liquid phantoms in an infinite geometry and compare the results with Monte Carlo simulations of the setup. Different data evaluation schemes and the possibilities to separate absorption and scattering properties of the sample using the single-fiber approach are discussed.

Optical measurement of sound using laser speckles

T. S. Leung, S. Jiang, J. C. Hebden, Univ. College London (United Kingdom)

Laser Doppler Vibrometry can measure sounds radiating from a surface without direct contact. It is based on the Doppler shift introduced by the tiny vibration of the surface and the interferometry principle to derive the velocity of the vibration. It has been applied to measure heart and lung sounds. In this work, we present an alternative approach to measure sounds by capturing the dynamics of the laser speckles on the surface without contact. This novel technique does not measure vibrations on the surface directly. It involves pointing a pulsed laser beam on the surface of a turbid medium and using a CCD camera to capture the time varying laser speckles. Acoustic waves induce local changes in density and refractive index within the turbid medium. When photons pass through such medium, their optical pathlength are altered in a systematic manner. As these photons reach the surface, they interfere with one another resulting in a laser speckle pattern which blinks at the acoustic frequency, a phenomenon known as the acousto-optic effect. We will demonstrate the principle using tissue mimicking phantoms with similar acoustic and optical properties to human tissues. The experiment involved measuring sounds up to 2 kHz using a 633nm HeNe laser. This technique can potentially be applied to measure turbulent noise of the brain which is an indication of blocked cerebral artery or aneurism. In this particular application, conventional acoustic detection is severely limited by the high attenuation of acoustic waves through the skull.

3D modeling of noncontact fiber-based approach for time-resolved diffuse optical tomography

F. Nouizi, M. Torregrossa, O. Genevax, R. Chabrier, P. Poulet, Univ. de Strasbourg (France)
Diffuse optical tomography (DOT) in vivo provides tissue functional information and 3D spatiotemporal maps of its optical properties by tomographically measuring near-infrared diffusive light along the boundary. We developed time-resolved diffuse optical tomography system that enables performing noncontact 3D DOT measurements of irregular shapes which is appropriate for small animal imaging. To retrieve the surface mesh, a noncontact holographic setup using a sensor and an XY optical scanning system was used. 4 picosecond diodes with different wavelengths in the near-infrared range were used to excite the sample and 8 TCSPC modules were used to register signals provided by an 8-anode Micro-Channel Plate Photo-Multiplier Tube used as detector. DOT image reconstruction quality highly depends on the accuracy of the modeling used to solve the forward problem. We develop a noncontact modeling approach that consists on computing Time Profile Spread Functions of diffused photons detected taking into account the free space propagation from or to the fibers disposed around the animal at some distance from its surface to reconstruct the 3D maps of the optical properties of the animal. A 3D Finite Element Method was used to solve the diffusion equation chosen to model the light propagation in the high scattering medium.

Two reconstruction methods were performed by iteratively minimising an error expression between treated measured data and simulated ones. First one is based on the optimisation of the moments of the temporal intensity distribution computed from the measured data and the simulated ones. Second optimises the entire points of the TPSF.

7896-36, Session 8

Wavelength and code-division multiplexing in diffuse optical imaging

L. Ascarì, G. Berrettini, S. Iannaccone, Scuola Superiore Sant’Anna (Italy); M. Giacalone, Consorzio Nazionale Interuniversitario per le Telecomunicazioni (Italy); D. Contini, L. Spinelli, Politecnico di Milano (Italy); G. Trivella, Consiglio Nazionale delle Ricerche (Italy); L. ’Abbate, Scuola Superiore Sant’Anna (Italy); L. Potì, Consorzio Nazionale Interuniversitario per le Telecomunicazioni (Italy)

We recently applied time domain near infrared diffuse optical spectroscopy (TD-NIRS) to monitor hemodynamics of the cardiac wall (oxy and desoxyhemoglobin concentration, saturation, oedema) on anesthetized swine models. Published results prove that NIRS signal can provide information on myocardial hemodynamic parameters not obtainable with conventional diagnostic clinical tools. Nevertheless, the high cost of equipment, acquisition length, sensitivity to ambient light are factors limiting its clinical adoption.

This paper introduces a novel approach, based on the use of wavelength and code division multiplexing, applicable to TD-NIRS as well as diffuse optical imaging systems (both topography and tomography); the approach, called WS-CDM (wavelength and space code division multiplexing), essentially consists of a double stage intensity modulation of multiwavelength CW laser sources using orthogonal codes and their parallel correlation-based decoding after propagation in the tissue; it promises better signal to noise ratio (SNR), higher acquisition speed and lower costs compared to both the conventional systems and the more recent spread spectrum approach based on single modulation with pseudo-random bit sequences (PRBS). Parallel acquisition of several wavelengths and from several locations is achievable.

TD-NIRS experimental results guided Matlab-based simulations aimed at correlating different coding sequences, lengths, spectrum spreading factor, with the WS-CDM performances on such tissues (achievable SNR, acquisition and reconstruction speed, robustness to channel inequalization, ...).

Simulations results and preliminary validation on phantom confirm the significant improvements that WS-CDM could bring to diffuse optical imaging (not limited to cardiac functional imaging).

7896-37, Session 8

Radio-frequency circuit design and performance evaluation for small animal, frequency domain, NIR fluorescence optical tomography

C. D. Darne, B. Zhu, Y. Lu, I. Tan, J. C. Rasmussen, E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

Herein we report on hardware development and evaluation for frequency domain photon migration (FDPM) technique that is miniaturized for incorporation into a micro-CT gantry for hybrid CT/NIR/PET imaging. Immunity to endogenous optical properties and enhanced contrast associated with fluorophore lifetime is inherent to the FDPM measurements and enables unique opportunities for quantitative tomography when compared to the time independent (continuous wave) approach. A miniaturized radiofrequency (rf) circuitry has been developed in our laboratory for homodyne FDPM measurements that makes use of a single 100MHz oscillator to simultaneously launch optically modulated excitation light into a small animal as well as to modulate an NIR sensitive image intensifier for collection of fluorescent signals. The use of a single oscillator not only eliminates signal drift that otherwise results from the use of multiple oscillators individually driving both source and detector, but also reduces the circuit footprint for incorporation into the CT gantry. Herein, overall system performance parameters of signal-to-noise ratio, measurement precision, spatial resolution, modulation depth (ac/dc), excitation light rejection, and clinically relevant data acquisition times are presented for mouse phantom data. Image reconstruction of phantom data and integration of circuitry for hybrid CT/NIR/PET imaging is also presented towards the ultimate validation of NIR optical tomography using PET imaging as a “gold-standard” for small animal measurement.

7896-38, Session 8

Time-resolved optical system for an early detection of prostate tumor

L. Hervé, A. Laidevant, M. Deboureau, J. Boutet, J. Dinten, Commissariat à l’Énergie Atomique (France)

We developed an endorectal time-resolved optical probe aiming an early detection of prostate tumors targeted by fluorescent markers. Optical fibers are embedded inside a clinical available ultrasound endorectal probe. Excitation light is driven sequentially from a femtosecond laser (775 nm) into 6 source fibers. 4 detection fibers collect the medium responses at the excitation and fluorescence wavelength (850 nm) by the mean of 4 photomultipliers associated with a 4 channel time-correlated single photon counting card.

Fluorescence optical detection of prostate tumor is challenging in a lot of aspects, including the strong absorption parameters leading to weak fluorescent signals, the reflectance geometry enhancing parasite signal and early stage tumor size (1 mm) requiring adequate reconstruction performances. Furthermore, acquisitions must be performed fast (seconds) for the patient and the practicing physician comfort.

To evaluate our system performance, we acquired measurements of a 40 μL ICG inclusion (10 μmol.L-1) at various lateral and depth locations in a phantom. The forward model was numerically computed by solving the diffusion equation on a mesh taking into account the probe geometry. A dedicated reconstruction algorithm uses intensity and mean time of the optical signals and assumes a point-like inclusion.

Analysis of results showed we correctly reconstructed the fluorophore for the lateral positions (16 mm range) and for depths up to 2.5 cm. Precision of localization was found to be around 1 mm which complies well with precision specifications needed for the clinical application. Our system and these results will be presented at the BIOS2011 symposium.
Monitoring neo-adjuvant chemotherapy response using optical tomography guided by ultrasound

Q. Zhu, Univ. of Connecticut (United States); P. DeFusco, Hartford Hospital (United States); S. Tannenbaum, Univ. of Connecticut Health Ctr. (United States); Y. Xu, B. Tavakoli, Univ. of Connecticut (United States); E. Cronin, Hartford Hospital (United States)

Neoadjuvant chemotherapy is being used frequently for patients with locally advanced breast cancers. When used prior to surgery, it often allows for breast conservation by reducing tumor size. An additional benefit of neoadjuvant chemotherapy is the opportunity to assess the chemo-responsiveness of the tumor in vivo. Moreover, when a pathologically complete response is achieved, patients have increased their chances of being disease-free and their overall survival. The objective of this study is to assess early vascular changes during neoadjuvant chemotherapy and correlate early vascular changes with the tumor pathological response.

Patients who underwent neoadjuvant treatment were recruited from two hospitals and their tumor vascular content was assessed with a combined imager consisting of a commercially available US system coupled to a NIR imager (NIR/US). Patients were imaged before their treatment, at the end of every treatment cycle and before their definitive surgery. The co-registered US was used to localize the tumor and to guide the NIR imaging reconstruction; while the NIR imager was used to map the tumor vascular distribution which was assessed based on a percentage total hemoglobin (%Hb) concentration normalized to the pre-treatment level. A total of 27 patients have been recruited since December 2007. Initial results have showed that all complete or near-complete responders showed a rapid reduction in %Hb at the end of cycle two or three; while all non-responders showed no change or small changes in %Hb throughout the treatment. On average, partial responders showed changes in between.

In-vivo cancer therapy monitoring with diffuse optical techniques

R. Choe, S. Pathak, S. H. Chung, Univ. of Pennsylvania (United States); T. Durduvan, ICFO - Instituto de Ciencias Fótòunicas (Spain); H. Y. Ban, D. R. Busch, T. Averna, E. M. Buckley, M. N. Kim, Univ. of Pennsylvania (United States); A. DeMichele, C. Mies, M. A. Rosen, M. D. Schnall, The Univ. of Pennsylvania Health System (United States); A. G. Yodh, Univ. of Pennsylvania (United States)

Diffuse optical techniques such as diffuse optical and diffuse correlation spectroscopy/tomography can provide oxy-, deoxy-hemoglobin concentrations and blood flow information at the microvascular level. Especially, there is a great interest in detecting early metabolic changes which may precede morphological changes. The usage of non-ionizing radiation as light source and low cost instrumentation make diffuse optics suitable for frequent monitoring of cancer progression or therapeutic response. To this end, we have utilized a hybrid system consisting of a frequency-domain diffuse optical instrument and a diffuse correlation spectroscopy instrument to probe oxygen metabolism of cancer response to the therapy. Breast cancer patients (N=10) undergoing neoadjuvant chemotherapy have been measured typically at 4 time points: pre-treatment, 1-2 days after the first chemotherapy, inter-regimen, and post-treatment time points. Hemodynamic responses at different time points will be categorized with respect to histopathological response and presented. Preliminary analysis on patients who participated in a more frequent monitoring study show that blood flow and hemoglobin concentration changes exhibit correlating features with pathologic responses measured at the end of therapy. In addition to breast cancer therapy, the results from monitoring chemo/radiation therapy in human head and neck patients and the effects of molecular therapy on a xenograft mouse model of head and neck cancer will be presented.

Diffuse optical spectroscopic imaging biomarkers as therapeutic endpoints in breast cancer patients treated with neo-adjuvant therapy

A. E. Cerussi, V. W. Tanani, A. F. Durkin, Beckman Laser Institute and Medical Clinic (United States); D. J. Hsiang, J. A. Butler, R. S. Mehta, Univ. of California, Irvine (United States); B. J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

Diffuse Optical Spectroscopic Imaging (DOSI) non-invasively and quantitatively measures tissue hemoglobin, water, and lipid. Pilot studies in small groups of patients demonstrate that DOSI may be useful for longitudinal monitoring and predicting breast cancer neo-adjuvant chemotherapy pathological response. Pathological response is important because it has been shown to be a potential therapeutic endpoint that correlates with 5 and 10 year survival.

In this study, we update with our latest clinical results by comparing changes in DOSI-measured tissue optical properties and final pathological response. Thirty-six stage II/III breast cancers (34 patients) were measured in vivo with DOSI prior to, in the middle of and after the completion of pre-surgical neoadjuvant chemotherapy. Changes in DOSI-measured parameters at each timepoint were compared against final surgical pathology. Absolute changes in the tumor-to-normal (T/N) ratio of tissue deoxy-hemoglobin concentration (ctHHb) and relative changes in the T/N ratio of a tissue optical index (TOI) were the most sensitive correlates to pathological response. Changes in ctHHb and TOI were significantly different between tumors that achieved pathological complete response (pCR) vs. non-pCR.

We further report on the importance of spectroscopy to enhance response evaluation, the importance of standardization, as well as necessary next steps to address current limitations for further translation of DOSI into clinical use.

Monitoring early tumor response to drug therapy using optical tomography

M. L. Flexman, H. Kim, Columbia Univ. (United States); S. L. Hernandez, J. Huang, T. Johung, Columbia Univ. Medical Ctr. (United States); J. H. Lee, F. Vlachos, Columbia Univ. (United States); D. J. Yamashiro, J. J. Kandel, Columbia Univ. Medical Ctr. (United States); A. H. Hielscher, Columbia Univ. Medical Ctr. (United States); J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

It is well acknowledged that treatment efficacy could be increased and unnecessary toxicities reduced if a rapid assessment strategy were available to allow individual tailoring of cancer therapy. Optical tomographic imaging has emerged as a potential candidate for the early detection of treatment response. In this work we focus on using optical imaging to detect tumor response to anti-angiogenic treatment within the first 5 days of therapy. Anti-angiogenic drugs, such as bevacizumab, have shown promising results in cancer therapy but have also shown a large variability in effectiveness. For this study we implanted two different types of cells lines (SK-NEP and NGP) into the kidney of NCR nude mice and grew the resulting tumor to about 1g. Optical tomographic imaging with a dual-wavelength (765nm and 830nm) continuous wave
Diffuse optical spectroscopic imaging (DOSI) for very early prediction of response to neo-adjuvant chemotherapy in breast cancer patients

D. M. Roblyer, S. Ueda, A. E. Cerussi, W. V. Tanamai, A. F. Durkin, Beckman Laser Institute and Medical Clinic (United States); R. S. Mehta, D. J. Hsiang, J. A. Butler, Univ. of California, Irvine (United States); B. J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

Breast cancer patients who do not respond to chemotherapy may undergo months of unwarranted side effects. Early, non-invasive markers of response would provide physicians a valuable tool to make evidence-based changes to treatment strategies. Diffuse Optical Spectroscopic Imaging (DOSI) was used to measure tumors and surrounding normal tissue in 23 breast cancer patients daily during the first week of neo-adjuvant chemotherapy. DOSI utilizes temporally modulated near-infrared light (50Mhz - 600Mhz) from 6 laser diodes in combination with broadband excitation to recover broadband absorption and scattering values from thick tissues. Tissue concentrations of oxyhemoglobin, deoxyhemoglobin, as well as water and lipids are fit to the recovered absorption spectra. Measurements are made using a simple handheld probe placed on the skin. Functional 2D chromophore images are created by interpolation between measurement points. Overall response to therapy was determined by the decrease in anatomic tumor size. A flare in tumoral oxyhemoglobin concentration (average 40%) and spatial extent occurred on day 1 after the start of chemotherapy in patients achieving a partial or pathologic complete response. Non-responding patients exhibited no flare and an average decrease in concentration of -22.5% was observed. This flare was sufficient to perfectly discriminate patients who were partial or pathologic complete responders from those who were non-responders. Non-responders and partial responders had statistically higher ctO2Hb (p = 0.007), ctTHb (p = 0.04), and higher stO2 (p < 0.0001) than those with non-pCR. Ki67 was positively correlated with ctO2Hb levels (r = 0.67, p = 0.0002), ctTHb (r = 0.61, p = 0.0008), and stO2 (r = 0.57, p = 0.002). Glut-1 positive tumors exhibited higher ctO2Hb (p = 0.04), ctTHb (p = 0.05), and higher stO2 (p = 0.02) compared to Glut-1 negative tumors. These results suggest that highly proliferative tumors have an increased vascular supply, which likely contribute to response to chemotherapy. We have demonstrated that non-invasive DOSI measurements are useful as not only predictors of pCR, but also potential surrogate markers for tumor proliferation and glucose metabolism.

Assessing dynamic vascular changes in breast tissue in response to subject-specific hyperoxic and hypercarbic gas inhalation based upon end-tidal expiration

S. Jiang, B. W. Pogue, M. A. Mastanduno, K. E. Michaelson, Dartmouth College (United States); T. E. Frazee, Dartmouth Hitchcock Medical Ctr. (United States); K. D. Paulsen, Dartmouth College (United States); S. P. Poplack, W. A. Wells, R. M. diFlorio-Alexander, P. A. Kaufman, Dartmouth Hitchcock Medical Ctr. (United States)

Frequency Domain Near-infrared Spectral tomography appears to be feasible and may provide easily-acquired, low cost image signatures by assessing dynamic vascular changes of tissue such as total hemoglobin (HbT) and blood oxygen saturation (StO2) in the breast. In this preliminary clinical study, a gas sequencer and breathing circuit were used to prospectively target and sustain end-tidal pO2 and pCO2 values independently of each other for each subject. This approach was designed based upon several previous studies which indicate that this reduces inter-subject variability in observed tissue response. The end-tidal pCO2 and pO2 of each subject was targeted to 40 mmHg and 100 mmHg (respectively) for 2 minutes while we obtained 6 sets of data on the baseline response. Then, the targeted end-tidal pCO2 were increased to 45 mmHg for 4 minutes to induce blood oxygen changes and increases in blood flow due to pCO2-induced vasodilation. After a 10 minute recovery period, the targeted end-tidal pO2 are increased to 500 mmHg for 4 minutes to increase blood oxygen. Finally, 2 minutes of recovery imaging are recorded while the subject’s end-tidal pO2 and pO2 were targeted to 40 mmHg and 100 mmHg, respectively. Dynamic vascular changes are imaged with 10-20 second time resolution. By analyzing the resulting images and testing for correlation with their clinical parameters, we obtain information on (1) the variation in dynamic vascular changes in the normal breast as a function of age and breast density, (2) time dependence of dynamic changes in StO2 as well as HbT within the breast during hyperoxia, hypercarbia and recovery periods.
Quantitative assessment of indocyanine green enrichment in breast tumors

A. J. Hagen, D. Grosenick, Physikalisch-Technische Bundesanstalt (Germany); A. Pöllinger, S. Burock, P. M. Schlag, Charité Universitätssmedizin Berlin (Germany); H. H. Rinneberg, R. Macdonald, Physikalisch-Technische Bundesanstalt (Germany)

Using indocyanine green (ICG) bound to plasma proteins to serve as macromolecular contrast agent, we recorded time-resolved fluorescence projection mammograms on twenty breast cancer patients in order to assess permeability of tumor microvasculature. Highest fluorescence contrast between carcinomas and surrounding tissue was achieved after ICG was mostly cleared from the blood by the liver (extravascular phase). Inhomogeneous absorption by structures with higher blood content were largely eliminated after dividing fluorescence mammograms by mammograms recorded simultaneously at the excitation wavelength. The resulting ratio images showing the net-fluorescence of extravasated ICG revealed carcinomas at high contrast on a fairly homogeneous background. In order to assess ICG enrichment caused by the higher vessel permeability in cancerous tissue relative to healthy breast tissue these ratio images were analyzed by applying the model of diffraction of fluorescence photon density waves by a spherical object. Tumor position and size, both entering the analytical model, were estimated from transmission measurements with lateral source-detector offsets of 2 cm using a modified tomosynthesis algorithm. Results obtained by fitting time integrated photon counts along a profile including healthy and diseased tissue were in good agreement with those obtained from fits to the corresponding distributions of times of flight of photons and were successfully validated on breast-like fluorescent phantoms. From our analysis of the in vivo data, ICG enrichment of up to 10 and even higher was found for carcinomas in the extracellular phase.

A correlation study between DOT-measured hemoglobin concentrations and histopathological proliferation parameter Ki67 and CD34 stained microvessel density in breast cancer

S. H. Chung, Univ. of Pennsylvania (United States); M. D. Feldman, Hospital of the Univ. of Pennsylvania (United States); R. Choe, S. Pathak, Univ. of Pennsylvania (United States); F. Valdivieso, Hospital of the Univ. of Pennsylvania (United States); D. Martinez, The Children’s Hospital of Philadelphia (United States); A. G. Yodh, Univ. of Pennsylvania (United States)

We investigate the relationship between macroscopically measured hemoglobin concentration and oxygenation of breast tissues, and Ki67 and CD34 stained micro-vessel density. The latter quantities provide microscopic information about breast cancer pathology and physiology. DOT was employed to measure 20 infiltrating ductal carcinoma patients. The Ki67 and micro-vessel density were measured at several ROIs on each slide and a weighted mean by the area was taken for each slide. The percent of nuclei stained by antigen Ki67 (0.89-25.3%, median=5.67%, N=16) was positively correlated with HbO2 (relative oxygen-hemoglobin concentration, R=0.44, p-value: 0.087, Spearman rank correlation) and rST02R=0.36, p-value: 0.165, Spearman). CD34 stained micro-vessel density (number of vessels in unit area (mm²), 1.38±0.5 - 52.72±0.5/mm², median=13.75±0.5/mm², N=20) was positively correlated with HbO2 (R=0.43, p-value: 0.058, Pearson correlation) and rTHC (R=0.34, p-value: 0.148, Pearson). The positive correlations between HbO2 and both Ki67 and micro-vessel density suggest that in tissues wherein more cells are proliferative, more oxygenated blood is supplied through micro-vessels. This result indicates that mitosis (measured by Ki67) requires aerobic conditions. The differences between the measurement tissue volume, i.e., between macroscopic tissue volumes measured by DOT and microscopic tissue quantification of Ki67/CD34, is probably one of the reasons for the relatively low correlation between the two methods. Regardless of the scope difference, however, the results of this study suggest that DOT can indirectly measure proliferation of cancer cells by quantifying oxygenated hemoglobin in cancer tissues.

Changes in endogenous tissue chromophores during tumor growth and post cyclophosphamide treatment on rat breast tumors

J. G. Kim, T. B. Rice, S. Ueda, Beckman Laser Institute and Medical Clinic (United States); E. L. Nelson, Univ. of California, Irvine (United States); A. E. Cerussi, B. J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

Previous clinical studies have shown that there are significant changes in optical signals after chemotherapy treatment within a few days. In this study, we have inoculated 1 million of 13762 MAT B III murine mammary tumor cells in the breast of Fisher 344 rats. When the tumor diameter reached ~1cm (~0.5cm³), the rats were divided into the control (n=3) and treatment (n=5) group. Treatment group rats were treated with a single dose of cyclophosphamide (100 mg/kg, i.p.) while control group were administered with the same amount of saline. Tumor size and body weight were measured to monitor the therapeutic or side effects of cyclophosphamide treatment. A spatially modulated imaging system was employed to obtain oxy-, deoxy-, total hemoglobin concentration, tissue oxygen saturation, fat, and water of tumors during their growth and after cyclophosphamide administration. Oxy-, total hemoglobin concentration, and water increased as tumor grows while deoxyhemoglobin concentration initially decreased then increased as tumor grows. After cyclophosphamide treatment, tumors continued to grow two more days then regressed. However, oxy-, deoxy, and total hemoglobin concentrations reached their maximum at 1 day post cyclophosphamide treatment then gradually decreased along with tumor regression. For control tumors, we let them to grow ~2cm (~4.2cm³) in diameter and watched optical signals changes. Oxy- and total hemoglobin increased until tumor volume is ~1.5cm³ then decreased as tumors continue to grow while deoxyhemoglobin continued to increase. As a conclusion, we found that endogenous tissue chromophores respond to chemotherapy earlier than physical tumor size change which supports clinical reports.

Instrumentation of a 3rd generation frequency domain multispectral DOT breast imager

H. Y. Ban, Univ. of Pennsylvania (United States); S. D. Konecky, Univ. of California, Irvine (United States); H. Chung, Univ. of Pennsylvania (United States); F. Moscatelli, Swarthmore College (United States); D. R. Busch, S. Pathak, R. Choe, A. G. Yodh, Univ. of Pennsylvania (United States)

We describe the implementation of our 3rd generation Diffuse Optical Tomography (DOT) breast imaging device. Building on previous experiences with our current (2nd gen) clinical device and bench-top experiments, we have designed a system with higher absorption and scattering quantification and image resolution. New features include increased number of source-detector pairs (10^7) for the largest clinical breast imaging data yet reported, high speed image acquisition for pharmacokinetic imaging, and multi-spectral measurements in the Frequency Domain (FD) to decrease absorption and scattering crosstalk.
In this system, FD measurements are made with heterodyne detection; intensity modulated source (70 MHz) and gain modulated CCD mounted image intensifier (70 MHz, +1 Hz) produce a cross correlation frequency signal (1 Hz) slow enough to be measured with a CCD. Multiple exposures of this 1 Hz signal is fit to a sinewave to obtain the DC intensity, amplitude, and phase of the diffusing photon wave. Multi-spectral (6 wavelengths) data is taken in the parallel-plane geometry with 209 source positions and detected by 512x512 CCD pixels. The breast tank and CCD are now mounted on a rotating arm, allowing subjects to be imaged in the cranio-caudal and/or mediolateral orientation, permitting multiple views of the same cancer and optimization of viewing angle based on cancer position. Results from characterization of the instrument with phantom experiment will be presented.

Method yields oxygenation maps. We have now implemented depth discrimination capabilities by introducing two additional collection optical fibers, one placed 1.3 cm off-axis in the x direction and one placed 1 cm off-axis in the y direction. These off-axis collection optical fibers allow for measurements of the depth coordinate (z) of optical inhomogeneities by a weighted spatial cross-correlation analysis between on-axis and x/y off-axis second-derivative images. Validation of the new depth discrimination capabilities has been performed on tissue-like phantoms. We report depth resolved images of healthy human breasts that show the effectiveness of this imaging system in detecting blood vasculature within the breast and in assigning the depth within breast tissue of detected blood vessels. We also report our initial clinical results on cancer-bearing breasts that show the potential of our spectral oxygenation imaging approach in detecting breast cancer.

Handheld video-rate fluorescence diffuse optical tomography for mapping sentinel lymph nodes


We have developed a fiber-based handheld video-rate fluorescence molecular tomography (FMT) system for noninvasive in vivo sentinel lymph node (SLN) mapping. FMT provides molecular contrast with potentially higher sensitivity and more accurate deep tissue localization (> 3 mm) compared to reflectance fluorescence imaging. While most FMT systems image on the time scales of minutes to hours, FMT has the potential for imaging at speeds above the respiratory and cardiac fluctuations to capture pharmacokinetics and pharmacodynamics of contrast agents. Currently, most FMT systems are CCD-camera based and subsequently have inherent limitations of low dynamic range and slow scan times. Here, we demonstrate the feasibility of implementing an avalanche photodiode based handheld fluorescence diffuse optical tomography (DOT) system with high dynamic range (106) and cross-talk rejection (10-6) and 30 Hz DOT frame rate. The system is composed of a grid of alternating 12 sources and 13 detectors. We acquire frequency encoded fluorescent emission and reference transmission light levels at each detector through individual bandpass filters optimized for fluorescence emission. The data is then reconstructed using a normalized Born approach. Accurate localization was established by imaging fluorescent targets embedded in agarose phantoms at depths up to 22 mm. We have also confirmed in vivo performance by imaging the accumulation of a N IR dye into a lymphnode above the shoulder of a rat after injection into the forepaw. These results suggest that handheld video-rate fluorescent DOT has potential as a clinical tool for noninvasive SLN mapping to monitoring cancer therapy progress.

3D tomographic breast imaging using a handheld optical imager

S. Erickson, S. Martinez, J. Gonzalez, M. Roman, A. Nunez, A. Godavarty, Florida International Univ. (United States)

Hand-held optical imagers are currently developed toward clinical imaging of breast tissue. However, the hand-held optical devices developed to date have been used towards spectroscopic measurements rather than 3D tomographic imaging since they are not able to coregister the image to the breast tissue geometry. In our Optical Imaging Laboratory, we have developed a hand-held optical imager which has demonstrated 3D tomography in tissue phantoms with simple geometries using manual coregistration. Automated coregistration facilities were implemented with the device in order to perform coregistered imaging on breast tissue of different curved geometries. Preliminary studies were carried out to validate coregistered imaging in normal human subjects. Herein, extensive studies are performed to improve the accuracy of coregistered imaging of human subjects with different tissue volumes and geometries. Normal female volunteers age 21 and above were recruited for the IRB approved study. Spherical targets were placed noninvasively under the flap of the breast tissue and imaged through the tissue. The targets were filled with 1% Liposyn combined with appropriate absorption or fluorescence contrast agents for diffuse or fluorescence imaging studies, respectively. Optical images were collected and coregistered to the breast tissue geometry using MATLAB/LabVIEW software developed in house. Currently 3D tomography analysis is carried out using the coregistered optical images of the breast tissue and an approximate extended Kalman filter based reconstruction algorithm. The results will demonstrate the potential to perform automated coregistered imaging and feasibility of 3D tomographic imaging in vivo.

Near-infrared optical mammography with broadband spectral imaging and depth discrimination

Y. Yu, A. Sassaroli, Tufts Univ. (United States); M. J. Homer, R. A. Graham, Tufts Medical Ctr. (United States); S. Fantini, Tufts Univ. (United States)

We have previously reported the development of an instrument for diffuse spectral imaging of the human breast operating over the wavelength range 650-900 nm. This instrument images the slightly compressed human breast in a planar geometry by introducing a cross correlation frequency filter on the x-y plane, of a 4 mm illumination optical fiber and a 5 mm collection optical fiber that are collinear and located on opposite sides of the breast. An edge-correction algorithm accounts for breast thickness variability over the x-y plane, a second-derivative imaging algorithm enhances the image resolution, and a paired-wavelength spectral method yields oxygenation maps. We have now implemented depth discrimination capabilities by introducing two additional collection optical fibers, one placed 1.3 cm off-axis in the x direction and one placed 1 cm off-axis in the y direction. These off-axis collection optical fibers allow for measurements of the depth coordinate (z) of optical inhomogeneities by a weighted spatial cross-correlation analysis between on-axis and x/y off-axis second-derivative images. Validation of the new depth discrimination capabilities has been performed on tissue-like phantoms. We report depth resolved images of healthy human breasts that show the effectiveness of this imaging system in detecting blood vasculature within the breast and in assigning the depth within breast tissue of detected blood vessels. We also report our initial clinical results on cancer-bearing breasts that show the potential of our spectral oxygenation imaging approach in detecting breast cancer.

Multimodal compressed breast imaging

S. A. Carp, Q. Fang, C. Wanyo, S. J. Isakoff, D. A. Boas, Massachusetts General Hospital (United States)

Diffuse optical tomography (DOT) is an emerging technology targeted at imaging tissue vascular and metabolic markers and has shown great promise for cancer detection and therapeutic monitoring. Time-resolved measurements of breast tissue changes that occur either intrinsically or in response to external gaseous or mechanical stimulation may lead to the development of new optical disease markers to complement those derived from steady state measurements. In particular, breast tissue hemodynamics following external compression are governed by the interplay of tissue bio-mechanics and metabolic activity, thus offering access to a rich information content related to these mechanical and metabolic characteristics. We have built multi-wavelength dynamic optical tomography instrumentation for both standalone compressed breast measurements, as well as a co-registered optical MRI breast compression platform that integrates a compression mechanism, an optical interface, and a custom phased-array breast coil system for
Dynamic breast imaging with a digital optical tomography system

M. L. Flexman, Columbia Univ. (United States); R. M. Al Abdi, SUNY Downstate Medical Ctr. (United States); B. Reig, Columbia Univ. Medical Ctr. (United States); C. J. Fong, J. M. Masciotti, Columbia Univ. (United States); D. Hershman, E. Desperito, Columbia Univ. Medical Ctr. (United States); R. L. Barbour, SUNY Downstate Medical Ctr. (United States); A. H. Hielscher, Columbia Univ. (United States)

Continuous wave optical tomography is non-ionizing, uses endogenous contrast, and can be performed quickly and at low cost which makes it a suitable imaging modality for breast cancer screening. Using multiple wavelengths of light to illuminate the breast at various angles, three-dimensional images of the distribution of chromophores such as oxy- and deoxy-hemoglobin can help identify cancerous tissue. Dynamic optical imaging can provide additional insight into cancer characteristics such as angiogenesis and metastasis. Dynamic imaging of the breast has been performed using stimuli such as an injection of indocyanine green (ICG), the application of pressure, respiration of gases, and through a breath hold. Here we present the first clinical data acquired with our novel digital breast imaging system. This system is based upon a Digital Signal Processor (DSP) architecture that leverages the immediate digitization of acquired analog data to reduce noise and quickly process large amounts of data. Employing this new instrument we have investigated the dynamic changes due to a breath hold and its potential for use in breast cancer screening. Over the course of a breath hold, images have been collected simultaneously from both breasts at a rate of more than 1.5 frames per second with 32 sources and 64 detectors per breast and four wavelengths of light at 765, 805, 827, and 905nm. Initial results involving 5 healthy volunteers and 5 breast cancer patients support the potential use of dynamic imaging for breast cancer detection.

Continuous-wave and frequency domain optimization in breast tomosynthesis-guided diffuse spectroscopy

K. E. Michaelsen, V. Krishnaswamy, B. W. Pogue, K. D. Paulsen, Dartmouth College (United States)

Multiple wavelengths of near infrared light can be used to assess information about tissue characteristics including hemoglobin, water and lipid concentrations. Combined with breast tomosynthesis, it provides both physiological and spatial information that may be useful in clinical situations. Tomosynthesis images have been shown to be of equal or superior quality to mammography, and provide excellent spatial visualization of the tissue, with special value for imaging radio-dense tissues.

The goal of this study was to optimize frequency domain and continuous wave components of the spectroscopy system within spatial, time and monetary constraints. Breast tomosynthesis imaging is performed under compression so minimization of exam time is an important consideration for a combined system attaining complete co-registration of the two images. Patient tomosynthesis images were segmented into adipose, fibroglandular and malignant tissue types. Broadband near infrared illumination was simulated at ten different wavelengths in a projection geometry. Guided by the segmented image, the detector measurements were used to produce spatial maps of hemoglobin, water and lipid concentrations in the tissue. The frequency domain system is necessary for determining the light scattering within the tissue. The continuous wave components determine the tissue’s light absorption in a more time efficient manner. This study compares reconstruction accuracy of several frequency domain and continuous wave configurations. This analysis will help determine the optimum hardware components for a combined system that is currently being built, in order to achieve the best possible accuracy in quantifying tissue properties within the constraints of a multimodality imaging system.

MRI-guided imaging pulse-oximetry for visualization of breast hemodynamics

Z. Li, S. Jiang, V. Krishnaswamy, S. C. Davis, K. D. Paulsen, B. W. Pogue, Dartmouth College (United States)

High frame rate Near-infrared (NIR) tomography of tissue can be used to measure functional properties, providing potentially useful diagnostic information, especially in tumors where optical contrast caused by hemoglobin content and oxygen saturation differences are large. A NIR tomography system with parallel spectral-encoded source arrays at dual-wavelength bands has been built to quantify the temporal NIR contrast available for imaging thick tissues of several centimeters across inside a 3Tesla MRI. The systems were integrated through a customized breast MR coil interface to provide tissue structural information for improved image reconstruction. An MR-compatible pulse oximeter was synchronized to the NIR system to provide heartbeat frequency range during imaging, and frequency lock for post-processing of the signals. Three healthy subjects have been imaged in this hybrid system, and each subject was imaged twice at different dates. The right breast of each subject was imaged by both the NIR system and the MR system at the same time, and the pulse oximeter was attached to the subject’s finger. The acquired NIR data was preprocessed and band-pass filtered at to the heartbeat range before calibration and reconstruction. The breast tissue of each subject was segmented according to the coronal MR images into an adipose region and a fibroglandular region to perform region-based reconstruction. The periodic variation of the absorption coefficients
and oxygen saturation in two regions were compared, and their time relevance was also analyzed to quantify the variation of the system and the signal to noise level of imaging regions of the breast.

7896-59, Session 12

Breast tumor hypoxia mapping using ultrasound guided diffuse optical tomography

N. C. Biswal, Y. Xu, Q. Zhu, Univ. of Connecticut (United States)

Tumor hypoxia is an important indicator of tumor metabolism and tumor response to various forms of therapy. Currently, no imaging modality exists that can directly map tumor hypoxia non-invasively. We present an ultrasound guided diffuse optical imaging technique for precisely measuring the tumor oxygenation. The approach employs ultrasound structural information as a-priori knowledge for diffuse optical imaging. Hypoxia mapping is achieved using endogenous chromophores such as oxy- and deoxy- hemoglobin in the tissue. Because oxy- and deoxy- hemoglobin respond differently at different wavelengths, four different laser diodes of wavelengths 740 nm, 780 nm, 808 nm and 830 nm are used for mapping tumor hypoxia by diffuse optical imaging. Hypoxia model experiments are performed using phantoms at different oxygenation conditions (Hemoglobin oxygen saturation: 6%-100%) representing the hemoglobin oxygenation range in tissue. Targets of different sizes mimicking different development stages of breast tumors, 1.0 cm to 2.5 cm diameter in 0.5 cm steps, are tested to validate the oxygen saturation measurement accuracy with target size. The reconstructed hemoglobin oxygen saturation is within 90-96 % of the true oxygen measurements (by using an invasive oxygen probe) for oxygen saturation 25%-100% and the accuracy is lower for less than 25% oxygenation. Since the oxy-hemoglobin has stronger absorption at 830 nm, for combined targets with deoxy-hemoglobin at the center, we see a low contrast at the center of the image and a high contrast at the periphery of diffuse images. A strong correlation is found between phantom experiments and observations seen from advanced breast cancers.

7896-60, Session 13

Optimization of time-gated small animal fluorescence tomography

M. J. Niedre, N. Valim, Z. Li, J. L. Brock, Northeastern Univ. (United States)

Among the major technical challenges associated with small animal fluorescence mediated tomography (FMT) is the relatively poor resolution due to the high degree of light scatter in vivo. We and others have shown previously that time-gated detection of “early photons” (EPs) - i.e. photons that undergo preferentially less scattering than un gated photons - can be used to improve FMT imaging resolution in mice. However, it is also known that measurement of EPs can degrade instrument noise performance and therefore reduce sensitivity and quantitative accuracy versus ungated photons. In this presentation, we describe the results of several of our recent studies designed to optimize imaging performance with time-gated FMT. First, we experimentally measured instrument photon density sensitivity functions (PDSFs) with a picosecond pulsed laser, time-correlated single photon counting and an absorption perturbation approach. It was determined that the volume of PDSFs of early photons were reduced by a factor of 6 to 9 versus ungated photons over a range of optical properties and pathlengths useful for small animal imaging. The optimal operating point was determined by comparing the improvement in imaging PDSFs versus instrument noise performance at different time gates. Next, we developed a novel image reconstruction strategy that combined EPs and ungated photons in a ‘hybrid’ approach. We found that by using this approach the respective advantages of both - i.e. imaging resolution and noise performance - were retained. This was demonstrated in simulation studies and experimentally in nude mice with fluorescent (Alexa-Fluor 750) inclusions.

7896-61, Session 13

Hyperspectral fluorescence tomography of quantum dots

A. D. Klose, Columbia Univ. (United States)

Hyperspectral excitation-resolved fluorescence tomography (HEFT) offers an alternative way how fluorescence tomography (FT) of quantum dots could be performed. It only uses a single excitation source with tunable wavelength selection for fluorescence stimulation. No spatial source-detector multiplexing is required for the purpose of three-dimensional image reconstruction. This approach suggests a technologically simpler design, but still providing image reconstruction results comparable to images obtained by conventional FT. The excitation field is a function of tissue absorption that varies over three orders of magnitudes between 560 nm and 660 nm. The relative amount of excited quantum dots is, therefore, wavelength-dependent as well and the emission strength of the fluorescent reporter probes will encode for their location inside tissue. Subsequently, the fluorescence light is measured on the surface at only a single wavelength, but for different partially overlapping wavelength bands of the excitation spectrum. The partial current on the tissue surface is cast into an algebraic system of equations, which is solved for the unknown quantum dot distribution with an expectation-maximization (EM) method. Last, the light propagation model for predicting the boundary current is based on two coupled diffusion equations of a SP3 radiative transfer model with partially reflective boundaries. It is solved with a parallel blocking-off finite-difference method on an eight core Intel processor computer using the message passing interface (MPI). The image reconstruction is achieved in less than five minutes. In vivo and ex vivo image reconstruction results will be presented.

7896-62, Session 13

Time-domain fluorescence diffuse optical tomography for live animals by total-light algorithm

G. Nishimura, K. Awasthi, K. Locharoenrat, Hokkaido Univ. (Japan); S. Okawa, Y. Yamada, The Univ. of Electro-Communications (Japan)

We are reporting the first trial image reconstruction of an implanted fluorescent target into a live rat abdomen. We used the temporal profiles of the excitation and fluorescence light to reconstruct the image by a simplified fluorescence diffuse optical tomography (FDOT) algorithm, so-called the total-light algorithm. The idea of this technique is to construct the virtual optical signal without the fluorescence targets from the fluorescence and excitation light intensities. As the result, the problem of FDOT is reduced to two separate DOT problems, yielding the absorption images with and without the fluorescence targets. We made a glass capillary target (1.6mm I.D. and 3-4cm length) containing indocyanine green (ICG) and Intralipid mixture solution and implanted into the abdomen of an anesthetized rat. We attached several source-detection pairs around the abdomen to obtain time-resolved transmitted intensities by a time-resolved instrument. Then, using the mean transit time (MTT) of the time-resolved intensity profile, we reconstructed two 2D tomographic images of the absorption coefficient with and without the fluorescence targets. It is difficult to identify the anatomical structure or the blood distributions in either one of the two absorption images reconstructed from the virtual light and excitation light. Interestingly, however, the difference of the images highlights the target. This suggests that this algorithm is robust to the artifacts of the reconstructed images under the uncontrollable environment of in vivo measurements. We will discuss the absorption images with different subjects.
Tumor hypoxia fluorescence imaging using 2-nitroimidazole bis-carboxylic acid indocyanine dye conjugate

N. C. Biswal, C. Pavlik, M. B. Smith, Univ. of Connecticut (United States); L. T. Kuhn, K. P. Claffey, Univ. of Connecticut Health Ctr. (United States); Q. Zhu, Univ. of Connecticut (United States)

We present tumor hypoxia mapping by diffuse optical fluorescence tomography and fluorescence spectroscopy. A novel 2-nitroimidazole bis-carboxylic acid indocyanine dye conjugate has been developed for tumor-targeted hypoxia fluorescence imaging. The hypoxia probe has been evaluated in-vitro using 4T1 tumor cell lines and in-vivo tumor targeting in mice. In-vivo tumor targeting in six mice demonstrated that a measured half-life of 2-nitroimidazole-indocyanine dye wash out in the tumor was significantly longer (112±32.37 minutes) than that of bis-carboxylic acid indocyanine dye (69.75±14.01 minutes). The bis-carboxylic acid indocyanine dye was completely washed out from the tumor site after 5 hours, but 2-nitroimidazole-ICG remained for more than 21 hours in the tumor site. Near infrared fluorescence images of mice tumors showed a 2.6-fold contrast of dye uptake with hypoxic conjugate injection (7.46±1.68 µM) compared to that with indocyanine dye injection (2.9±0.60 µM). Significant differences in fluorescence emission and dye wash out half-life between the hypoxia probe and unconjugated indocyanine dye were observed in all tumors of size from 9 mm to 13 mm in the largest dimension. The in-vitro cell studies were performed to assess fluorescence labeling comparing hypoxia to normoxia conditions. A fluorescence emission ratio of 2.5-fold was found between the cells treated with the 2-nitroimidazole-indocyanine dye and incubated under hypoxia compared to the cells in normoxia condition. Hypoxia specificity was also confirmed when compared to cells treated with unconjugated indocyanine dye alone.

Multisite and multidepth tumors localization enhancement after autofluorescence removal

A. Montcuquet, F. P. Navarro, L. Hervé, Commissariat à l’Énergie Atomique (France); J. I. Mars, Institut National Polytechnique de Grenoble (France); J. Dinten, Commissariat à l’Énergie Atomique (France)

Fluorescence imaging in diffusive media detects or reconstructs tumors tagged by injected fluorescent biomarkers. In reflectance geometry, the fluorescence signal of markers exponentially decreases with light travel distance. Despite near infrared light is used to maximize tissue penetration and minimize natural fluorescence of biological tissues (autofluorescence), deep embedded markers detection is compromised by autofluorescence unwanted detection.

Therefore, to explore thick media (breast, prostate for example), autofluorescence removal is a sine qua non condition. Only a few methods have been explored to remove autofluorescence in NIR, and to provide adequate signals for further FDOT reconstructions.

We suggest using spectroscopic acquisitions processed by Non-negative Matrix Factorization (NMF) method to discriminate fluorescence sources according to their fluorescence spectra, and thus only retain fluorescence signal of interest for FDOT reconstruction.

To get spectrally resolved acquisitions, object of interest is scanned with a laser at 690 nm, and emitted back fluorescence signal is collected by an imaging spectrometer coupled with a CCD camera.

NMF processing on fluorescence acquisitions of mice with a single subcutaneous tumor allowed doubling tumor/healthy tissue ratio. Experiment was then run with two capillary tubes filled with ICG-LNP markers simultaneously inserted in mice 1mm and 6mm deep in tissues. Even if deepest markers signal was mixed up with autofluorescence signal and masked by intensive 1mm deep markers contribution, NMF algorithm succeeded to eradicate autofluorescence and detected deepest markers.

Unmixing and autofluorescence removal results on multi-site and multi-depths mice cancer will be presented at BIOS 2011 symposium.

Fluorescence lifetime molecular tomography of a genetically expressed FRET construct in a mouse

J. A. McGinty, Imperial College London (United Kingdom); V. Y. Soloviev, Univ. College London (United Kingdom); D. W. Stuckey, K. B. Tahir, R. Laine, Imperial College London (United Kingdom); D. J. Wells, The Royal Veterinary College (United Kingdom); J. V. Hajnal, A. Sardini, Imperial College London (United Kingdom); S. R. Arridge, Univ. College London (United Kingdom); P. M. W. French, Imperial College London (United Kingdom)

Fluorescence molecular tomography (FMT) aims to reconstruct the 3-D optical and fluorescence properties of turbid (highly scattering) media. We demonstrate a tomographic imaging system with wide-field time-gated detection applied to fluorescence lifetime imaging (FLIM) of a genetically expressed Förster resonance energy transfer (FRET) construct in a mouse. A cytosolic preparation of the FRET construct, consisting of the fluorescent proteins eGFP and mCherry linked by a 6 amino acid flexible linker, was characterised in a time-resolved spectrophotometer and compared to a mixture of eGFP and mCherry (not linked) and eGFP alone. The plasmds coding for these fluorescent proteins were then electrooptically into the anterior tibialis of mice. The mice were sacrificed five days post-electroporation (at the peak of protein expression). Transmitted excitation and fluorescence signals were acquired using a wide-field time-gated intensifier system at 10 degree rotation steps over a full revolution. An inverse scattering algorithm, based on a diffusion analogue of the back-projection algorithm obtained by minimizing an appropriate cost functional, was used to reconstruct the optical properties of the mouse leg including the position, fluorescence lifetime and quantum yield of eGFP for all constructs. In both spectrofluorimeter measurements and tomographic image reconstructions, there was a significant reduction in the fluorescence lifetime of eGFP in the FRET construct compared to the controls. A reduction in the relative quantum yield was observed alongside the reduction in the lifetime, confirming that we have demonstrated ex vivo tomographic reconstruction of fluorescence parameters reporting a FRET interaction.

Imaging sub-nanomolar concentrations through more than five centimeters of tissue with time-domain diffuse fluorescence tomography

F. Leblond, F. El-Ghussein, B. W. Pogue, Dartmouth College (United States); K. M. Tichauer, Dartmouth College (Canada); R. W. Holt, Dartmouth College (United States)

Photodetection based on time-correlated single-photon counting technology is used to demonstrate that diffuse fluorescence tomography can detect fluorophores in transmission through more than five centimeters in tissue-simulating phantoms, and that this can be achieved for concentrations lower than $C = 1 \text{ nM}$ with dyes commonly used for in vivo pre-clinical biological studies. The results that are presented demonstrate that an unprecedented level of sensitivity can be achieved with time-domain fluorescence tomography allowing this technology to be used for applications involving animals larger than mice as well as applications where limited contrast is available. A multi-modal system combining microcomputed tomography (microCT) and time-domain fluorescence tomography is used to image a 5 cm-diameter cylindrical tumor.
phantom containing a 5 mm-diameter inclusion where a mixture of fluorophore and intralipid has been injected. The optical properties of the phantom (absorption and reduced scattering coefficient) were characterized and shown to be consistent with optical properties of rodents such as mice and rats. Fluorescence tomography images were reconstructed using the structural information obtained from the microCT (outer surface of the interrogated sample) as prior information for light transport modeling. Results are presented showing that optical images can be reconstructed down to sub-nanomolar concentrations and that the system response is linear to concentration variations. In vivo reconstruction results are presented supporting the fact that the system can be used to image biological models associated with rats.

7896-67, Session 14

Autofluorescence insensitive fluorescence diffuse optical tomography with multispectral priori regularization

P. Svenmarker, C. T. Xu, H. Liu, S. Andersson-Engels, Lund Univ. (Sweden)

Fluorescence diffuse optical tomography has many strengths, but also weaknesses such as tissue autofluorescence and a mathematically ill-conditioned inverse problem. Tissue autofluorescence typically sets the limit for the sensitivity. The unstable inverse problem yields multiple non-unique solutions. Upconverting nanoparticles (NaYF4 : Er3+ /Tm3+), which emits anti-stokes shifted fluorescent photons at two different wavelengths (650 nm and 800 nm) upon near-infrared excitation (975 nm), make autofluorescence insensitive tomography possible. We propose to use these particles to stabilize the inverse problem through multispectral priori regularization.

Complete autofluorescence free tomography was achieved by detecting the fluorescence signal at a shorter wavelength than the excitation wavelength, a spectral region which does not contain any autofluorescence. Inverse problem stabilization was accomplished by rendering a multispectral spatially varying regularization map, based on the fluorescence emission migration and its tissue optical property dependence. The wide spectral distance between the two emission lines helps to create a high contrast regularization map.

Finite element computer simulations and tissue phantom measurements were made. Two different regularization methods were compared: the multispectral versus Tikhonov regularization. To evaluate their performance, the minimum number of projection needed for a given image quality was found. The computer simulations shows that the multispectral regularization needs far less projection, in comparisons to Tikhonov regularization, for creating equal quality reconstructions. Ongoing tissue phantom reconstructions suggest a confirmation of the simulation results. In conclusion, upconverting nanoparticles can be used to image biological models associated with rats.

7896-68, Session 14

Autofluorescence suppression in fluorescence tomography of quantum dots using time-gated detection and ultrafast pulsed laser

X. Zhang, C. Badea, G. A. Johnson, Duke Univ. (United States)

Quantum dots (QDs) are being used widely in fluorescence tomography because they are highly quantum-efficient, photostable, engineerable, and conjugatable. Despite these advantages of QDs for in vivo animal imaging, autofluorescence is still one of the most fundamental limitations in optical data acquisition. This limitation is particularly detrimental to image reconstruction for low-light imaging, e.g., free-space fluorescence tomography. In animals studies, fluorescence emission from exogenous fluorescent probes (QDs) cannot be effectively differentiated from endogenous broad-spectral substances (mostly proteins) using optical filters. Instead, we made use of another intrinsic optical characteristic of the QDs, very long light life-time compared to proteins, to suppress the background noise due to autofluorescence. Fluorescent emission from the QDs was excited using an ultrafast pulsed laser, and was detected using a time-gated imaging intensifier. A tissue-simulating imaging phantom was used to demonstrate the effectiveness of this method. The reconstruction was significantly improved compared to non-time-gated acquisition. This study was funded by NIH/NCRR P41 RR05959.

7896-69, Session 14

Unmixing heterogeneous fluorophore lifetimes with frequency domain diffuse optical tomography

R. E. Nothdurft, S. Achilefu, J. P. Culver, Washington Univ. in St. Louis (United States)

A critical task in imaging complex biological interactions is unmixing contributions from multiple sources within a single voxel or pixel. This task may arise when multiplexing molecular probes or when examining the graded response of a molecular probe to a changing local environment. The fluorescence lifetime (FLT) concept has been well established in fluorescence lifetime microscopy (FLIM) for these unmixing tasks (e.g. to visualize local variations in biological samples such as pH, polarity and the presence of different binding sites). With recent advances in diffuse optical tomography (DOT) the potential exists to extend FLT contrasts to whole body in vivo imaging. Previous work with Time-domain DOT has shown unmixing when a priori knowledge of the lifetime states is available. Unmixing with frequency-domain data in a small animal DOT scanner has not received the same attention. The frequency domain approach has the advantage of using a simple linear model for image reconstruction. Furthermore, frequency domain data (reconstructed using a normalized Born method), has been shown to have high precision phase measurements capable of distinguishing small (<100 ps) differences in the average lifetime of a voxel. In this work we evaluate the use of a multi-frequency domain FLT-DOT system to unmix samples with co-localized lifetimes. Via simulated data we demonstrate how the mechanism works in principle, allowing unmixing with or without a priori knowledge of lifetime. Using experimental data we then separate mixed tissue phantoms. The implications of these results on small animal FLT-DOT will be discussed.

7896-70, Session 15

Can a one-layer optical skin model including melanin and inhomogeneously distributed blood explain spatially resolved diffuse reflectance spectra?

H. Karlsson, Linköping Univ. (Sweden); A. Pettersson, Perimed AB (Sweden); M. Larsson, T. Strömberg, Linköping Univ. (Sweden)

Calibrated diffuse reflectance spectroscopy (DRS) can be used for determining oxygenation and concentration of chromophores in tissue. The estimation of these parameters depends, however, on the selected optical model. The aim of this study was to assess the effect of including melanin and inhomogeneously distributed blood in a one-layer optical skin model. Data from four humans were collected during provocations with local heating, oxygen breathing, venous and systolic occlusion. A two-channel fiber optic probe with source-detector distances 0.4 and 1.2 mm was used. Spectra, in the visible wavelength range, were white-normalized and calibrated using a characterized microsphere suspension. Spectral fitting involved inverse Monte Carlo and Levenberg-Marquardt techniques. Data were analyzed as temporal means for all recordings. Inclusion of melanin decreased the spectral fitting rms-error (3% to...
Detecting peripheral artery disease in the lower extremities using DOT

M. Khalil, H. Kim, Columbia Univ. (United States); I. Kim, R. Dayal, New York Presbyterian Hospital (United States); A. H. Hielscher, Columbia Univ. (United States)

In this study we explore the potential of dynamic diffuse optical tomography (DOT) for the diagnosis and monitoring of peripheral artery disease (PAD) within the lower extremities. PAD affects 8 to 12 million individuals in the United States and is associated with significant morbidity and mortality. The current diagnosis of PAD requires invasive methods, the use of contrast agents and ionizing radiation, or that the patients have compressible arteries. These approaches typically do not allow for effective monitoring of disease progression and co-morbidities such as diabetic neuropathy often render the arteries incompressible and often lead to misdiagnosis. DOT imaging is independent of the compressibility of the arteries, and does not require the use of contrast agents to obtain valuable information about the hemodynamics within the foot. In this particular study we target the response of the cross section of the foot that encompasses the dorsalis pedis artery which is one of the major arteries in the foot. Patients diagnosed with PAD place their foot into a specially designed imaging head and imaging data is acquired as a pressure cuff is applied to the patient’s thigh. The responses to the occlusion and release of the cuff are repeatedly recorded over a 5 minute period. Comparing the data obtained from 5 patients with data from 5 healthy volunteers, we observe difference in the dynamic responses. In general it appears that the vasculature of patients with PAD responses slower and weaker to the occlusion. Therefore the amplitude and time constants of hemodynamic responses differ significantly in patients with PAD as compared to healthy patients.

Real-time assessment of blood volume and blood oxygenation in the skin using multispectral imaging and spatial priors

J. M. Kainerstorfer, J. D. Riley, M. Ehler, L. Najafizadeh, F. Amyot, M. Hassan, R. H. Pursley, National Institutes of Health (United States); S. G. Demos, Lawrence Livermore National Lab. (United States); V. V. Chernomordik, National Institutes of Health (United States); C. K. Hitzenberger, Medizinische Univ. Wien (Austria); A. H. Gandjbakhche, National Institutes of Health (United States)

Diffuse multi-spectral imaging on the skin combined with reconstruction algorithms can retrieve spatial distributions of blood volume and blood oxygenation. Most algorithms use some form of pixel-wise or volumetric reconstruction method, which is computationally expensive and thus time consuming. Multi-spectral images from healthy volunteers’ lower forearm were acquired before, during, and after arterial occlusion, applying 180 mmHg of pressure. Reconstruction of blood volume and oxygenation was performed using a two-layered analytical skin model, the first layer being the epidermis, second one being the dermis. We have shown previously that Principal Component Analysis (PCA) can be used for mapping blood volume and oxygenation in real-time and found that the first and second eigenvector correspond to blood volume and oxygenation, respectively. However, a subject dependent shift was also observed. In order to evaluate the epidermal thickness variations between subjects and its influence on PCA results, we created numerical phantoms with varying thickness. We found that the correlation between eigenvector 1 and 2 with blood volume and oxygenation is distorted if the epidermal thickness is not taken into account, leading to errors as large as 60%. This error could be corrected for, if the thickness is known at every image pixel. We thus performed Optical Coherence Tomography (OCT) measurements on the same area of the forearm as measured with multi-spectral imaging and extracted the epidermal thickness. Using this additional spatial information, we compared results from the reconstruction algorithm and PCA, taking the epidermal thickness into account.

Respiratory challenges to detect cyanide toxicity extent in a sublethal rabbit model

J. G. Kim, J. Lee, S. B. Mahon, D. S. Mukai, Beckman Laser Institute and Medical Clinic (United States); W. C. Blackledge, Univ. of California, San Diego (United States); S. Patterson, Univ. of Minnesota, Twin Cities (United States); G. R. Boss, Univ. of California, San Diego (United States); B. J. Tromberg, Beckman Laser Institute and Medical Clinic (United States); M. Brenner, Univ. of California, Irvine (United States)

Accidental exposure to cyanide during fires and industrial accidents is a continual concern and intentional cyanide poisoning by terrorists could lead to mass casualties. Cyanide poisoning induces lethal histotoxic anoxia and stops aerobic cell metabolism by disabling the function of cytochrome c oxidase in mitochondria. As a result, cells consume less oxygen and therefore, more oxygen becomes available in blood. In this study, we tested our hypothesis that the amplitudes of oxy- and deoxyhemoglobin concentration changes during a respiratory challenge from 100% to 21% oxygen will be smaller as cyanide toxicity increases. For the measurements, New Zealand male white rabbits (~4kg) were administered 10mg of NaCN in 60ml normal saline via the left femoral vein at a rate of 1cc/min. After CN infusion, additional 90min of recovery time was monitored. Respiratory challenges were applied before, during, and post cyanide infusion and changes in oxy- and deoxyhemoglobin concentration throughout the entire experiment were measured from both brain and forearm muscles using two continuous wave near infrared spectroscopy (NIRS) probes. A switch from 100% oxygen to air caused a drop of oxyhemoglobin and increase of deoxyhemoglobin in both brain and forearm muscle. However, the amplitudes of both lessened during cyanide infusion and returned to the baseline level at the end of recovery. These results prove our hypothesis and show that NIRS combined with a respiratory challenge can be a useful non invasive tool for estimating cyanide toxicity extent in vivo.
implanted devices, a two-wavelength, single-distance, continuous-wave iNIRS has been evaluated in-vivo. A weighted difference of the changes in attenuation at two wavelengths, across the isoelectric point of the hemoglobin spectra, was taken to be the microvascular oxygenation trend indicator (O2 Index). Although the exact weight depends on the local vascular distribution and their oxygen levels, the hypothesis that a constant weight may be adequate for hemodynamic trends during short arrhythmic episodes was tested. The sensor was implanted subcutaneously both on fresh tissue and inside scar tissue that formed around a pre-existing implant. In 3 animals each. Attenuations were recorded at 660 and 890 nm during normal sinus rhythm (NSR) and induced ventricular fibrillation (VF). The slope of the O2 Index over 10 seconds was computed for 7 NSR and 8 VF episodes in fresh and 13 NSR and 15 VF episodes in scar tissue pockets. The mean O2 Index slope was significantly different (p<0.0001) between NSR and VF rhythms for both the fresh and scar tissue pockets. Therefore iNIRS may be useful for preventing inappropriate detection of VF during electromagnetic interference, double counting of ECG T-wave as an R-wave, ICD lead failure, electrocardiographic aberancy etc.

7896-75, Session 16

Hyperspectral imaging for monitoring temporal development and healing of diabetic foot ulcer

D. Yudovsky, L. Pilon, Univ. of California, Los Angeles (United States); A. Nouvong, Western Univ. of Health Sciences (United States)

Foot ulceration is a debilitating comorbidity of diabetes that may result in loss of mobility and amputation. This study aims to observe temporal changes in local epidermal thickness and oxyhemoglobin concentration over the entire plantar of the foot and to gain insight into the mechanism of foot ulcer formation and healing. In fact, inflammation and necrosis preemt ulceration and can result in vascular and structural changes in the skin prior to ulceration. It can also occur and complicate ulcer healing. Previous studies estimated oxyhemoglobin and deoxyhemoglobin concentrations around pre-ulcerative and ulcer sites on the diabetic foot using commercially available hyperspectral imaging systems. These measurements were successfully used to detect tissue at risk of ulceration and predict the healing potential of ulcers. The present study combines non-invasive hyperspectral imaging with a two-layer optical model and inverse algorithm - previously validated with experimental from in vivo measurements on human skin - to monitor ulcer development and healing on diabetic feet. It shows epidermal thickening and decrease in oxyhemoglobin concentration can also be detected prior to ulceration at pre-ulcerative sites. In addition, thick calluses were observed around formed ulcers. The method was also able to observe reduction in the epidermal thickness combined with an increase in oxyhemoglobin concentration around the ulcer as it healed and closed. These observations can be used for early prediction of diabetic foot ulceration in a clinical setting.

7896-76, Session 16

Deep illumination angular domain spectroscopic imaging: tissue-mimicking phantom study

Y. Zhang, F. Vasefi, Simon Fraser Univ. (Canada); E. Ng, A. Charnson-Reig, Lawson Health Research Institute (Canada); B. Kaminska, Simon Fraser Univ. (Canada); J. Carson, Lawson Health Research Institute (Canada)

The Angular Filter Array (AFA) is a silicon micro-machined optical collimator. It has proven to be an effective device for imaging turbid samples since it only accepts image-forming ballistic and quasi-ballistic photons. This paper describes a novel Angular Domain Spectroscopic Imaging (ADSI) technique that utilizes deep illumination from the front surface of the sample and an AFA to optically filter the back-scattered photons emitted from the sample. The approach permits spectroscopic imaging of turbid samples too thick for trans-illumination imaging. An ADSI system was constructed from a broadband near infrared (NIR)light source, a high speed monochromator, two line illuminators, an AFA, and a line camera. The two incident illumination line patterns were focused on the surface of a tissue-mimicking phantom at 45° and 135°, respectively, with the incident patterns 1.1 mm apart. The AFA was placed between the line illuminators and collected scattered photons that were emitted normal (90°) to the surface of the phantom. Each phantom contained three groups of indocyanine green (ICG) doped inclusions at depths from 1 to 3 mm. By scanning the AFA/illumination system over the sample, the intensity of the scattered light along the normal direction of the surface was acquired as a function of location and wavelength, Spectral images of each inclusion were successfully captured and analyzed, demonstrating that ADSI could detect subsurface features that differed in absorption and/or scattering properties from the surrounding medium. The results suggested that deep illumination ADSI could be implemented as a non-invasive tissue imaging and spectral mapping method.

7896-77, Session 16

Hierarchical segmentation for improved image reconstruction in diffuse optical tomography of human prostate cancer

V. C. Kavuri, Z. Lin, H. Liu, The Univ. of Texas at Arlington (United States)

The inclusion of anatomical prior information in reconstruction algorithms can improve the quality of reconstructed images in near infrared diffuse optical tomography (DOT). The prior information on possible locations of human prostate cancer from trans-rectal ultrasound (TRUS) is very limited and could be false negative, which may lead to biased reconstructed images. The prior information can be obtained by simultaneous measurements or individual measurements from two or more modalities. But in case of TRUS-coupled, DOT probe, the inverse problem could be worse due to a limited number of measurements. Motivated by these shortcomings, we have focused in our study on (1) the development of a hierarchical segmentation approach to improve the reconstruction algorithm for DOT and (2) to compare the reconstructed optical properties using a TRUS-NIR-combined probe and NIR-standalone probe. With computer simulations, we generated a mesh which resembled the rectal wall and had the anomaity at 2-cm depth below the rectum. The optodes were placed on the surface of the rectal wall, resembling the probe array touching the rectal wall. Furthermore, the experimental data were taken using a DOT probe array, similar to that used in the numerical simulation. A laboratory phantom was made of gelatin-intralipid for the laboratory experiments with two different probe geometries (rows x columns: 8x2, 6x3 for TRUS-NIR, NIR standalone), respectively. A continuous wave system was used in both simulations and experiments to determine the optical properties. Reconstructed images from both probe configurations demonstrate that hierarchical segmentation is an effective means to improve image reconstruction in DOT.

7896-78, Session 16

Characterization of 2D surface imaging of tissue optical properties using a submillimeter fiber optic probe

V. Sharma, H. Liu, The Univ. of Texas at Arlington (United States)

Broad-band light reflectance spectroscopy (LRS) of tissue with sub-millimeter fiber optic probes in the visible and near-infrared range has been used for differentiation of tissue types, identification of cancer, and measurement of stimulus-induced physiological responses. So far, single point measurement has been the major setup to determine local optical
properties of tissue. However, in many applications, it is often of interest to form a two-dimensional (2D) image of tissue rather than just to obtain a parameter at a single point, as in cases of cancer margin detection or intraoperative perfusion measurement. It is thus imperative to expand the LRS technique to multipoint measurement covering a larger surface area. In this study, we utilize a bifurcated optical fiber probe with a diameter of 100 µm for both source and detector (100 µm source-detector separation) to perform 2D scanning of a surface region with 1mm pixel width, in order to characterize the lateral resolution and penetration depth of such fiber optic probes. The sensing device consists of a broad-band light source and a CCD array spectrometer. Tissue-simulating phantoms were constructed by mixing blood with intralipid as the surrounding medium and having blood-contained glass capillaries embedded in the medium. The data are analyzed using two methods: (1) the reflectance model to calculate absolute values of scattering and absorption, and (2) the modified Beer-Lambert law for calculation of relative changes in oxy- and deoxy-hemoglobin concentrations. The results indicate that the lateral resolution is 1mm or better, with a penetration depth of more than 2mm from the surface.

7896-79, Session 16

A quantitative analysis on image quality in an analytical diffuse optical tomography system

J. Ho, J. Dong, K. Lee, Nanyang Technological Univ. (Singapore)

In a diffuse optical tomography (DOT) system where system parameters are pre-defined, three key factors determine image quality: the size of heterogeneities, their relative locations in the homogeneous medium, as well as the contrast between the heterogeneities and the surrounding medium.

In this paper, we present the first implementation of an analytical DOT system in cylindrical geometry, together with a detailed quantitative analysis on the reconstructed images. In the experimental setup, objects representing a range of sizes, locations and contrast are hung in a cylinder filled with highly scattering medium. Two mirrors are installed beside the cylinder to provide additional data at different projections on the CCD image for the same rotation angle. Surface CCD data is then collected for various source positions and used for reconstructing the absorption coefficient.

For image analysis, the evaluation of image quality is divided into two parts: spatial resolution assessment and contrast-detail analysis. Under the assessment of spatial resolution, the point spread function corresponding to objects of different sizes and locations will be compared, as it has different characteristics for objects of various sizes and can be easily distorted by tissue boundaries. As for contrast-detail analysis, it will be useful in determining the minimum detectable range of contrasts for all sizes, and also demonstrating the difference in sensitivity for objects located at the center and near the edge of the medium.
Comparison of thermal and mechanical effects in tissue depending on laser parameters of Er:Cr:YSGG and Er:YAG lasers using high-speed thermal optical thermography

R. M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); V. G. Lemberg, Optomix (United States)

The Er:Cr:YSGG (2.79 μm) and Er:YAG (2.9 μm) lasers have become accepted as useful instruments in a variety of different medical applications from dentistry, surgery to dermatology. With slightly different wavelengths around a steep water absorption peak and pulse shapes, there might be a substantial difference in ablation and thermal effects during laser-tissue interaction between the Erbium laser systems in soft tissues.

In this study, specialized imaging techniques were used to visualize the ablation, heating and heat dissipation during laser tissue interaction. Using a high speed thermal imaging setup based on color Schlieren techniques, a relative comparison between parameters as energy, pulse durations were obtained in a tissue model of polyacrylamide gel with a composition of 90% water, providing a target similar to soft tissue. Significant differences in the ablation depth as well as residual thermal effects were observed by changing the pulse duration and the repetition rates for fixed energy densities. These results can have an considerable impact on the clinical performance of the Er:Cr:YSGG and Er:YAG lasers. The high speed thermal imaging technique provides a tool to find the optimized laser parameters for specific medical applications.

Influence of laser parameters and staining on femtosecond laser-based intracellular nanosurgery

K. Kuetemeyer, R. Rezgui, H. Lubatschowski, A. Heisterkamp, Laser Zentrum Hannover e.V. (Germany)

Femtosecond laser-based intracellular nanosurgery has become an important tool in cell biology, albeit the mechanisms in the so-called low-density plasma regime are largely unknown. Previous calculations of free-electron densities for intracellular surgery used water as a model substance for biological media and neglected the presence of dye and biomolecules. In addition, it is still unclear on which time scales free-electron and free-radical induced chemical effects take place in a cellular environment. Here, we present our experimental study on the influence of laser parameters and staining on the intracellular ablation threshold in the low-density plasma regime. Hoechst-stained cell nuclei, used as a model substance for intracellular surgery, were irradiated in arbitrarily selected areas while the extent of apparent photodamage was evaluated using two-photon microscopy. We found that the ablation effect of femtosecond laser pulse trains resulted from the accumulation of single-shot multiphoton-induced photochemical effects finished within a few nanoseconds. At the threshold, the number of applied pulses was inversely proportional to a higher order of the irradiance, depending on the laser repetition rate and wavelength. Furthermore, fluorescence staining of subcellular structures before surgery significantly decreased the ablation threshold. Based on our findings, we propose that dye molecules are the major source for providing seed electrons for the ionization cascade because of their significantly lower ionization energy (~5 eV) compared to water (~6.5 eV) and high absorption cross-section. Consequently, future calculations of free-electron densities for intracellular surgery have to take them into account, especially in the calculations of the multiphoton ionization rates.
Endovenous laser ablation with TM-fiber laser

M. F. Somunyudan, N. Topaloglu, Bogaziçi Univ. (Turkey); M. Ü. Ergenoglu, Yeditepe Univ. (Turkey); M. Gülsoy, Bogaziçi Univ. (Turkey)

Endovenous Laser Ablation (EVLA) has become a popular minimally invasive alternative to stripping in the treatment of saphenous vein reflux. Several wavelengths have been proposed; of which 810, 940 and 980-nm are the most commonly used. Thermal shrinkage of collagenous tissue during EVLA plays a significant role in the early and late results of the treatment. Longer wavelengths (>1000-nm) show greater water absorption than shorter wavelengths and may have some advantages for EVLA. However, the most appropriate wavelength is still the subject of debate. The aim of this study is to compare the efficacy of 980 and 1940-nm laser wavelengths in the treatment of varicose veins. In this study, 980 and 1940-nm lasers were used to irradiate stripped human veins. Different power settings (8/10W for 980-nm, 2/3W for 1940-nm) were used to compare their effects. Before and after the laser application, the outer and inner diameters were measured and results were compared. Veins were cut longitudinally after the ablation procedure to assess carbonization effects and thermal damage. The most prominent contraction and narrowing in outer and inner diameter were observed with the 1940-nm at 2W, following 980-nm at 8W, 1940-nm at 3W and finally 980-nm at 10W. The minimum carbonization was observed with the 1940-nm at 2W. 1940-nm Tm-fiber laser has a significant effect in the management of varicose veins due to more selective energy absorption in water and consequently in the vein. As a conclusion, 1940-nm Tm-fiber laser is a promising method in the management of varicose veins.

Non-invasive optical modulation of local vascular permeability

M. Choi, C. Choi, KAIST (Korea, Republic of)

For a systemically administered drug to act, it first needs to cross the vascular wall. This step represents a bottleneck for drug development, especially in the brain or retina, where tight junctions between endothelial cells form physiological barriers. Here, we demonstrate that femtosecond pulsed laser irradiation focused on the blood vessel wall induces transient permeabilization of plasma. Nonlinear absorption of the pulsed laser enabled the noninvasive modulation of vascular permeability with high spatial selectivity in three dimensions. By combining this method with systemic injection, we could locally deliver molecular probes in various tissues, such as brain cortex, meninges, striated muscle, and bone. We suggest this method as a novel delivery tool for molecular probes or drugs.

Laser-induced detachment and re-orientation of cells

L. Gu, N. D. Ingle, S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Re-orientation of a targeted single adhering cell with respect to another cell has not been yet possible, thus limiting study of controlled interaction between cells. In the past, laser catapulting has enabled non-contact isolation and analysis of single cell(s) by use of pulsed laser beam. Here, we report cell detachment using a near-infrared laser beam. HeLa cells were cultured on the surface of plastic petridish and stained with propidium iodide (PI) to check their viability. A tunable Ti: Sapphire laser was used to selectively disrupt the apical surface of individual epithelial cells in rat lung slices (~250 micron thick), which contain small airways (<300 micron in diameter) comprised of epithelial cells, subepithelial matrix (lamina propria), and smooth muscle cells. Laser ablation of single epithelial cells immediately triggered global airway contraction, which appeared to be induced by smooth muscle shortening. We infused the airways with 1.5% agarose gel embedded with 1 µm red fluorescent beads to track deformation. We then estimated the airway contraction force using a finite element model. The force induced by laser ablation of a single airway epithelial cell was comparable to that generated by acetylcholine, an inflammatory agonist commonly used to induce acute asthma. Future studies will focus on determining the precise mechanism of how laser ablation of epithelial cells generates airway contraction force.

Small airway epithelial cells form a continuous sheet covering the conducting airways, which serves as a physical barrier to protect the underlying tissue. In asthma, loss of epithelial cells can occur during bronchoconstriction, which may exacerbate airway hyperreactivity. However, it is not clear whether disruption of the epithelial cell barrier can lead directly to an increase in contractile force. To investigate the role of epithelial cells in the mechanical property of the airway wall, a nanosecond Nd:YAG pulsed laser (λ=1321nm, 2ns pulse) was used to precisely disrupt the apical surface of individual epithelial cells in rat lung slices (~250 micron thick), which contain small airways (<300 micron in diameter) comprised of epithelial cells, subepithelial matrix (lamina propria), and smooth muscle cells. Laser ablation of single epithelial cells immediately triggered global airway contraction, which appeared to be induced by smooth muscle shortening. We infused the airways with 1.5% agarose gel embedded with 1 µm red fluorescent beads to track deformation. We then estimated the airway contraction force using a finite element model. The force induced by laser ablation of a single airway epithelial cell was comparable to that generated by acetylcholine, an inflammatory agonist commonly used to induce acute asthma. Future studies will focus on determining the precise mechanism of how laser ablation of epithelial cells generates airway contraction force.
inside the media using the same laser beam by Gravito-optical trap. The detached cell could then be repositioned by movement of the sample stage. The height at which the cell was stably held was found to depend on the laser beam power. The cell could be brought back to the substrate by reducing the laser beam power using a polarizer or blocking the laser beam. Viability of the detached and manipulated cell was found not to be compromised as confirmed by PI fluorescence exclusion assay. The re-oriented cell was allowed to re-attach to the substrate at a controlled distance and orientation with respect to other cells. The cell was found to retain its shape even after multiple detachments and manipulation using the laser beam. This technique opens up new avenues for non-contact modification of cellular orientations that will enable study of inter-cellular interactions and design of engineered tissue. We will present results of detachment and reorientation of the selected cell with respect to other cells.

7897-11, Session 3

Terahertz pulsed imaging in vivo

E. Pickwell-MacPherson, Hong Kong Univ. of Science and Technology (Hong Kong, China)

There are strong water absorptions in the terahertz (10–1000 Hz) region of the electromagnetic spectrum. This can be construed both as a disadvantage and an advantage. The disadvantage is that biological tissues typically have a high water content and so the strong water absorptions will limit the penetration depth of the terahertz light through the sample. However, the sensitivity of terahertz light to water can be manipulated to be an advantage - the terahertz signal is able to detect subtle differences in water content. Changes in water content in biological tissues can be indicative of disease and thus terahertz imaging could for instance be used to determine the extent of tumor. Thus to overcome the low penetration depth, we perform terahertz imaging in a reflection geometry to acquire detailed information about the sample surface.

Skin is the most easily accessible and vast organ of the body. We therefore use skin as the first sample to investigate using terahertz in vivo imaging. We extend our previous work by using a prototype terahertz probe that enables us to access more regions of skin - we are no longer limited to just the skin on the hand. The hand-held probe can be wanded over areas of interest - using the probe gives more flexibility, but it also increases the noise and requires existing processing methods to be improved.

In this paper we present in vivo measurements of human skin using the prototype terahertz probe and discuss the processing techniques that we have developed to analyze the results.

7897-12, Session 3

THz techniques for human skin measurement

Y. Guan, T. Shibuya, K. Suizu, Nagoya Univ. (Japan); S. Hayashi, RIKEN (Japan); K. Kawase, Nagoya Univ. (Japan) and RIKEN (Japan)

Our group has been conducting research activities in several directions within the THz field. We introduced many types of THz-wave sources, and we also suggested wide range of real-life applications using THz-imaging techniques. Recently, we are developing several novel THz techniques for skin measurement.

A high-resolution tomographic imaging was demonstrated using a reflection-type terahertz time-domain spectroscopy. Broadband terahertz waves up to 25 THz were generated with the combination of optical fibers and a nonlinear crystal, but it also increases the noise and requires existing processing methods to be improved.

Secondly, we have demonstrated a sensitive imaging method combined a terahertz time-domain spectroscopy and an interference effect for label-free protein detection on a polyvinylidene difluoride membrane.
GPU performed 3-D simulations roughly 90 times faster than the CPU.

For the in vivo THz exposures, using a six-layer skin model, we found that the GPU and CPU were statistically insignificant in terms of accuracy. For that the GPU ran the simulations 100 times faster than the CPU, and the accuracy of comparable simulations on a GPU (Tesla C1060) and a CPU speedup provided by GPUs, we then compared the performance (speed, accuracy) of comparable simulations on a GPU (Tesla C1060) and a CPU.

In recent years, several Terahertz time-domain spectrometers (THz-TDS) have been developed. These spectrometers are exceptional laboratory tools for measuring the optical properties of liquids and biological tissues. However, due to their size and weight, they are impractical for use in battlefield hospitals, medical evacuation aircrafts, or other settings with limited space. In this study, we developed a compact, light, and portable THz-TDS device. We used this system to collect spectra from 0.1-1.6 THz for liquids (ethanol, water, saline), freshly harvested porcine tissues (muscle, adipose, and skin), and frozen/thawed porcine skin. For all samples tested, we found that the index of refraction (n) decreases with frequency, while the absorption coefficient (μa) increases with frequency. Muscle, adipose, and frozen/thawed skin exhibited comparable μ values ranging from 2.5 at 0.1 THz to 2.0 at 1.6 THz, whereas the n values for freshly harvested skin were ~40% lower. In addition, fresh skin samples exhibited μa values ranging from 50 to 300 cm⁻¹. Overall, the optical properties measured with this system were in agreement with values measured using conventional spectrometers. These results suggest that this THz-TDS provides accurate skin measurements, and therefore is an excellent tool for assessing skin health in battlefield settings.

The model also accounts for the water dependence of tissue properties (both thermal and optical), and variations in blood perfusion rates based on local tissue injury. Our calculations show that water diffusion would mitigate local refractive index variations, and hence influence the phenomenon of thermal lensing.

Development of a compact terahertz time-domain spectrometer for the measurement of the optical properties of biological tissues

G. J. Wilming, B. L. Ibay, Air Force Research Lab. (United States); T. D. Tongue, B. J. Schulkin, N. Lam, Zornega Terahertz Corp. (United States); X. G. Peralta, The Univ. of Texas at San Antonio (United States); C. C. Roth, B. D. Rivest, W. P. Roach, Air Force Research Lab. (United States)

In this study the corneal temperature rise from multiple 2.01 micron Tm:YAG laser pulses was investigated using ex-vivo rabbit eyes. Two separate thermal-measurement data sets were simultaneously collected while an embedded type T (Copper-Constantan) micro-thermocouple detectors captured surface temperature rises resulting from laser pulses while an embedded type T (Copper-Constantan) micro-thermocouple measured temperature increases below the surface at various depths within the rabbit eye. Single 10 ms pulses as well as two, three, and four pulse sequences were utilized while the total energy delivered to irradiate human Red blood cells. Red blood cells are separated from human whole blood using centrifugation method (time=10 min., temperature=15οC and RPM=3000) and then exposed to HeNe laser radiation. Laser exposure time is varied from 10 min. to 40min for Red blood cells.

Absorption spectrum, FTIR and fluorescence spectra of RBC are compared before and after HeNe laser irradiation. The absorption spectrum of RBC after exposure to HeNe laser shows a significant decrease in absorbance. The FTIR spectrum of non irradiated RBC clearly show the peaks due to O-H (free group), C=O (amide I group), N=O (nitro group), C-O (anhydride group) and C-H (aromatic group). Laser radiation changes in transmittance in FTIR spectra related to C=O group and percentage of transmittance increases for O-H, C=O, N=O, C-O and C-H group.

Temperature increase of ex-vivo corneas from multiple 2.01-micron incident laser pulses

E. Kelly, T. E. Johnson, Colorado State Univ. (United States)

Current laser safety standards for multiple pulse lasers are based primarily on modeling and the results of single pulse studies. Previous thermal effects studies have focused on histological and visible endpoints, with only a few studies examining the actual temperatures achieved. The goal of this research was to probe the actual vertical temperature profile produced by 2.01 micron laser pulses in the cornea. In this study the corneal temperature rise from multiple 2.01 micron Tm:YAG laser pulses was investigated using ex-vivo rabbit eyes. Two separate thermal-measurement data sets were simultaneously collected and compared. An infrared thermal camera employing microbolometer detectors captured surface temperature rises resulting from laser pulses while an embedded type T (Copper-Constantan) micro-thermocouple measured temperature increases below the surface at various depths within the rabbit eye. Single 10 ms pulses as well as two, three, and four pulse sequences were utilized while the total energy delivered was held constant. A comparison of the data to temperatures required for denaturing proteins and the current laser safety guidelines will be presented.

Bioheat model evaluations of laser effects on tissues: role of water evaporation and diffusion

R. P. Joshi, D. Naglapally, Old Dominion Univ. (United States); R. J. Thomas, Air Force Research Lab. (United States)

A two-dimensional, time-dependent bioheat model is applied to evaluate changes in temperature and water content in tissues subjected to laser irradiation. Our approach takes account of liquid-to-vapor phase changes and a simple diffusive flow of water diffusion within the biotissue. An energy balance equation considers blood perfusion, metabolic heat generation, laser absorption, and water evaporation. The model also accounts for the water dependence of tissue properties (both thermal and optical), and variations in blood perfusion rates based on local tissue injury. Our calculations show that water diffusion would reduce the local temperature increases and hot spots in comparison to simple models with water evaporation alone. Also, the reduced suppression of perfusion rates due to tissue heating and damage with water diffusion affect the necrotic depth. Two-dimensional results for the dynamic temperature, water content, and damage distributions will be presented for skin simulations. It is argued that reduction in temperature gradients due to water diffusion would mitigate local refractive index variations, and hence influence the phenomenon of thermal lensing.

Characterizing temperature-dependent photo-oxidation to explain the abrupt transition from thermal to non-thermal laser damage mechanisms at 413 nm

M. L. Denton, C. D. Clark III, G. D. Nookin, K. J. Schuster, TASC, Inc. (United States); C. W. Burney, B. A. Rockwell, R. J. Thomas,
7897-20, Session 4

Effect of 1125-nm laser radiation on porcine skin

K. Mcmillan, gRadiant Research, LLC (United States)

Commonly used deeply penetrating laser wavelengths are preferentially absorbed by blood; consequently, tissue response is affected by inhomogeneities in vascular density, and iatrogenic vessel damage is a risk. When the objective of treatment is to cause deep and homogeneous heating of soft tissue, the 1125 nm wavelength may be advantageous. In this work, a new prototype 1125 nm quantum dot diode laser is tested on porcine skin and subcutaneous tissue. Fresh ex vivo tissue specimens are irradiated and the depth and extent of heating and thermal injury is characterized by temperature monitoring and vital staining with nitroblue tetrazolium chloride. Irradiation times from subsecond range to tens of seconds are employed, along with surface cooling at various temperatures. The ability to localize thermal damage at dermal and subcutaneous depths by varying laser irradiation parameters is characterized, and compared to the results of mathematical modeling using literature values for intrinsic optical constants and thermal properties. Potential applications of the 1125 nm laser in dermatology are discussed.

7897-55, Poster Session

Laser ultrasound characterization of normal and decayed teeth by measuring elastic properties of surface layers

Y. H. El-Sharkawy, Cairo Univ. (Egypt)

We firstly investigate the mechanic and acoustic properties of human teeth by using the laser generation of surface acoustic wave (SAW) technique. The materials investigated included normal and decayed teeth, which have the same grain size and different thickness, are used as the samples. The tissue responds to the laser-induced stress by thermoelastic expansion. We can obtain the shape of acoustic pulse and the phase velocity was determined for the teeth system and extract information on the teeth thickness, density, and transverse sound velocity that could be used as diagnostic parameters.

7897-56, Poster Session

VEGFC as a survival factor for retinal pigment epithelial cells under thermal stress

B. J. Lavey, U.S. Air Force (United States); K. E. Sheldon, Air Force Research Lab. (United States); L. E. Estlack, Conceptual MindWorks, Inc. (United States); K. J. Schuster, TASC, Inc. (United States); M. D. Barnhart, U.S. Air Force Academy (United States)

Vascular endothelial growth factor (VEGF) is known for its role in neovascularization and cellular signaling pathways of subthreshold retinal lesions. The objective of this study was to elucidate potential protection mechanisms to laser-induced heat stress utilizing an in vitro retinal model. The cell line was characterized to determine the relative abundance of VEGF-C protein. Cells, preconditioned via water bath and controls, were then exposed to 2 µm laser radiation to assess whether increases in protein production following preconditioning could confer any protection. There was no significant increase in threshold damage irradiance (ED50) in the preconditioned cells versus control.

7897-57, Poster Session

Analysis on unevenness of skin color using the melanin and hemoglobin components separated by independent component analysis of skin color image

N. Ojima, KAO Corp. (Japan); I. Fujiwara, Chiba Univ. (Japan); Y. Inoue, KAO Corp. (Japan); N. Tsumura, T. Nakaguchi, Chiba Univ. (Japan); K. Iwata, KAO Corp. (Japan)

Unevenness of skin color is one of the biggest concerns about appearance of skin. Recently several techniques to analyze skin color have been introduced by separating skin color information into chromophore components, such as melanin and hemoglobin. However, there are not many reports on unevenness of skin color considering chromophore, size, and concentration. We propose a new image analysis and simulation method based on chromophore analysis and spatial frequency analysis. This method is mainly composed of three techniques: independent component analysis (ICA) to extract hemoglobin and melanin chromophores from a single skin color image, an image pyramid method which decomposes each chromophore into multi-resolution images, which can be used for identifying different sizes of clusters or spatial frequencies, and analysis of the histogram obtained from each multi-resolution image to extract unevenness parameters. As the application of the method, we also introduce an image processing technique to change unevenness of melanin component. As the result, the method showed a high capability to analyze unevenness of each skin chromophore: 1) Vague unevenness on skin could be discriminated from noticeable pigmentation such as freckles or acne. 2) By analyzing the unevenness parameters obtained from each multi-resolution image for Japanese ladies, age-related changes were observed in the parameters of middle spatial frequency. 3) An image processing system modulating the parameters was proposed to change unevenness of skin images along the axis of the obtained age-related change in real time.

7897-58, Poster Session

Monte Carlo simulation for light propagation in 3D tooth model

Y. Fu, Sharp Labs. of America, Inc. (United States); S. L. Jacques, Oregon Health & Science Univ. (United States)

The goal of this research is to estimate the light energy deposition in the target region of tooth with given light source information, tooth optical properties and tooth structure. Monte Carlo (MC) simulation was implemented in a three dimensional tooth model to simulate the light propagation in the tooth for antibiotic photodynamic therapy and other laser therapy. Two use cases are presented to demonstrate the application of this model. With this model, we can find the proper light source, irradiation dosage and incident point to meet the photodynamic therapy (or other laser therapy) dosage requirement. The oral bacteria do not always grow in the surface of tooth; sometimes it may also grow in the crack or the cavity of tooth. Some novel designs of PDT light source based on optical fiber has been designed for light
power delivery. However, in some cases the fiber still can’t access the bacteria. The light needs to irradiate on the surface and penetrate into the tooth.

Monte Carlo tooth model is developed as a tool for clinician application. Light propagation into the tooth geometry model is simulated statistically. According to the results in the photodynamic therapy dosage, two use cases are presented to demonstrate the light source type comparison and incident point comparison. This program could be feasible to design a clinical photodynamic therapy plan in the dentist’s office.

7897-59, Poster Session

Biophotonics and laser-optics technologies for controlling gas-exchange processes in biological tissue

M. M. Asimov, B.I. Stepanov Institute of Physics (Belarus); R. Asimau, Sensotronica Ltd. (Belarus); A. N. Rubinov, B.I. Stepanov Institute of Physics (Belarus)

Laser-tissue interaction at low intensity (cold) laser radiation and especially the light impact on gas exchange in biological tissue is a one of interesting aspects of modern photomedicine and photobiology. The results of in vivo investigations of laser-induced photodissociation of oxyhemoglobin in cutaneous blood vessels and its role in tissue oxygenation are presented. Laser-induced photodissociation of oxyhemoglobin in vivo experimentally investigated by two independent methods: pulsed oximetry and polarographic transcutaneous oxygen monitoring. Unique possibility of enhancing cell metabolism due to the local increase of the molecular oxygen concentration in tissue is demonstrated. New method of dosimetry in phototherapy as well as laser therapy based on local tissue oxygenation and stimulation of aerobic cell metabolism is developed. Novel optical method of photodecomposition of blood carboxyhemoglobin and elimination of poisoning effect of carbon monoxide is developed. It is experimentally shown that light induced photodissociation of carboxyhemoglobin decreases the time of restoring the normal function of hemoglobin to carry of oxygen approximately by order of magnitude. Laser-optical method of local tissue oxygenation based on laser-induced in vivo photodissociation of oxyhemoglobin in cutaneous blood vessels is proposed. The results of experimental study and computer modeling of the kinetics of tissue oxygen distribution from blood plasma are presented. Different biomedical applications the effect of light and cold laser radiation on controlling gas exchange in biological tissue are discussed.

7897-60, Poster Session

A study of light fluence rate distribution for intracavitary PDT using MC simulation

J. Sandell, The Univ. of Pennsylvania Health System (United States)

In photodynamic therapy (PDT), it is essential to accurately determine the light fluence rate distribution from the known treatment geometry and optical properties. The light distribution calculation for intracavitary PDT is a complex problem because the light near the tissue surface inside the cavity is influenced by the geometry of the surrounding tissue, by multiply scattered light inside the cavity, and by possible attenuation of the fluid contained within the cavity. To address this problem we use Monte Carlo simulations as a gold standard to calculate the light fluence in a spherical cavity, both with homogenous and inhomogeneous optical property distributions. The MC code is developed in the Matlab platform with the Fresnel reflection well defined at the tissue-medium boundary, allowing the number of times a photon can be scattered within the cavity to be user specified. We find that increasing the attenuation, causes a decrease the light fluence rate for the same total light power from a point source. The resulting fluence rate is then compared with our empirical model based on diffusion. Preliminary results of comparisons of the MC simulation and the empirical model will be presented. We conclude that the empirical model is adequate for modeling light fluence within the cavity.

7897-61, Poster Session

Angular-domain spectroscopic imaging of turbid media: derivative analysis

F. Vasefi, M. Najiminaini, Simon Fraser Univ. (Canada); E. Ng, The Univ. of Western Ontario (Canada); B. Kaminska, Simon Fraser Univ. (Canada); J. J. Carson, The Univ. of Western Ontario (Canada)

Angular Domain Spectroscopic Imaging is a novel technology that employs an array of micro-channels to perform angular filtering of light that traverses a turbid sample. Angular filtration enables rejection of scattered light at moderate levels of scattering (i.e. up to 20 mean free paths) that would normally be detected as a noise during spectral measurements. An ADSI system in trans-illumination mode was constructed from a halogen light source, an Angular Filter Array (AFA), and a pushbroom imaging spectrometer. The free-space collimated broadband light source was used to trans-illuminate a turbid sample over a wide range of wavelengths (650 nm - 950 nm). The imaging spectrometer decomposed the output of the AFA into hyperspectral images representative of spatial location and wavelength. It collected and angularly filtered a line image from the object onto the CCD camera with the spatial information displayed along one axis and wavelength information along the other.

In this paper, we experimentally characterized the ADSI system by capturing transmission spectra of absorbing indocyanine green (ICG) targets at a range of concentrations (5 - 20 µM) as well as different Intralipid™ concentrations. The spectral signature of each sample and the first and second derivative were computed. The library of zero-order transmission spectra and the derivatives was used to estimate the concentration of ICG in a scattering medium for an unknown sample. The experimental results provided an estimate of the capabilities and limitations of ADSI for quantitative spectroscopic imaging of ex vivo tissue samples up to 4 mm thick.

7897-62, Poster Session

Effect of low-level GaAs laser irradiation on the proliferation rate of human periodontal ligament fibroblast: an in vitro study

S. Ahuja, P. Madhukar, Maharana Pratap College of Dentistry & Research Ctr. (India)

Regeneration of Periodontal tissue lost because of the progression of destructive periodontal diseases remains the ideal results of periodontal therapy. A number of treatments have been proposed to modify the root surface and enhances its biocompatibility including mechanical, chemical as more recently physical methods, such as Laser radiations. One of the main cell in this regenerative process is the periodontal ligament fibroblast. The aim of this in vitro study was to evaluate a potential stimulatory effect of low level laser irradiation on the proliferation of (PDLF) Human periodontal ligament fibroblast.

PDL cells were obtained during 3rd molar impaction surgery, these cells were cultured under standard conditions. Sub confluent monolayers were irradiated with an 904nm GaAs laser operated at a power out of 14 mW in a continuous wave mode at energy fluences of 4.02/J/cm², frequency 10,000 Hz. Time of exposure was 300 sec per well and number of irradiation were 3. After every laser treatment, the culture was incubated for 24hrs. The proliferation rate of lased and control culture was determined by non radioactive assay containing redox indicator with Alamar Blue Dye after 24, 48 and 72 hrs. The number of cells were counted under neubar counting chamber to compare the proliferation rate of lased and control group after 24, 48 and 72 hrs. The irradiated cells revealed a considerably higher proliferation activity.
than the controls. The differences were highly significant up to 72 hrs after irradiation (Mann Whitney U test, p<0.001).

7897-63, Poster Session

FDTD multi-GPU implementation of Maxwell’s equations in dispersive media

M. R. Zunoubi, State Univ. of New York at New Paltz (United States); J. A. Payne, M. Knight, Air Force Research Lab. (United States)

The absorbed dose of dispersive media exposed to non-ionizing ultra-wideband (UWB) electromagnetic pulses of nanosecond duration is critical for establishing dose-response curves for such exposures. Due to its straightforward implementation and its ability to model a broad range of exposure conditions, the finite-difference time-domain (FDTD) method has gained considerable popularity for simulation of various electromagnetic problems. However, many practical applications require simulation of realistic models such as absorption of EM waves by a complete human body that could require hundreds of millions of grids resulting in prohibitively expensive computational scenarios. Traditionally, a computer cluster has been used to circumvent this problem, which itself needs a relatively large space, is expensive, requires occasional maintenance, and is shared among various research teams. Alternatively, researchers have recently focused on implementing the FDTD technique on Graphic Processing Units (GPUs), which possess inherent attributes for threaded computing and can be easily integrated into a standalone desktop with minimal extra cost and space. In this study, we present the first multi-GPU FDTD implementation of Maxwell’s equations in dispersive media that uses the OpenMP API to synchronize the operation of GPUs and their corresponding CPUs. By taking advantage of CUDA programming model, we present a unique implementation of the FDTD scheme that exploits the memory hierarchy of the CUDA technology that includes the global, texture, constant, and shared memory, which enables us to tackle problems that are otherwise computationally prohibitive. Practical results will be presented along with a measure of speedup factors achieved when using multiple GPUs.

7897-64, Poster Session

Human skin auto-fluorescence decay as a function of irradiance and skin type

M. P. Debreczeny, MPD Consulting (United States); R. Bates, R. M. Fitch, K. P. Galen, J. Ge, R. B. Dorshow, Covidiën Pharmaceuticals (United States)

Diagnostic optical measurements made through human skin often rely on the assumption that the skin is relatively un-perturbed by the optical interaction. The aim of this work was to establish measurement conditions under which endogenous skin fluorescence (“auto-fluorescence”) is relatively invariant, so that changes in exogenous agents can be accurately determined. Fluorescence emission was measured on the volar forearm of 36 subjects, chosen to be equally representative of all Fitzpatrick skin types. All subjects were exposed to ~40 minutes of optical excitation at 450 and 500 nm with 4 irradiances between 0.3 and 9 mW/cm².

The lowest irradiance measurements were used to normalize all higher irradiance measurements. This procedure revealed that the optically-induced auto-fluorescence decay was independent of skin type when excited at 450 nm, but significantly dependent on skin type when excited at 500 nm. Further, the extent of decay over time was linearly related to irradiance at 500 nm, but at 450 nm was non-linear, with the extent of decay rolling off between 2 and 9 mW/cm². The magnitude of the auto-fluorescence decay interference will depend on the exogenous agent being probed, but as an example, we find that in order to maintain the auto-fluorescence signal within 95% of its original value over a 30 minute period, the excitation at 450 nm needs to be limited to 1.5 mW/cm², while excitation at 500 nm should be limited to 5 mW/cm². The results will also be discussed with reference to previously proposed mechanisms for photo-bleaching and visible-light-induced skin darkening.

7897-65, Poster Session

What happens in the rat brain locally exposed to a shock wave? real-time optical diagnosis

S. Sato, S. Kawauchi, Y. Uozumi, H. Nawashiro, M. Kikuchi, H. Ashida, National Defense Medical College (Japan)

Blast-induced traumatic brain injury (bTBI) has recently been a major concern both in civil and military medicine. However, the mechanism and symptoms of bTBI are still not well understood, partially due to the lack of reliable animal model for laboratory use. We are proposing the use of laser-induced shock wave (LISW) to simulate bTBI in small animals. Advantages of this method include safety, easy handling and high spatiotemporal controllability of shock wave energy. In this study, we made optical diagnosis of rat brain that was locally exposed to a laser-induced shock wave. A laser target was placed on the parietal bone; an optical fiber pair was set in the vicinity of the target for diffuse reflectance measurements. The target was irradiated with a nanosecond laser pulse at 1 J/cm² with a spot diameter of 4 mm, producing a shock wave with a peak pressure of ~96 MPa. Oxygen saturation (SpO2) was monitored at the hindlimb, and blood pressure and heart rate were measured at the root of the tail. The local application of LISW to the brain did not cause any changes either in SpO2, blood pressure or heart rate. On the other hand, optical measurement of the brain showed drastic changes. Light scattering dynamically changed for ~4 min after LISW application, indicating the occurrence of strong depolarization waves. Both the total hemoglobin and oxygen saturation increased in this period and thereafter, both turned to decrease, showing a prolonged hypoxia. Optical signal relating to mitochondrial energy metabolism was also analyzed.

7897-66, Poster Session

Traumatic brain injury caused by laser-induced shock wave in rats: a novel laboratory model for studying blast-induced traumatic brain injury

B. Hatano, Y. Matsumoto, Japan Self-Defense Force (Japan); N. Otani, D. Saitoh, S. Tokuno, Y. Satoh, H. Nawashiro, National Defense Medical College (Japan); Y. Matsushita, Japan Self-Defense Force (Japan); S. Sato, National Defense Medical College (Japan)

Blast-induced traumatic brain injury (bTBI) is characterized by cerebral hemorrhage in the cortex and subcortical region, as well as widespread white matter fiber degeneration. However, the detailed mechanism of bTBI has not been revealed. Due to the danger and limited controllability of actual explosion-based experiments, reliable laboratory animal models for bTBI are strongly desired. In this study, we used laser-induced shock wave (LISW) to cause TBI in rats and investigated the histopathological similarities to those of actual bTBI. After craniotomy, rat brain was exposed to a single shot of LISW at various laser fluences; peak pressure and impulse of LISW linearly increased with increasing laser fluences. After a certain period of time, perfusion fixation was performed and the extracted brain was sectioned. The sections were stained with hematoxylin-eosin, caspase-3 antibody and Bodian’s silver. Evans Blue (EB) staining was also used to evaluate disruption of blood brain barrier. At certain laser fluence levels, neural cell injury and hemorrhagic lesion were observed in the cortex and subcortical region. However, injury was limited in the tissue region that interacted with LISW. Axonal injury was observed around the tissue exposed to LISW, while expression of caspase-3 was not evident in the present model. Fluorescence originating from EB was diffusely observed in the injuries at the high
fluence levels. Due to the grade and spatial controllability of injuries and the common histological observations to those of actual bTBI, brain injuries caused by LISWs would be a useful model to study bTBI.

7897-67, Poster Session
Error analysis of tissue optical properties determined by double-integrating sphere system and inverse Monte Carlo method
T. Terada, Osaka Univ. (Japan) and Japan Science and Technology Agency (Japan); T. Nanjo, N. Honda, K. Ishii, Osaka Univ. (Japan); K. Awazu, Osaka Univ. (Japan) and Japan Science and Technology Agency (Japan) and Univ. of Fukui (Japan)
For safe laser-treatment, quantitative knowledge about laser-tissue interaction is important. Laser-tissue interactions are regulated by optical properties of tissues. In our previous study, the determination system of absorption coefficients and reduced scattering coefficients from 350 nm to 1000 nm was developed, which was composed of double-integrating sphere system and inverse Monte Carlo method. Optical properties of a gel with 4mg/ml hemoglobin were determined by this system, when the positive peak of the absorption coefficient spectra was detected about 405 nm. The peak was also detected at about 405 nm in the reduced scattering coefficient spectra. In the general scattering theory, the scattering intensity is proportional to the fourth power of the reciprocal wavelength. Therefore, the reduced scattering coefficient spectra must have no peaks. This phenomenon is an important issue in the studies of the determination of tissue optical properties. The predictable reasons of this issue are measurement errors by double-integrating sphere system and calculation errors by inverse Monte Carlo method. According to the things that this issue is caused by other calculation methods and the calculated reduced scattering spectra is continuous, calculation errors have small effects. Measurement errors include the inaccurate reflectance and the tiny transmittance. After changing experimental conditions such as the sample thickness, the port diameter of integrating sphere and sample concentration, it is proved that the inaccuracy of the reflectance measurement have the most effect.

7897-68, Poster Session
Optical imaging through non-transparent small aquatic creatures with angular-domain imaging
R. Cheng, P. B. Tsui, G. Chiang, G. H. Chapman, Simon Fraser Univ. (Canada)
When imaging through small aquatic creatures, scattered photons produce problems in image quality and resolution. Angular Domain Imaging (ADI) reduces scattered photons and improves the image quality and resolution. ADI is an imaging technique which utilizes the angular spectrum of photons to filter multiple-scattered photons and accepts only photons with small angular deviation from their original trajectory. Advantages of the ADI technique are that it is insensitive to wavelength and the sources are not required to be high optical quality, coherent, or pulsed, as with OCT or time domain. Our target is to image a small species called Branchiostoma lanceolatum, a lancet that is 5-8cm long and 5mm thick, by using ADI to remove the scattering in order to image internal structures. A laser illuminates the lancet in a water-filled container and a spatiofrequency filter removes the scattered before the image. Experimentally, a coherent Nd:Yag second harmonic (533nm) laser creates images but also optical interference occurs within the internal structures of the lancet. Conversely, an incoherent broadband white light source eliminates the structural interference effect; however, the wavelength variation of the scattering coefficient combined with the limitation of the image sensor's dynamic range limit the ability to distinguish the internal structures in many areas. Thus, an IR diode laser (850nm) is used to lower the scattering coefficient as compared to conventional visible light source and to diminish the interference effects due to its shorter coherence length.

7897-69, Poster Session
Effect of porphyrins bound to tubulin dimers
B. McMicken, J. E. Parker III, L. Brancaloe, The Univ. of Texas at San Antonio (United States)
Photosensitizers are photoactive molecules that when irradiated with UV or visible light initiate photochemical or photophysical reactions that may affect the environment surrounding them, including proteins to which they are attached. Our photosensitizers of interest are the anionic porphyrin, mesotetraakis (sulfonatophenyl) porphyrin (TPPS) and the neutral protoporphyrin IX (PPIX), which bind noncovalently to Tubulin dimers. This is significant since we can then irradiate the porphyrin and cause a change in the geometry of the tubulin to specifically affect its function. What has yet to be fully understood is the mechanism of the photochemical reaction and unfolding of the protein after irradiation. A combination of various spectroscopic methods can give us insight into the structural changes of the photosensitizer and the protein and characterize the conformational changes produced in the protein. The study is completed by computational simulations of the docking as well as the unfolding of the protein.

7897-70, Poster Session
Evaluating changes in optical properties of biological cells due to histological staining
L. Cherkezyan, H. Subramaniam, Northwestern Univ. (United States); V. Konda, The Univ. of Chicago Medical Ctr. (United States); C. Chang, D. Damania, Northwestern Univ. (United States); I. Waxman, The Univ. of Chicago Medical Ctr. (United States); V. Backman, Northwestern Univ. (United States)
Various staining techniques are commonly used in a variety of biophotonics and medical applications to investigate intracellular morphology. The staining process introduces light absorption in the visible wavelength range in order to visualize subcellular detail. However, the effect of staining on the optical properties of biological samples, such as its effect on the refractive index of the cell, is not completely understood. Here, we present a method for evaluating the changes in refractive index introduced by staining. The effects of commercially available haematoxylin (absorption peak at 550 nm) and cytostain (peaks at 525 and 620 nm) were studied with squamous epithelial cells. A 2-D map of the cell is constructed though a pair of measurements: first the cells were measured using optical profilometry to determine the thickness at each pixel, second partial-wave spectroscopic (PWS) microscopy was used to determine the absorption spectrum at each corresponding pixel. From this 2D map, the spectral profile of the imaginary part refractive index is calculated. We then apply Kramers-Kronig relationship to extract the change in the real part of the refractive index as a function of wavelength in the visible range (400nm-700nm). We show the results of experimental measurements and compare to the predicted characteristics. We implemented the effect of staining on squamous epithelial cells from normal proximal esophageal tissue in PWS esophageal cancer detection studies.

7897-22, Session 5
Is tissue Raman spectrum really a linear combination of its constituent spectra?
I. Barman, N. C. Dingari, C. Kong, J. W. Kang, R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)
Failure to adequately regulate blood glucose may lead to disorders of...
glucose homeostasis, a leading cause of mortality. Near-infrared Raman spectroscopy has been proposed for continuous non-invasive monitoring that significantly reduces patient trauma. However, despite encouraging in vitro results, the search for a clinically accurate and robust algorithm capable of prospective prediction in a human population has been elusive. Multiple factors can be attributed to the current difficulty in achieving successful calibration transfer including variations in tissue absorption and scattering, autofluorescence levels and associated quenching, differences in glucose concentration in the blood and interstitial fluid compartments, and spurious correlations.

To develop robustness in the calibration models, we propose the application of kernel-based non-linear support vector regression. This shift from the conventional linear calibration schemes is based on the understanding that the linearity assumption between the spectral and concentration datasets may fail under the influence of fluctuations in the aforementioned process and system variables. In this talk, we will present our first results showing that support vector regression provides a significant improvement over the linear models with an increase in correlation between the actual and predicted concentrations by at least 30% and a concomitant decrease in the prediction error values by a factor of two or more, when multiple human subjects are included in the analysis. Furthermore, we show that application of support vector regression enables robust glucose concentration datasets to be clinically accurate predictions in the hypoglycemic range in human subjects. Finally, we investigate the root causes for the exhibited non-linearity.

7897-23, Session 5

Playing catch-up between the two-glucose compartments with spectroscopy
C. Kong, I. Barman, N. C. Dingari, J. W. Kang, R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

Frequent monitoring of glucose levels is essential for effective diabetes management, and a non-invasive glucose measurement method would alleviate much of the patient pain and inconvenience currently associated with the finger-prick testing method. However, as gaining direct access to the bloodstream requires invasive probes, transcutaneous glucose monitoring techniques encounter the physiological lag problem between blood and interstitial fluid (ISF) glucose components. Optical techniques, for example, predominantly probe glucose in the ISF compartment, although blood glucose measurements are used as reference. Specifically, it has been reported that glucose concentration in the subcutaneous ISF compartment lags behind that of the blood stream. This mismatch creates inconsistencies in the calibration model and leads to inaccuracies in the predicted glucose levels. In this talk, we propose a dynamic correction scheme to improve the prediction accuracy and precision for Raman spectroscopy-based transcutaneous glucose monitoring. By using the information of the rate of change in glucose levels, this calibration scheme inter-converts between the glucose concentrations in the two compartments. This provides consistency between the glucose concentration values represented by the acquired spectra and the measured reference blood glucose. By analyzing clinical Raman spectra obtained from human volunteers undergoing oral glucose tolerance tests, we show that the new spectroscopic calibration scheme significantly improves glucose concentration measurement accuracy by an average of nearly 20%. In addition, we discuss the effect of such application on the prediction uncertainty, which is a function of spectral noise and the (unknown) rate of change of concentration in the prediction subject.

7897-24, Session 5

Detection of familial adenomatous polyposis with polarized spectroscopic imaging and oral vascular density
A. Basiri, The Catholic Univ. of America (United States); D. Edelstein, F. Giardiello, The Johns Hopkins Univ. (United States); J. C. Ramella-Roman, The Catholic Univ. of America (United States)

Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterized by hundreds of colorectal adenomas in teenagers and progression to colorectal cancer if colectomy is not performed. We investigated the ability of two novel phenotypic markers- oral mucosal vascular density (OMVD) and oral mucosal reflectance (OMR) to identify individuals with FAP.

Thirty-three patients with FAP from 29 unrelated pedigrees with APC gene mutation and 50 population controls were evaluated for OMVD and OMR utilizing a photographic/spectrophotometric system capturing images and reflectance at various wavelengths. The OMVD was calculated from a 300 x 600 pixel portion of each image, utilizing an automatic tracing algorithm. A binary map of the traced vessels was generated and quantified by Kolmogorov Complexity, calculating an oral vascular density score for each subject. The OMR was also calculated from the same 300 x 600 pixel portion of each image. The average value of all pixels was calculated, and correlated to the total normal reflectance.

A statistically significant difference in OMVD between patients with FAP and controls was noted, p < 0.001. The sensitivity and specificity of oral mucosal vascular density for FAP was 91% and 90%, respectively. No association between this marker and age or gender was found. The positive and negative predictive values for OMVD for FAP were 86% and 94%, respectively. No significant difference in OMR between the two subject groups was noted.

7897-25, Session 5

Determining the optical properties in a fibrous turbid medium
A. S. Shuaib, G. Yao, Univ. of Missouri-Columbia (United States)

Light propagation in fibrous biological tissue is quite different from that in isotropic medium. Several studies showed that the measured reduced scattering coefficient strongly depends on the measurement direction relative to the fiber direction. In this study, we investigated the possibility of retrieving optical properties in anisotropic tissue using time-domain measurements. A Monte Carlo model was used to simulate light propagation in a fibrous tissue consisting of aligned cylinders and spherical shape scatterers. An isotropic diffuse model was then used to determine the optical properties from simulated time-resolved reflectance. When the measurement position was parallel to the fiber direction, the derived reduced scattering coefficient had good agreement with the true background scattering coefficient values with a less than 10% error. In contrast, when measuring in a direction perpendicular to the cylinders, the derived reduced scattering coefficient was close to the summation of the reduced scattering coefficients of both cylinders and background only in samples with small cylinder diameters. If the fiber size in the medium is known, the reduced scattering coefficient associated with the cylinders can be derived by using a correction coefficient.

7897-26, Session 5

Detection of cancer cells in prostate tissue with time-resolved fluorescence spectroscopy
C. Gerich, J. L. Opitz, Fraunhofer-Institut für Zerstörungsfreie Prüfverfahren (Germany); S. Füssel, M. Toma, M. Sergon, Universitätsklinikum Carl Gustav Carus Dresden (Germany); R. Nanke, J. Fehre, Siemens AG (Germany); G. Baretton, M. Wirth, Universitätsklinikum Carl Gustav Carus Dresden (Germany); J. Schreiber, Fraunhofer-Institut für Zerstörungsfreie Prüfverfahren (Germany)

Goals: One of the biggest challenges of tumour diagnostics is the...
precise differentiation between benign and malignant tissue. Due to these difficulties, pathologists and physicians strongly need a diagnostic system that facilitates the decision which tissue has to be removed. In previous studies many attempts were made to use tissue fluorescence for cancer recognition. However, no clear correlation was found between tissue type and fluorescence parameters like time and wavelength dependent fluorescence intensity (I(t,λ)). The present study is focused on cooperative behaviour of cells in benign or malignant prostates tissue reflecting differences in their metabolism.

Material and Methods: Each 6 punch biopsies from 50 prostates were obtained directly after radical prostatectomy. Time-resolved fluorescence spectra were recorded for 4 different measure points for each biopsy. Distribution of malignant and non-malignant tissue areas was assessed on the afterwards formalin-fixed paraffin-embedded specimens. An algorithm was developed to determine a relevant parameter of the time dependent fluorescence data.

Results: Analyzing 1200 measurements in total, optimal conditions for discrimination between malignant and non-malignant tissue areas were found resulting in a certain threshold. Although diagnostic performance is already appropriate, the proposed approach needs further optimization.

Conclusion: Nevertheless the new method seems to offer a very helpful diagnostic tool for pathologists as well as for surgery.

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7897-27, Session 5

Behavior of optical properties of coagulated blood sample at 633-nm wavelength

B. Morales, S. Vazquez-Montiel, J. A. Delgado Atencio, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico)

Determination of tissue optical parameters is fundamental for application of light in either diagnostics or therapeutic procedures. However, in samples of biological tissue in vitro, the optical properties are modified by cellular death or cellular agglomeration that can not be avoided. This phenomena change the propagation of light within the biological sample. Optical properties of human blood tissue were investigated in vitro at 633 nm using an optical setup that includes a double integrating sphere system. We measure the diffuse transmittance and diffuse reflectance of the blood sample and compare these physical properties with those obtained by Monte Carlo Multi-Layered (MCML). The extraction of the optical parameters: absorption coefficient μa, scattering coefficient μs and anisotropic factor g from the measurements were carried out using a Genetic Algorithm, in which the search procedure is based in the evolution of a population due by selection of the best individual, evaluated by a function that compares the diffuse transmittance and diffuse reflectance of those individuals with the experimental ones. The algorithm converges rapidly to the best individual, extracting the optical parameters of the sample. We compare our results with those obtained by using other retrieval procedures. We found that the scattering coefficient and the anisotropic factor change dramatically due to the formation of clusters.

7897-28, Session 6

Vibrational spectroscopy characterization of low-level laser therapy on mammary culture cells: an micro-FTIR study


Low level laser therapy (LLLT) is an emerging therapeutic approach for pain treatment, wound healing; tuberculosis; temporomandibular joint disorders; and several musculoskeletal conditions. The clinical effects induced by LLLT presumably go from the photobiostimulation/photobiokinibition at cellular level. However, the detailed mechanism underlying this effect is obscure. The present work is dedicated to quantify some relevant aspects of LLLT related to cell proliferation and apoptosis of. This goal was attached by exposing malignant breast cells (MCF7) to spatially filtered light of a He-Ne laser (633 nm). The parameters of the study were the laser power density (0.32, 0.65 and 0.97 mW/cm²), treatment time (1 minute), and cell adaptation time 6-24h. The cell viability was evaluated by microscopic observation using Triplan Blue. The vibrational spectra of each experimental group (micro-FTIR technique on dry cells) was used to identify the relevant biochemical alterations occurred in the process. It was found necrotic and apoptotic characteristic signals wich had statistically correlation to the high fluence experimental group.

7897-29, Session 6

Detection of pre-charring optical behavior at a laser catheter-tip in blood: ex vivo and in vivo study

M. Takahashi, A. Ito, T. Kajihara, T. Arai, Keio Univ. (Japan)

We studied an optical behavior of blood at a laser catheter-tip during a red laser irradiation (663 nm, CW) with around 50 W/cm² in blood. The aim of this study is to detect pre-charring optical behavior at the laser catheter-tip to prevent charring during the therapeutic laser irradiation in blood.

Red blood cell (RBC) shapes were observed after the laser irradiation ex vivo. A round formation, aggregation, and hemolysis were found until blood charring. We measured a time-history of diffuse-reflected light power and transmitted light power from the blood model consisted of major blood components during the laser irradiation with a microscope optics. The diffuse-reflected-light power decreased following a gentle peak before the charring. This decrease indicated the pre-charring behavior which may be induced by scattering and absorption coefficient changes due to RBC degenerations described above. We detected the pre-charring behavior in a backscattering-light power change via the laser catheter located in porcine right atrium.

To prevent the charring, we performed the laser power control to 80% at the pre-charring behavior detection. The charring free laser irradiation was achieved with this laser power control even though the energy input was at least 4.3 times as much as without power control.

We think that the backscattering light power measurement and laser power control via the laser catheter might be useful to detect pre-charring behavior, and to prevent the charring for therapeutic laser irradiation in blood under catheterization such as arrhythmya treatment with photodynamic therapy.

7897-30, Session 6

Three-dimensional angular-domain optical projection tomography

E. Ng, F. Vasefi, The Univ. of Western Ontario (Canada); B. Kaminska, Simon Fraser Univ. (Canada); J. J. Carson, The Univ. of Western Ontario (Canada)

Angular domain imaging (ADI) has been previously demonstrated to generate projection images of attenuating targets embedded within a turbid medium. The imaging system employs a silicon micro-tunnel array positioned between the sample and the detection system to reject scattered photons that have deviated from the initial propagation direction and to select for ballistic and quasi-ballistic photons that have retained their forward trajectory. Two dimensional tomographic images can be reconstructed from ADI projections collected at a multitude of angles. The objective of this work was to extend the system to three dimensions by collecting several tomographic images and stacking the reconstructed slices to generate a three dimensional volume representative of the imaging target. A diode laser (808nm, CW, ThorLabs) with a beam expander was used to illuminate the sample cuvette. An Angular Filter Array (AFA) of 80 μm x 80 μm square-shaped
Angular filtration on scattered photons. Images were detected with a linear CCD (16-bit, Mightex). Our approach was to use a SCARA robot (Epson E2S531S) to rotate and translate the sample to collect sufficient projections to reconstruct a three dimensional volume. Imaging targets consisted of graphite rods of various sizes and capillary tubes filled with absorbers at different optical densities. Graphite rods were then used to evaluate the signal contribution from absorbers outside of the imaging plane.

Angular-domain imaging of fluorescence sources within tissue phantoms
R. Cheng, P. B. Tsui, G. H. Chapman, N. Pfeiffer, B. Kaminska, Simon Fraser Univ. (Canada)

Conventional fluorescence imaging often does not have a mechanism to remove the scattering effect in biological tissue. We use Angular Domain Imaging (ADI) to improve the detection of smaller structures in fluorescence layer over that can be provided by existing systems. ADI is a high resolution, ballistic imaging method that utilizes the angular spectrum of photons to filter multiple-scattered photons and accepts only photons with small angular deviation from their original trajectory. Advantages of the ADI technique are that it is insensitive to wavelength and the sources are not required to be high quality, coherent, or pulse, as with OCT or time domain. Our target is to perform fluorescence ADI at shallow tissue such as skin (=1mm) with a buried collagen layer. To experimentally model shallow tissue with phantoms, a thin layer of scattering medium with similar scattering characteristic (μs = 200cm⁻¹, g = 0.85) is placed on top of fluorescence sources such as R6G (533nm excitation, = 570nm emission) and fluorescence plastic (415nm excitation, = 490nm emission) which is patterned by strips of non-scattering medium with small angular deviation from their original trajectory.

Experimental optical technique for the investigation of light transport within irradiated tissues
R. Ankri, D. Fixler, H. Taitelbaum, Bar-Ilan Univ. (Israel)

Our research main goal is to develop an experimental non-invasive optical method for tissue structure and physiological state investigation. This technique enables to identify the optical parameters of an irradiated tissue, such as its scattering coefficient, and its absorption parameter, by measuring the reflected light intensity profile of the tissue. We will present for the first time results that support the claim that reflected light intensity can be used as a fingerprint for the identification of tissue structures and their physiological condition.

Subsurface temperature imaging techniques during infrared laser-tissue interactions
R. M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); S. Been, J. H. Klaessens, Univ. Medical Ctr. Utrecht (Netherlands)

Thermal cameras can be used to study the thermal effects during IR laser tissue interaction, however, it is limited to the surface of a tissue and the typical video rates are 25 to 50 frames/s. A new strategy has been developed to enable visualization of the absolute temperature distribution below the surface by sandwiching biological tissue between ZincSelenide windows. A thermo camera, enhanced with close-up optics, looks through the window from aside during laser exposure at the surface. In addition, high speed imaging up to 10,000 frames is used to visualize the temperature gradients below the surface in a transparent tissue model based on color Schlieren techniques.

The basic temperature distribution and dynamics underneath the surface of biological tissues were studied with various IR laser systems: 810 nm continuous wave Diode, 2.0 µm continuous wave Thulium (288 µJ/pulse), Er:YSGSG, continuous wave and pulsed 10.6 µm CO2. Depending on the wavelength, designated optical fibers were used to deliver the IR light to the tissue: silica fibers, hollow waveguides, silver halide fibers and photonic bandgap fibers. The laser beam was either in a static position or scanned over the surface. The thermal imaging was simultaneously recorded with normal video for comparison.

The thermal, high speed and normal imaging techniques showed to be both compatible and complementary. The subsurface thermal imaging enable comparison and better understanding of the tissue effects between various continuous wave and pulsed IR laser systems and delivery systems.
for molecular imaging and selective photothermal therapy of human acute leukemia cells using a near-infrared laser. Gold Nanorods (GNR) are synthesized and conjugated to CD33. We report significantly increased accumulation of GNR-CD33 complexes on the surface of HL-60 cells, when compared with only GNR-PEG. The same effect was observed for chronic human leukemia (K-562 cells). The number of GNR on the surface of cells was determined with silver enhancement staining. After peylation, or conjugation with CD33 antibody, GNR were non toxic for leukemia cells: the measure of cytotoxicity was analyzed by lactate dehydrogenase release from the cells to the culture medium. Binding for GNR-antibody conjugates was higher for acute leukemia cells (HL-60), and therefore these cells were used for thermootherapy experiments. HL-60 cells were treated for 45 min with GNR conjugated with mAb CD33, or with GNR-PEG particles. We used a Quanta System q-switched titanium sapphire laser emitting at a center wavelength of 765 nm. Each sample was illuminated with 1 or 3 laser shots at either high or low fluence. Both laser modes (1 or 3 shots) were used in 3 independent cells probes. After laser application, the cells were resuspended and analyzed to cell viability with Trypan blue exclusion assay.

GNR-CD33 conjugates significantly increase the percentage of cell death as compare with a control group after laser illumination: even for single laser shot 3 fold increases, and near 4 times fold increase for 3 laser shots.

7897-35, Session 7
Skin damage thresholds with continuous-wave laser exposures at the infrared wavelength of 1.3 µm

J. W. Oliver, S. S. Kumru, R. J. Thomas, B. A. Rockwell, Air Force Research Lab. (United States); C. A. Harbert, G. D. Noojin, I. Noojin, K. J. Schuster, A. D. Shingledecker, TASC, Inc. (United States)

Damage thresholds (ED50) for skin using Yucatan mini-pig (Sus scrofa domestica) have been determined at the operational wavelength of 1.3 µm with beam diameters of 0.61 cm and 0.95 cm. Exposure durations of 0.25, 1.0, 2.5 and 10 seconds were used to determine trends in damage threshold with respect to exposure time and beam diameter at this moderately penetrating wavelength. A relatively narrow range of total radiant exposure from 19.3 J/cm² to 30.5 J/cm² was observed for threshold damage with laser parameters encompassing a factor of two in beam area and a factor of forty in exposure duration.

7897-37, Session 8
Amplified photodynamic effect on skin cells in-vitro exploiting the surface plasmon resonance effect of metal nanoparticles

B. Bauer, Göteborg Univ. (Sweden); S. Chen, M. Käll, L. Gunnarsson, Chalmers Univ. of Technology (Sweden); M. B. Ericson, Göteborg Univ. (Sweden)

Our investigations aim to increase efficiency of photodynamic therapy (PDT) by exploiting the surface plasmon resonance effect of metal nanoparticles. In PDT photosensitizers generate singlet oxygen at the tumour site upon exposure to visible light. Although PDT is a well established treatment for skin cancer, a major drawback is low singlet-oxygen quantum yield, creating a need for novel methods to enhance singlet oxygen production during treatment. In this light, we study the photodynamic effect on cultured human skin cells in the presence of metal nanoparticles, which, by enhancing the electromagnetic field, induce localized amplified photoactivation. In experiments, cultured skin cells are exposed to protoporphyrin IX and gold nanoparticles and subsequently illuminated with red light. We investigate the differences in cell viability by tuning different parameters such as incubation and illumination times. To find optimal parameters for specific targeting of tumour cells, we compare normal human epidermal keratinocytes with a human squamous cell carcinoma cell line. Results indicate significantly enhanced cell death in the presence of nanoparticles, and differences in treatment efficiency between normal and tumour cells, showing promise for further development of nanoparticle inclusion in clinical treatment.

7897-38, Session 8
Laser injury and in-vivo multimodal imaging using a mouse model

G. M. Pocock, Air Force Research Lab. (United States); A. Boretsky, P. Gupta, The Univ. of Texas Medical Branch (United States); J. W. Oliver, Air Force Research Lab. (United States); M. Motamedi, The Univ. of Texas Medical Branch (United States)

A murine model was used to perform in vivo experiments of laser-induced thermal damage to the retina. The expression kinetics and protein localization of biomarkers after laser induced injury to the retina and skin would provide a better understanding of the laser exposure levels at which the tissue becomes “stressed” and may be useful to detect sub-threshold laser damage that may or may not manifest as a visible lesion. A Heidelberg Spectralis HRA with a spectral domain optical coherence tomographer (OCT) was used to obtain images of the fundus and obtain cross-sectional views of the retina. Subthreshold and threshold lesions were observed using the OCT, infrared (IR) reflectance, and autofluorescence imaging modalities to observe lesion appearance. Volumetric representations of the retina were created to visualize lesion localization of damage.

7897-39, Session 8
Optical control of urination in neurogenic bladder using femtosecond-pulsed laser

J. Yoon, M. Choi, C. Choi, KAIST (Korea, Republic of)

Even though catheterization or electric stimulation are used for treatment of neurogenic bladder, invasiveness and inconvenience of these approaches prompt us to develop a new possible therapeutic method to control urination by using optical stimulation. The optical method using femtosecond pulsed laser (FSPL) has advantages of focused and subsurface stimulation. Irradiation of FSPL induced a rapid increase of intracellular calcium level followed by contraction of primary cultured human bladder smooth muscle cells. Short exposure of bladder detrusor ex-vivo to FSPL also induced a controlled contraction of detrusor. Collectively, we propose that FSPL can be considered as a potential therapeutic approach for intractable neurogenic bladder.

7897-40, Session 8
Low-energy laser irradiation promotes synovial fibroblast proliferation

D. Taniguchi, Ayabe City Hospital (Japan); P. Dai, Y. Harada, Y. Yamaoka, Kyoto Prefectural Univ. of Medicine (Japan); T. Hojo, Doshisha Univ. (Japan); T. Takamatsu, Kyoto Prefectural Univ. of Medicine (Japan)

Low-energy laser irradiation (LELI) has been found to modulate various biological effects, especially those involved in promoting cell proliferation. Synovial fibroblasts are important in maintaining the homeostasis of articular joints and have strong chondrogenetic capacity. Here, we investigated the effect and molecular basis of LELI on synovial fibroblast proliferation. HIG-82 rabbit synovial fibroblasts were cultured, and laser irradiation (660 nm) was applied at the power density of 40 mW/cm² for 2 minutes, corresponding to laser fluence of 4.8 J/cm². The effect of LELI on cell proliferation, cell cycle progression, and expression of cyclin-
dependent kinase inhibitors (CKIs) were investigated. We also examined whether the effects of LELI on HIG-B2 cell proliferation were affected by cAMP content, which is known to influence the cell cycle via inducing CKIs. LELI promoted HIG-B2 synovial fibroblast proliferation and induced cytoplasmic localization of cyclin-dependent kinase inhibitor p15 (INK4B/CDKN2B). Moreover, the proliferation of HIG-B2 synovial fibroblasts was reduced by cAMP, while cAMP inhibitor, SQ22536, induced p15 cytoplasmic localization and as a result, elevated synovial fibroblast proliferation was observed. In addition, the promotive effect of LELI-induced HIG-B2 synovial fibroblast proliferation was abolished by cAMP treatment. Our findings suggest that cAMP may be involved in the effect of LELI on synovial fibroblast proliferation. We revealed the effect and molecular link involved in synovial fibroblast proliferation induced by 660-nm LELI. Our study provides new insights into the mechanisms by which LELI has biological effects on synovial fibroblast proliferation.

No effect of femtosecond laser pulses on DNA, protein, M13, or E. coli
J. C. Wigle, E. A. Holwitt, K. E. Sheldon, U.S. Air Force (United States); C. D. Trinh, Conceptual MindWorks, Inc. (United States); A. M. Brincko, U.S. Air Force (United States)

Following published methods we have been unable to reproduce inactivation results, or to show any interaction whatsoever, between 90 femtosecond (fs) pulses of 850 nm or 425 nm laser radiation and buffer/water, DNA, protein, M13 or E. coli. Data showing what is postulated to be Impulse Stimulated Raman Scattering (ISRS) induced inactivation of M13 bacteriophage, Tobacco Mosaic Virus, Escherichia coli (E. coli), and human Jurkat T-cells following exposure to 80 femtosecond (fs) pulses of 850 nm or 425 nm laser radiation have been published. Interest in the physical/chemical mechanism of this phenomenon led us to attempt to reproduce the published results for M13 and E. coli. Irradiations at which inactivation of M13 and E. coli were reported to occur were ~55 MW/cm² and ~700 MW/cm², respectively, after 1 hr exposures in a stirred suspension. We exposed purified plasmid DNA (pUC19), bovine serum albumin, and M13 to irradiances of up to 120 MW/cm² for 1 hr without effect, as measured by agarose gel electrophoresis (AGE), denaturing polyacrylamide gel electrophoresis (PAGE) and plaque forming efficiency in soft agar, respectively. We also measured no effect on DNA or coat proteins extracted from irradiated M13 as measured with AGE or PAGE. Finally, exposures of up 1 GW/cm² at 850 nm had no effect on the viability of E. coli as evaluated by a colony forming assay in soft agar. Peroxynitrite, known to be toxic, to cause single strand breaks in DNA, and to fragment proteins in vitro gave positive results in all the assays.

Correlating computational docking predictions with Raman spectroscopy for beta-lactoglobulin-porphyrin complexes
J. E. Parker III, L. Brancalone, The Univ. of Texas at San Antonio (United States)

Computational molecular docking simulations (Dock and AutoDock) may provide a wealth of structural information related to the bound configuration of protein-ligand complexes, but they require verification to ensure their predictions reflect reality. Resonance Raman spectroscopy data has been collected to correlate normal mode vibrations observed in the bound structure to computationally generated structures in order to determine the best match between the computational model and experiment. This methodology was used to determine the bound structures at an atomistic level of beta-lactoglobulin (BLG) and mesotetrakis (p-sulfonatophenyl) porphyrin (TSPF) in aqueous solutions at pH 7 and 9. Comparisons of Raman spectra of TSPF before and after binding to BLG yield line shifts that are related to the distortions in the future molecule that are presumed to be generated by the non-covalent binding of the ligand to the protein. Previous studies have shown that the Tanford transition in BLG, which occurs above pH 7.9, destabilizes the protein, allowing it to undergo a laser-induced structural change when bound to TSPF and illuminated by at least 0.3 J of laser energy. By examining the structures at pH above and below the transition, we hope to reveal the mechanism of action that initiates the laser-induced changes in the protein. Future studies will use the computed bound configuration as an initial condition for molecular dynamics simulations of the laser and protein-complex to predict the final state of the protein after irradiation.

Rotating wall vessel designed for fluorescent imaging
T. J. Tayag, Texas Christian Univ. (United States); D. Dimitrijevich, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); L. C. Del Gallego, P. Kumar, Texas Christian Univ. (United States)

Fluorescent imaging of cells and tissues cultured within a rotating wall vessel (RWV) bioreactor offers quantitative assessment of the 3-dimensional aggregation of cells into tissue constructs. Tissues cultivated in RWVs are typically removed from the culture process and analyzed with a variety of optical techniques, such as conventional microscopy, fluorescence microscopy, and flow cytometry. In each of these analysis techniques, the culture process must be halted and the cells or tissues removed from the bioreactor for analysis. In this paper, we present the design and characterization of an optimized fluorescent imaging system for real-time tissue analysis within a RWV bioreactor. This system is capable of dynamic fluorescence imaging using a mercury arc lamp excitation source as well as a laser excitation source. We demonstrate the fluorescent imaging of porcine pancreatic islets stained with DAPI after fixation. The trajectory of the islets within the RWV bioreactor is consistent with our hydrodynamic model of particle motion in the bioreactor. Modulation transfer function (MTF) characterization shows that our system provides an image resolution which is significantly improved over the commercially-available RWVs. The impact of this improvement will be realized as sophisticated digital image processing algorithms are developed for tracking the size and growth rate of 3-dimensional cultured tissues. The ability to non-intrusively monitor and assess cellular processes in real-time using fluorescence technologies will benefit tissue engineering techniques.
attenuation of the polymer across a temperature range of 0-70 deg C. The glass transition is clearly observed as a discontinuity in the 1st derivative of the speed of sound and a decrease in the acoustic attenuation. Although acoustics alone is useful in characterization the scaffolds, useful information can be obtained from optical techniques on the growth of cells within the scaffold. Light scattering is of course a significant problem for relatively thick engineered tissue (~5mm). The acoustic approach has therefore been extended to include laser illumination and detection of the ultrasound modulated optical pulse. Images of both gel-based tissue phantoms and tissue scaffolds will be presented demonstrating that an optical resolution of ~100um can be achieved in highly scattering samples.

7897-45, Session 9
In-vitro Raman spectroscopic process monitoring of tissue engineered oral mucosa constructs
A. Ganguly, J. H. Cole, S. Kuo, C. L. Marcelo, Univ. of Michigan (United States); K. Izumi, Niigata Univ. (Japan); S. E. Feinberg, M. D. Morris, Univ. of Michigan (United States)

We will describe the use of Raman spectroscopy for in vitro process analysis of tissue engineered oral mucosa constructs. The objectives of our study are: 1) to investigate noninvasive Raman spectroscopy as a possible in-line process monitoring technique for oral mucosa tissue engineering and 2) to identify and derive metrics for normal vs. abnormal tissue development which will provide process control variables. The engineered tissue is a human oral mucosa, called Ex Vivo Produced Oral Mucosal Equivalent (EVPOME), and has been previously used successfully in clinical trials for intraoral grafting. To induce abnormalities in tissue development, the specimens are incubated at elevated temperature 43°C with 5% CO2 (stressed) prior to a Raman measurement. Otherwise, both stressed and non-stressed specimens are cultured at 37°C using our standard protocol. Raman spectroscopy is performed with 785nm excitation and with microprobe or fiber optic probes. We will discuss the conditions needed for successful use of non-confocal optics in this highly turbid system. We will describe the problems arising from non-uniform thickness of both the AlloDerm® substrate and the cultured constructs. We will also discuss sampling protocols needed to detect local stress and other problems that may arise in the construct, as well as protocols for removal of interfering Raman bands arising from the specialized culture dishes used.

7897-46, Session 9
Validation of artificial skin equivalents as in-vitro testing systems
R. Schmitt, RWTH Aachen (Germany) and Fraunhofer-Institut für Produktionstechnologie (Germany); U. Marx, Fraunhofer-Institut für Produktionstechnologie (Germany); H. Walles, A. Heymer, M. Kaufmann, Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik (Germany)

With the increasing complexity of the chemical composition of pharmaceuticals, cosmetics and everyday substances, the awareness of potential health issues and long term damages for humanoid organs is shifting into focus. Artificial in vitro testing systems play an important role in providing reliable test conditions and replacing precarious animal testing. Especially artificial skin equivalents ASE are used for a broad spectrum of studies like penetration, irritation and corrosion of substances. One major challenge in the tissue engineering of ASEs is the growth of a completely covering hornified stratum corneum surface layer. The presence of a continuous and intact stratum corneum is needed for in vitro testing and the imitation of the skin protection barrier. Therefore, the validation of each individual ASE as testing system with non invasive sensor technologies is required. We performed different studies to characterize the stratum corneum and its potential structural defects by selectively harming the surface and analysing the effects. As for ASE we used a two layer cellular system based on primary humanoid or HaCaT keratinocytes and a collagen - fibroblasts matrix. For the analysis we developed an fully automated optical coherence tomography FDOCT system that is capable for inline monitoring of ASEs. 3D-OCT tomograms and surface topography charts were compared to histologies and high resolution confocal microscopy regarding defects, surface roughness and layer structure. We found that optical coherence tomography is a well suited technology to characterize artificial skin equivalents and validate the application as testing system.

7897-47, Session 10
Discrimination of type I and type II collagen by nonlinear microscopy
P. Su, W. Chen, T. Li, C. Chou, T. Chen, Y. Ho, C. Huang, National Taiwan Univ. (Taiwan); S. Chang, I-Shou Univ. (Taiwan); Y. Huang, National Taiwan Univ. (Taiwan); H. Lee, National Taiwan Univ. Hospital (Taiwan); C. Dong, National Taiwan Univ. (Taiwan)

Using excitation polarization-resolved second harmonic generation (SHG) microscopy, pixel-resolved SHG intensity is measured as a function of the excitation polarization angle for type I and type II collagens. We found that the $\chi^{(2)}$ tensor ratios can be used to distinguish collagens I and II. Specifically, the second order susceptibility ratios $\chi^{(2)}_{xxz}/\chi^{(2)}_{zzx}$ and $\chi^{(2)}_{zzx}/\chi^{(2)}_{xxz}$ can be used to discriminate the two collagen types. By displaying the $\chi^{(2)}$ tensor ratios as images, variations in the $\chi^{(2)}$ tensor ratios can be used as a contrast mechanism for distinguishing type I and II collagens.

7897-48, Session 10
Advanced methods of quantifying electrospun fiber alignment
N. J. Schaub, S. J. Kirkpatrick, Michigan Technological Univ. (United States); R. Gilbert, Rensselaer Polytechnic Institute (United States)

The degree of electrospun fiber alignment has been shown to directly influence the degree of directed cellular migration. However, there are few robust methods that can quickly and accurately quantify fiber alignment. The goal of this work is to develop and assess a method of quantifying fiber alignment utilizing the Radon transform (RT). The RT method is compared to an assessment using a 2-dimensional Fast Fourier Transform (FFT), a common method for determining alignment. To evaluate the sensitivity of each method to variations in alignment and signal-to-noise ratio, a series of images with varying degrees of line angle variation and noise were generated. The application of each algorithm is demonstrated on SEM images of two types of electrospun fiber samples: Aligned, semi-aligned, and randomly oriented samples. Both the RT and FFT-based approach to assessing the distribution of fiber alignment yield a PDF of fiber orientations in the images. Using these PDFs, the degree of orientation of the fibers in each sample was then quantified by calculating the entropy in each image. Utilizing the RT for analysis of fiber alignment has potential in accurately quantifying fiber alignment.
Chemotaxis and migration of mutant and wild-type cells in 3D and 4D using ultra-high-resolution optical coherence tomography

S. M. Rey, Cardiff Univ. (United Kingdom); B. Považay, B. Hofer, Medizinische Univ. Wien (Austria); A. Harwood, Cardiff Univ. (United Kingdom); W. Drexler, Medizinische Univ. Wien (Austria)

Changes in cell chemotaxis can severely affect normal physiological function. Typically, monitoring of this behaviour is performed on 2D glass substrates for ease of visualization of unlabelled cells. However, cell substrates, for example usually opaque biomaterials, often significantly influence cell behaviour. Behaviour also differs within more natural three-dimensional constructs. Therefore conventional methods of investigating cell chemotaxis can reveal only limited information. Probing effects of the additional freedom of the third dimension on the cell generally requires use of chemical markers which can influence cellular processes.

Frequency domain optical coherence tomography (OCT) at 800nm, a high-speed label-free volumetric imaging technique, is used to image chemotaxing Dictyostelium discoideum cells in timelapse, during development and on exposure to a cAMP gradient. OCT enables visualization of cells in 2D on opaque substrates and when suspended within a 3D agarose gel. Ax2 (wild-type) cells are able to move within agarose and form streams. However, knockout mutants with structural or chemotactic pathway deficits respond differently. Cells lacking myosin (mhcA-), are able to migrate in 2D but are unable to move within the more challenging environment of agarose. Cells lacking adenylyl cyclase (ACA) are able to move in 3D but unable to respond to cAMP chemoattractant. Migratory and chemotactic changes in cells can be visualized using OCT and subsequently quantitatively analyzed. This enables a greater understanding of the mechanisms behind cell movement and in vivo cell biology in complex more natural environments, thereby potentially assisting the treatment of disease and the engineering of new and replacement tissues.

Dynamic in-vivo visualization of anastomosis between a prevascularized implantable tissue construct and host circulation

S. White, C. Hughes, B. Choi, S. C. George, Univ. of California, Irvine (United States)

The thickness of implantable engineered tissue is restricted by the relatively short diffusion path lengths of waste products and necessary nutrients such as oxygen. Convective flow by means of a vascular network can be used to overcome this limitation. Such networks can be created in vitro prior to tissue implantation, termed prevascularization. We have previously prevascularized fibrin tissues seeded with cord blood endothelial precursor cell-derived endothelial cells and normal human lung fibroblasts to demonstrate rapid (~1 day) anastomosis with the host circulation. In this study, we have implanted these tissues into mouse dorsal window chambers to facilitate dynamic imaging of anastomosis using laser speckle imaging (LSI), multispectral imaging (MSI), and multiphoton microscopy (MPM). Our preliminary LSI and MSI data suggest that anastomosis between the implanted tissue with the host circulation occurs as early as day 3, and subsequent changes in vessel architecture and hemodynamics can be measured. By day 21, functional vascular density had increased by 226% in the prevascularized implant as compared to 154% in the fibrin-only control. MPM confirmed the presence of blood-filled lumens within the implant and allowed observation of vessels as they were remodeled from immature to organized networks consistent with native capillaries. LSI, MSI, and MPM of a dorsal window chamber permits the visualization and quantification of dynamic in vivo anastomosis between prevascularized tissue implants and the host, and may be used to enhance the design of thick tissue engineered constructs, as well as mechanisms of anastomosis.

Optical methods for diagnostics and feedback control in laser-induced regeneration of spine disc and joint cartilages

E. Sobol, A. P. Sviridov, A. V. Omelchenko, O. I. Baum, Institute on Laser and Information Technologies (Russian Federation); A. V. Baskov, I. Borchshenko, V. S. Golubev, V. A. Baskov, Medical Ctr. for Vertebrology and Orthopedics (Russian Federation)

Optical techniques allow to enhance efficacy and safety of the novel low-invasive laser procedures to be used in orthopedics for the treatment cartilages of spine and joints.

The paper considers physical processes and mechanisms of laser regeneration, presents results of investigations aimed to optimize laser settings and to develop feedback control system for laser reconstruction of spine discs (LRD) based on the light scattering measurements. Since 2001 the LRD has been performed for 510 patients with 90% positive results. LRD differs from other techniques of low invasive treatment of spine diseases. (1) LRD effects on the nucleolus pulposus (NP) without any heating of annulus fibrosis (AF) since other techniques effect mainly on the AF and the nerves surrounding the disc. The heating of NP in the course of LRD is quite low (up to 50 C), the main effect of laser irradiation is thermomechanical activation of regeneration processes. (2) The positive dynamics of the results during six years follow up. Similar technology has been experimentally tested for reparation of traumatic and degenerative diseases in joint cartilage of 20 minipig. The results have shown that laser regeneration of cartilage allows feeling large (more than 5 mm) defects which usually never repair on one’s own. Optical techniques have been also used to monitor the dynamics of gas bubbles growth in the course of heterogeneous heating of cartilage under laser radiation. Since the size of gas bubbles and pores in cartilage is of great importance these measurements can be used for tissue diagnostics.

Optical methods for diagnostics and feedback control in laser-induced regeneration of spine disc and joint cartilages

A. S. Kurkov, A. M. Prokhorov General Physics Institute (Russian Federation); T. Genning, D. Arslanova, L. Belozerova, O. Voronova, V. Svetukhin, Ulyanovsk State Univ. (Russian Federation); E. Sholokhov, A. M. Prokhorov General Physics Institute (Russian Federation); T. Genning, D. Arslanova, L. Belozerova, O. Voronova, V. Svetukhin, Ulyanovsk State Univ. (Russian Federation)

Laser jet, created in GPI RAS with the help of specialists from UIUS with operating power up to 5 W inclusive and operating wavelength - 1265 nm was used in this work. Experiment was hold on Er of human beings and rats after irradiation of fiber RAMON-laser (duration of exposure was 1, 5, 10 and 20 min) with average irradiation intensity 0.2 W/sm2. Morphofunctional state was estimated by the level of peroxidation of lipids and activation of enzymatic link of antioxidant system by the rate of osmotic swelling and also by the topology and rigidity of membrane. Vividly expressed strong undulating dependence of changes of morphofunctional state and loss of Er under irradiation by RAMON-laser with mentioned parameters subject to duration of exposure was revealed in the experiment.
Red light emitting diode irradiation inhibits high glucose-enhanced osteoclastogenesis of rat bone marrow cells

W. Li, Chung Yuan Christian Univ. (Taiwan)

Hyperglycemia has been implicated in the pathogenesis of diabetic bone disease. By photo-modulating the biological functions of osteoclasts, LLLI (Low level light irradiation) may be applied in the clinical treatments for diabetic osteoporosis. Light irradiation was performed at radiant exposure of 2 J/cm², and single or multiple exposures using a light source composed of red light emitting diodes (LEDs) with wavelength of 660 nm and irradiance of 10 mW/cm². Our results found that LLLI led to decrease the number of osteoclasts differentiated from rat bone marrow monocytes. Cell cycle analysis revealed that the percentage of cells in G0/G1 phase increased and that in S phase decreased following LLLI. The gene expression of RANK and cathepsin K were found to decrease after LLLI. When rat bone marrow monocytes were cultured in a medium containing high concentration of glucose, decreased viability and increased level of lactate dehydrogenase release were found. Production of reactive oxygen species (ROS) and the gene expression level of RANK, TRAP and cathepsin K elevated during high glucose incubation. Whereas LLLI was shown to lessen the effects on rat monocytes from osmotic shock due to high glucose incubation by increasing cell viability and inhibiting ROS production as well as osteoclastic gene expression. In conclusion, our findings suggest that LLLI under specific parameters may accelerate the process of bone remodeling by inhibiting osteoclastogenesis and also alleviate the enhancement of osteoclastic differentiation resulted from high glucose incubation.

Stretching of red blood cells by optical tweezers quantified by digital holographic microscopy

N. Cardenas, W. Hanson, The Univ. of Texas at Arlington (United States); L. Yu, Nanoscope Technologies LLC (United States); S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Red blood cells (RBC) possess unique visco-elastic characteristics which allow them to pass through capillaries narrower than their size. Estimation of visco-elastic properties of RBC requires measurement of extent of deformation in RBC subjected to known force. Optical tweezers, being gentle and absolutely sterile, are emerging as the tool of choice for application of localized force on cells. However, axial deformations due to stretching of cells cannot be quantified by classical microscopic images. Here, we report realization of off-axis digital holographic microscopy (DHM) for highly sensitive axial changes in RBC shape due to stretching by optical tweezers. In order to evaluate the resolution of our method, the RBC was stretched in axial direction with nanometer precision by change of divergence of the trapping beam at back of the microscope objective. DHM revealed stress distribution over a larger region though the RBC membrane was stretched at a point. The obtained force vs deformation patterns were compared with numerical simulation. Since in diseases such as malaria and leukemia, change in rigidity of red blood cells occurs, this system could prove to be an important tool for diagnosis based on elasticity changes.
Ultra-high-sensitive optical microangiography reveals dynamic changes of depth-resolved microcirculations within skeletal muscles

Y. Jia, R. K. Wang, Oregon Health & Science Univ. (United States)

The primary pathophysiology of peripheral arterial disease (PAD) is associated with impaired perfusion to the muscle tissue in the lower extremities. The lack of effective pharmacologic agents to treat this disease by stimulating vessel collateralization emphasizes the need for an imaging method that can be used to dynamically visualize depth-resolved microcirculation within muscle tissues. Optical microangiography (OMAG) is a recently developed label-free imaging method capable of producing 3D images of dynamic blood perfusion within micro-circulatory tissue beds at an imaging depth up to ~2 mm, with an unprecedented imaging sensitivity to the blood flow at ~4 µm/s. In this study, we demonstrate the utility of OMAG in imaging the detailed blood flow distributions, at a capillary level resolution, within skeletal muscles in mice. By use of the mouse model of hind-limb ischemia, we show OMAG can yield the chronic assessment of time-dependent changes in muscle perfusion and perfusion reserve along tissue depth. These findings indicate that OMAG can represent a sensitive and consistent technique to effectively study pharmacologic therapies aimed at promoting the growth and development of collateral vessels.

Tissue structural characterization appropriate for Monte Carlo studies

D. D. Duncan, Portland State Univ. (United States); D. G. Fischer, NASA Glenn Research Ctr. (United States); A. L. Dayton, S. A. Prahl, Providence St. Vincent Medical Ctr. (United States)

Analysis of the first and second order statistical properties of light is powerful means of establishing the properties of a medium through which light has propagated. In turn, the first and second order statistical properties of the medium dictate the manner in which light interacts with the medium. Towards an understanding of the propagation of light through complex structures, such as biological tissue, one might choose the first or second viewpoint. Fundamental to the problem, however, is a physical parametric model of the propagation medium; a model that allows prediction of the measured effect or prediction of the parameters based on measurements. This is the objective of our study.

For studying propagation through random media, a common characterization of the medium is in terms the spatial power spectrum of its refractive index. Here, we choose a characterization in terms of the refractive index gradient. Such a characterization is convenient for use in Monte Carlo studies. We demonstrate acquisition of the requisite quantitative phase gradient information using a conventional, unmodified differential interference contrast microscope. Results are in terms of local maps of the polar and azimuthal scatter directions. Subsequently, first and second order statistics are computed. The concept is demonstrated for phantoms and some representative tissues.

Fluctuation imaging to screen for subcellular dynamics in living tissue

D. D. Nolte, R. An, J. J. Turek, Purdue Univ. (United States); K. Jeong, Korean Military Academy (Korea, Republic of)

Motility contrast imaging (MCI) is a coherence-domain volumetric imaging approach that uses subcellular dynamics as an endogenous contrast agent to image living tissue [1]. Fluctuation spectroscopy analysis of dynamic light scattering from three-dimensional tissue displays functional frequency bands related to organelle transport, membrane undulations and cell movements. Drugs and environmental perturbations affect the fluctuation spectral bands in specific ways that enable us to construct fingerprints that may be useful in early drug toxicity screening in drug discovery applications. To interpret these fingerprints, we study the specific action of perturbations and drugs on multicellular tumor spheroids.

Two equivalent fluctuation analysis approaches are temporal autocorrelation and spectral power density. Although mathematically equivalent, they place different emphasis on the interpretation of the scattering data. The simplest models for dynamic light scattering invoke pseudo-diffusive transport or directed transport (for organelles) that do not agree well with general 1/f behavior observed for the MCI datasets. On the other hand, several aspects of the spectral power density do relate to multiple scattering in tissue and to different dynamical mechanisms. These include a strong temperature response, transient responses to osmolarity and thermal shocks, and inhibited or enhanced motions caused by anti-mitotic drugs. Changes in fluctuation amplitudes and frequencies provide the underlying biological basis to interpret subcellular dynamic responses to these environmental or xenobiotic influences.

preservation porcine aorta. The change in the pore size can be attested to the swelling of the tissue, collagen dissociation, and/or protein degradation. By using OCT, we have noninvasively determined that hypothermic preservation in physiological solution essentially maintains the integrity of biological tissue for the first 3 days of cryostorage.

7898-06, Session 1
**Diffusion versus deterministic mass transport in live cells**

R. Wang, Z. Wang, R. Iyer, L. Millet, Univ. of Illinois at Urbana-Champaign (United States); A. Levine, Univ. of California, Los Angeles (United States); M. Gillette, G. Popescu, Univ. of Illinois at Urbana-Champaign (United States)

The live cell is a highly dynamical system with complicated biophysical and biochemical processes taking place at diverse spatial and temporal scales. Though it is well known that cytoskeletons, mainly microtubules and actin filaments, play important roles in intracellular transport, their dynamic behavior is not entirely understood. We propose a unified approach to studying transport in live cells. We used Spatial Light Interference Microscopy (SLIM) [1], a quantitative phase imaging method developed in our laboratory, to extract cell mass distributions over broad spatiotemporal scales, i.e. (0.3-100) microns spatially and 0.5 s to a day temporally. The dispersion relations for this transport dynamics, i.e. frequency bandwidth vs. spatial frequencies, reveal deterministic mass transport at large spatial scales (w~q) and diffusive transport at small spatial scales (w~q^2) [2]. At submicron scales, we observed a w~q^3 behavior, which indicates bending of protein filaments bundles. We performed control experiments where both the microtubule and actin polymerization was blocked and found that essentially actin governs the long spatial scales behavior and microtubules the short scales. Moreover, the blebbistatin was used to inhibit the Myosin II movement which dominates the region of large q on the dispersion curve. This label-free method enables us to access different components of cell dynamics and quantify diffusion coefficients (in microns/ min) and the band width of speed distribution of motor proteins (in nm/s).

Ref.

7898-07, Session 1
**The study of proteoglycan concentration on tendons’ optical properties by PS-OCT**

Y. Yang, M. Ismail, A. P. Weightman, I. Wimpenny, Keele Univ. (United Kingdom); P. Bagnaninchi, The Univ. of Edinburgh (United Kingdom); M. Ahearn, Keele Univ. (United Kingdom)

Tendons are load-bearing tissues consisting mainly of type-I collagen and various proteoglycans including decorin and versican. It is widely accepted that highly orientated collagen fibers in tendons a play critical role for transferring tensile stress and display birefringent optical properties. However, little is known about the influence proteoglycans have on tendons’ optical properties. Tendinopathy (defined as a syndrome of tendon pain, tenderness and swelling that affects function) is a common disease which can result from a sporting injury or degeneration. As the essential components of the tendon extracellular matrix, the composition of proteoglycans and the changes in their expression and compositions have been associated with tendinopathy. In this study, polarization sensitive optical coherence tomography (PS-OCT) has been used to reveal the relationship between proteoglycan concentration and tendons’ properties and functions, in terms of birefringence. Tendons dissected from freshly slaughtered rats and chicken were fixed on a supportive board with the two ends being tied with sutures, and scanned by PS-OCT. The proteoglycans were extracted using guanidine HCl following a well established protocol. Time lapse PS-OCT images were collected throughout the extraction processing. It was found that the birefringence of the tendons decreased systematically regardless of the species or the tendon location. The remaining tendons’ morphology analyzed histologically. By combining nanofibers and ground materials (hydrogel) with variations of the hydrogel concentration and nanofiber density, the correlation of tendon components and birefringence has been established, which demonstrates a great potential for using PS-OCT as a diagnostic tool to examine tendon abnormality.

7898-08, Session 1
**Design of nanoparticles for medical applications: key physical mechanisms of efficient optical contrast**

L. D. Shvartsman, B. Laikhtman, The Hebrew Univ. of Jerusalem (Israel)

Biomedical applications of noble metal nanoparticles (NP) are based on efficient contrast. These applications include various kinds of imaging and photo-thermal therapy. For all of them, in the required spectral range the contrast has to be increased by the optimal choice of NP geometry and electro-physical properties of NP core and coatings. Plasmon resonances are believed to be the key physical mechanism of formation of resonant structure in NP scattering and absorption. We present a different physical picture and claim that the mechanisms of radiation trapping may be at least of the same importance. Thus, the very approach to NP design may be changed. Practical examples are presented.

7898-09, Session 2
**Speckle-based measurement of the light scattering by red blood cells in vivo**

I. Fine, A. Kaminsky, Elfi-Tech Ltd (Israel)

No abstract available.

7898-10, Session 2
**Effects of multiple decorrelartion time constants in laser speckle contrast imaging**

S. J. Kirkpatrick, Michigan Technological Univ. (United States); D. D. Duncan, Portland State Univ. (United States)

Laser speckle contrast imaging and analysis usually assumes a single decorrelation time constant. However, in actual, in vivo situations, this is probably unlikely and there are usually multiple underlying time constants. A well known example of this would be when using LSCI to assess cranial blood flow or perfusion through an intact skull. In this scenario, one could envision several underlying time constants: those associated with motion within the skull itself, those associated with un-ordered motion in the brain tissue outside of blood vessels, those associated with motion within capillaries and capillary beds, and those associated with the ordered flow within larger vessels. Several authors in the past have addressed the influence of static speckle on speckle contrast. Herein, we address through numerical simulations the issues of multiple time constants and of varying the proportion of ‘scatterers’ with faster or slower time constants. The results indicate that the effects of multiple time constants has a profound effect on the observed speckle contrast and that a complete analysis of speckle contrast data is potential much more complicated than is typically presumed.
High-speed dynamic laser speckle imaging of changes of microcirculation in vivo

J. Qin, L. An, R. K. Wang, Oregon Health & Science Univ. (United States)

The conventional laser speckle imaging (LSI) method uses the speckle contrast to imply (or quantify) blood perfusion within highly scattering media. The lower the speckle contrast is referred to the higher the blood flow velocity. While this statement is generally true when the imaging speed of the system is slow (i.e., 30 fps), it does not reflect the real situation when the imaging speed is higher. This is particularly problematic when the study of capillary recruitment is of concern. This study is designed to experimentally elucidate what the main constraints are when the LSI imaging is applied. In this study, we used an 808 nm laser diode beam to illuminate the sample. The light backscattered from the sample is collected by a high-speed CMOS camera running at 500 fps (1024×1280 pixels per frame). The field of view of the system is ~4 mm×4 mm, with a pixel resolution ~7.0µm. Through well defined phantom experiments, we found that there exists a critical point that divides the velocity domain into two halves, one half that is below the critical point where the higher the velocity, the higher the speckle contrast is; and it is however opposite in the other half. In our experimental setup, the critical point is ~68 µm/sec. This finding provides us a novel explanation about the LSI imaging. Based on our new observations, we demonstrate the relative changes of dynamic microvascular blood flow through occluding the main blood vessels and then reperfusion in the mouse ear flap. The promising results show that the high speed LSI can give useful information as to transient and dynamic microcirculation during occlusion and reperfusion.

Measurement of ordered blood flow by laser speckle

E. R. Hirst, Industrial Research Ltd. (New Zealand); O. B. Thompson, Industrial Research Ltd. (New Zealand) and Univ. of Auckland (New Zealand); M. K. Andrews, Industrial Research Ltd. (New Zealand)

Recent success in reconciling laser Doppler and speckle measurements of dermal perfusion by the use of multi-exposure speckle has prompted an investigation of speckle effects arising from the directed blood flow which might be expected in the small blood vessels of the eye. Unlike dermal scatter, the blood in retinal vessels is surrounded by few small and stationary scatterers able to assist the return of light energy by large-angle scatter. Returning light is expected to come from multiple small angle scatter from the large red blood cells which dominate the fluid.

This work compares speckle measurements on highly scattering skin, with measurements on flow in a retinal phantom consisting of a glass capillary which is itself immersed in an index matching fluid to provide a flat air-phantom interface. Brownian motion dominated measurements when small easily levitated scatters were used, and flow was undetectable. With whole-blood, Brownian motion was small and directed flows in the expected region of tens of mm/s were detectable. The speckle-derived power spectra arising from perfusion and flow are compared. The nominal flow speed relates to the known pump rate; within the capillary the flow will have a profile reducing toward the walls.

Application of laser speckle contrast analysis for the study of dental resin composite polymerization kinetics

E. M. Wells-Gray, Oregon Health & Science Univ. (United States); S. J. Kirkpatrick, Michigan Technological Univ. (United States); D. D. Duncan, Portland State Univ. (United States)

Kinetic behavior during the photo-activated polymerization reaction of dental resin composite plays an important, though not well understood, role in the development of shrinkage stress and the resultant integrity of the final restoration. Here we use laser speckle contrast analysis (LSCA) to investigate the effect of curing lamp irradiance on polymerization kinetics. Thin layer samples are considered, and attention is given to the effect of sample thickness on speckle intensity decorrelation rate. We present results for intensity rate as a function of irradiance for two statistical models of scatterer motion: Lorentzian and Gaussian. Results indicate that the rate of scatterer motion varies with irradiance via a power law relationship, which agrees well with experimental and theoretical results in the literature. The LSCA method is most frequently used for monitoring changes in blood flow; technical and statistical implications of its use in studying composite polymerization kinetics are discussed.
A pilot experiment based on predefined PDT protocols, which uses clinical treatment. provides portability and cost-effectiveness, offering great potential for control was performed by LabVIEW. The simplicity in hardware setup on the raw signal, as well as flexible variation of the lag time. System hardware correlators, this method provides benefits of denoising effects in the DCS system, we employed a fast multi-tau software correlator to study variations in relative blood flow (rBF), hemoglobin concentration measurements during photodynamic therapy. LSCI might be used for studying human blood flow pulsation.

In this paper, we present a novel multi-modal portable instrument to study blood-flow pulsation using laser speckle contrast imaging.

**Study on blood-flow pulsation using laser speckle contrast imaging**

S. Yuan, The Univ. of Memphis (United States); Y. Chen, Univ. of Maryland, College Park (United States); C. Preza, The Univ. of Memphis (United States); C. Tang, Univ. of Maryland School of Medicine (United States)

Laser speckle contrast imaging (LSCI) is becoming an established method for full-field imaging of blood flow dynamics in animal models. Blood flow pulsation originated from heart beat affects blood flow measurement results of LSCI and it is considered as major physiology noise source for most biomedical applications. But in some biomedical applications, the details of the pulsation process might provide useful information for disease diagnostics. In this study, we investigated the ability as well as the limitation of LSCI in monitoring flow pulsation in phantom study. Both intralipid (2% - 5%) and human whole blood samples are used in phantom study. A syringe pump is controlled by a computer-programmable motor controller and liquid phantom is pushed through a 400 µm ID capillary tube by the pump at different pulsation patterns, varied in frequency (1-7 Hz), valley-to-peak ratio (10%-50%), acceleration/deceleration rate, etc. Speckle contrast images are acquired at 15-30 frames-per-seconds. Our results show: (1) it is very hard for LSCI to pick up signals from high frequency pulsation (5-7 Hz), which is close to the heart back frequency of rats. This might be caused by the nature of fluid dynamics of blood during pulsation. LSCI might not work well for animal models in detecting pulsation. (2) With low frequency pulsation (1 Hz, close to human normal pulsation rate), our experimental results show from most pulsation patterns, LSCI could catch the fine details of the blood flow change in a cycle. LSCI might be used for studying human blood flow pulsation.

**A pilot study on hemodynamics response monitoring during photodynamic therapy based on real-time diffuse optical measurements**

J. Dong, J. Ho, S. L. Ting, M. Tan, K. Lee, Nanyang Technological Univ. (Singapore)

Diffuse optical techniques, such as diffuse correlation spectroscopy (DCS) and diffuse optical spectroscopy (DOS), have been conducted in clinical settings such as photodynamic therapy (PDT) treatment, in order to study variations in relative blood flow (rBF), hemoglobin concentration and blood oxygen saturation. Such techniques provide real-time feedback on therapeutic efficacy.

In this paper, we present a novel multi-modal portable instrument incorporating both DCS and DOS, which enables us to concurrently monitor hemodynamic changes in deep tissue volumes in real-time. In the DCS system, we employed a fast multi-tau software correlator based on a simple autocorrelation algorithm. Compared to conventional hardware correlators, this method provides benefits of denoising effects on the raw signal, as well as flexible variation of the lag time. System control was performed by LabVIEW. The simplicity in hardware setup provides portability and cost-effectiveness, offering great potential for bedside monitoring of blood flow and oxygenation in deep tissues during clinical treatment.

A pilot experiment based on predefined PDT protocols, which uses Chlorine 6 as the photosensitizer, has also been performed to validate system performance. The hemodynamic parameters are compared before and after experiment and results are discussed in great detail.

**Role of microcomputed tomography in microvascular imaging**

E. L. Ritman, Mayo Clinic (United States)

Since the early 1980s high resolution computed tomography (micro-CT) has progressed from mainly imaging bone specimens at 20-50 micrometer resolution to the present in which 50-100 micrometer resolution scans of living small mammals and sub-micrometer resolution scans of tissue specimens are commercially available. The scans generate three-dimensional images consisting of the order of 10003 voxels (3D picture elements), each cubic voxel being sub-micron to 100 micrometer on a side. The gray-scale modulation within tomographic images reflects the local attenuation of the x-ray. This allows for differentiation of different tissues by virtue of their elemental content. As calcium rich bone attenuates x-ray much more than soft tissues this is the reason why micro-CT is popular in analysis of bone micro-structure and level of mineralization. However, the natural elements in blood vessel walls and within blood differ little from organ parenchyma, hence they are not readily detectable, unless the attenuation of blood is enhanced by injecting a heavy element (such as iodine) into the blood stream or by staining the vessel wall tissues with osmium tetroxide. The former method is applicable in living animals but the latter is useable only in post mortem specimens.

The power of 3D micro-CT is that it images a volume of (light-opaque tissue) large enough to include entire, intact, vascular trees without the need to destroy the 3D tissue specimen. Hence, the fluid dynamic and the perfusion territory size consequences, as well the micro-anatomic relationship of the vascular branching geometry and interconnectivity to parenchymal structures (e.g., nephron, hepatic lobule or cancer) can be readily appreciated and quantified. The permeability of microvasculature can also be imaged by virtue of the increased contrast resulting from the fraction of the injected contrast agent passing through the endothelium into the surrounding extravascular tissue.

In recent years micro-CT based on the imaging of coherent x-ray scatter and on x-ray phase shift of x-rays by local electron density distributions (reflecting molecular bond type in some cases) provide greater inherent image contrast than does x-ray attenuation. These new capabilities are now active avenues of research and development.
Smart velocity ranging quantifiable optical micro-angiography
Z. Zhi, Y. Jia, L. An, R. K. Wang, Oregon Health & Science Univ. (United States)

Optical microangiography (OMAG) is a novel extension of optical coherence tomography technology, capable of performing 3D dynamic imaging of blood perfusion within micro-circulatory tissue beds. However, the quantification of flow velocity using OMAG is not yet well studied. In this study, we introduce a new type of OMAG called Quantifiable Optical Microangiography (QOMAG) which is capable of performing quantitative flow imaging with smart velocity ranging. In order to extracting multi-range velocity, two three dimensional data sets need to be acquired at the same imaging area. One data set performs dense scanning in B-scan direction and Doppler analysis was done at the basis of subsequent A-scans, while the other data set performs dense scanning in C-scan direction and Doppler analysis was done at the basis of consecutive B-scan. Since the velocity ranging is determined by the time interval between consecutive measurements of the spectral fringes, longer time interval will give us smaller velocity range but higher sensitivity to slow velocity. By simultaneous acquiring data sets with different time intervals, we can perform smart velocity ranging quantification on blood flow characterized by different velocity values. The feasibility of QOMAG for quantitative flow imaging is evaluated using a well defined flow in a glass capillary. Further, in vivo studies were executed on cerebral blood flow of mouse model with the cranium left intact. Multi-range detailed blood flow velocity distribution within intracranial dura mater and cortex can be given by QOMAG simultaneously.

Multimodal approach for functional diagnostics and imaging of vascular network and blood microcirculation
I. Meglinski, Univ. of Otago (New Zealand); V. Kalchenko, A. Harmelin, Weizmann Institute of Science (Israel)

We report the results of combine use of the fluorescence imaging modalities and Dynamic Light Scattering-based techniques for simultaneous non-invasive imaging of lymph and blood vascular network and blood microcirculation. We demonstrate that the local blood micro-flows and blood microcirculation can be observed and analyzed quantitatively under the “biological zero”, i.e. when the arterial and venous flows are completely blocked. We show that the biological zero signal arises from the local blood micro-flows observed post-mortem up to 100 minutes. The use of this new dual-modal diagnostic system is particularly important and has a great potential to significantly expand the capabilities of vascular diagnostics providing synchroic in vivo images of blood and lymph vessels.

Correlation mapping: rapid method for retrieving microcirculation morphology from optical coherence tomography intensity images
E. Jonathan, J. G. Enfield, M. J. Leahy, Univ. of Limerick (Ireland)

The microcirculation plays a critical role in maintaining organ health and function by serving as vascular area where trophic metabolic exchanges between blood and tissue takes place. To facilitate regular assessment in vivo, non-invasive microcirculation imagers are required in clinics. Among this group of clinical devices, are those that render microcirculation morphology such as nailfold capillaroscopy, a common device for early diagnosis and monitoring of microangiopathies. However, depth ambiguity disqualify this and other similar techniques in medical tomography applications where due to the 3-D nature of biological organs, imagers that support depth-resolved 2-D imaging and 3-D image reconstruction are required. Here, we introduce correlation map OCT (cmOCT), a promising technique for microcirculation morphology imaging that combines standard optical coherence tomography and an agile imaging analysis software based on correlation statistic. Promising results are presented of the microcirculation morphology images of the brain region of a small animal model as well as measurements of vessel geometry at bifurcations, such as vessel diameters, branch angles. These data will be useful for obtaining cardiovascular related characteristics such as volumetric flow, velocity profile and vessel-wall shear stress for circulatory and respiration system.

Development of an absorption-based tomographic system for mapping the human microvasculature
P. M. McNamara, E. Jonathan, M. O’Connell, M. J. Leahy, Univ. of Limerick (Ireland)

There exist numerous surface imaging methods for mapping the human microvasculature. In medical diagnostics, tomography is preferred over surface imaging for the simple reason that biological organs are 3-dimensional in nature. The aim of this work is to create a novel technique to noninvasively map the concentration of red blood cells in the human skin microcirculation allowing 3-dimensional image reconstruction.

We propose a tomographic system which is based on absorption contrast imaging. This work details the design, construction and calibration procedure of the method and presents preliminary results of an experiment to determine the depth of photon-return for narrow-bandwidth wavelengths.

In-vivo three-dimensional Doppler variance imaging for tumor angiogenesis on chorioallantoic membrane
W. Qi, G. Liu, Z. Chen, Beckman Laser Institute and Medical Clinic (United States)

Non-invasive tumor microvasculature visualization and characterization play significant roles in the detection of tumors and importantly, for aiding in the development of therapeutic strategies. The feasibility and effectiveness of a Doppler variance (standard deviation) imaging method for tumor angiogenesis on chorioallantoic membrane were tested in vivo on a rat glioma F98 tumor spheroid. Utilizing a high resolution Doppler variance OCT system with A-line rate of 20kHz, three-dimensional mapping of a tumor with a total area of 4x3mm^2 with ten subareas of 1x2mm^2 (2048x128 axial scans) was completed within 1.7 minutes. The top-view image clearly visualized the complex vascular perfusion with the detection of capillaries as small as approximately 10µm. The results of the current study demonstrate the capability of the Doppler variance (standard deviation) imaging method as a non-invasive assessment of tumor angiogenesis, with the potential for its use in clinical settings.

Quantitative assessment of perfusion velocities with holographic laser Doppler
C. V. Magnain, B. Samson, M. Atlan, Ecole Superieure de Physique et de Chimie Industrielles (France)
We demonstrate that quantitative flow velocity in the 100 microns/s to 10 mm/s range can be derived from the analysis of Doppler spectral images recorded with holographic laser Doppler imaging. Holographic laser Doppler imaging relies on parallel heterodyne detection with a digital camera. It enables wide-field imaging of the Doppler spectrum of light fluctuations. It is achieved by mixing the backscattered radiation with a separate local oscillator field. The local oscillator beam is frequency-shifted sequentially with respect to the illumination beam to enable tunable detection of spectral broadenings in the 0-100 kHz range. An efficient noise rejection scheme involving a spatial and temporal modulation of the light beating signal enables high sensitivity measures, which are crucial for accurate determination of first-order fluctuation spectra. The resulting wide-field heterodyne Doppler images and spectra are used to assess momentum transfer between scattered light and moving diffusers. Radiofrequency fluctuation spectra measures of multiply-scattered light from intralipid flows in a tube at known velocities are in agreement with analytical behaviors derived from Diffusing-Wave Spectroscopy formalism. Since our approach enables measures of Doppler spectra in low-light of superficial microvascular blood flow in tissue, it paves the way to the design of robust tools intended to non-invasive, non-ionizing and quantitative hemodynamic parameters assessment without any contrast agent.

7898-24, Session 4

Influence of changes in hemoglobin concentration on the laser Doppler perfusion signal measured during postocclusive reactive hyperemia

S. Wojtkiewicz, A. Liebert, R. Maniewski, Institute of Biocybernetics and Biomedical Engineering (Poland)

Three-wavelength laser-Doppler (LD) in-vivo measurements were performed to show influence of oxymyoglobin and deoxyhemoglobin concentrations on the LD perfusion signal. Laser-Doppler signals were recorded with utilization of three semiconductor laser light sources (650 nm, 780 nm and 850 nm) and an home-made instrumentation allowing for analysis of the AC and DC components of the photocurrent. Three optodes consisting of source-detector fibers were fixed on fingertips of index, middle and ring fingers of the left hand of a healthy volunteer. Postocclusive hyperemia test was carried out by pumping a cuff located on the forearm to the pressure of 200 mmHg. In widely used signal processing algorithm the perfusion index is assessed by calculation of the first moment of power density spectrum of AC component of the photocurrent normalized by square of the detected light intensity (DC component of the photocurrent). It was observed that the light intensity is influenced by hemoglobin concentrations depending on the utilized light wavelength. This phenomena is well known and utilized in near infrared spectroscopy techniques. Furthermore, we observed that the normalization of perfusion signal leads to significant influence of the light intensity profile on the perfusion signal observed during the postocclusive reactive hyperemia. The return of the hemoglobin concentrations is significantly slower than the return of the blood circulation observed in the first moment of the LD power spectral density. Thus, the normalization of the perfusion signal by square of the detected light intensity causes that the perfusion signal is contaminated with the hemoglobin concentration changes.

7898-25, Session 4

Dynamic changes in brain hemodynamics and cerebral metabolic rate of oxygen during repeated squat-stand

H. Niu, L. Li, G. Suresh Bhave, The Univ. of Texas at Arlington (United States); R. Zhang, The Univ. of Texas at Dallas (United States); K. Behbehani, H. Liu, The Univ. of Texas at Arlington (United States)

Cerebral hemodynamics and cerebral metabolic rate of oxygen (CMRO2) in response to dynamic changes in perfusion is altered and can be quantified by a dual-modal functional brain imager. These quantified parameters may be used as biomarkers to diagnose and monitor early stage of autoregulation-related diseases, such as Alzheimer’s disease, mild cognitive impairment (MCI), or obstructive sleep apnea (OSA). In this study, we have utilized a mathematical model to associate CMRO2 with two measurable quantities that can be acquired by TCD (Trans-cranial Doppler) and DOT (Diffuse Optical Tomography) simultaneously as a combined brain imager. In the study, we have utilized a squat-stand protocol, which was specifically designed to investigate the brain adaptive mechanism that works to adjust the system’s response to stimuli. The dual-modal brain imager was used to perform human experiments on control subjects in response to dynamic changes in perfusion. From the temporal profiles and spatial maps of the corresponding cerebral hemodynamic parameters and CMRO2, a clear and significant change can be demonstrated. Since cerebral autoregulation plays an essential role in maintaining an appropriate blood pressure in the brain, the hypothesis is that the MCI patients or OSA patients may have a certain variation in the parameters, such as the amplitude or time length in the recovery period after over-perfusion. This study provides a good start for further proof or validation of this hypothesis.

7898-26, Session 4

The lag time between changes in blood and interstitial glucose levels in vivo by ATR-FTIR spectroscopy

N. Skrebova Elkje, MC Professional OÜ (Estonia)

Calculation of the lag time (LT) could be a method to calibrate measurements of glucose levels in vivo. Based on the previous reports on measuring intrstitial glucose levels in vivo by HATR-FTIR spectroscopy at about 1030-1041, 1080, 1118 and 1153 cm⁻¹, the LT was calculated between the blood and interstitial fluid compartments in the upper layer of the skin tissue during oral glucose tolerance test (OGTT). Calculated LT has been used to describe the changes of the glucose dynamics between healthy subjects and subjects with (pre-)diabetes at OGTT with different doses (5 g, 20 g, 75 g). The LT was not only dose-dependent in all subjects, but was also longer for healthy subjects at each measured dose of OGTT. Moreover, the LT was bi-phase in healthy subjects and a subject with type 2 diabetes, except for the subject with pre-diabetes, in whom the LT was multi-phase. Measurement of the LT has showed a potential to provide insight to the glucose dynamics between the blood and interstitial fluid (IF) compartments in the upper layer of the skin tissue by means of measured the LT during OGTT at different doses. As well, the LT might be a method to calibrate measurements of glucose levels in vivo.

7898-27, Session 5

Interaction of nanoparticles with the skin barrier: safety aspects and pharmaceutical potential

J. M. Lademann, H. Richter, W. Sterry, A. Patzelt, Charité Universitätsmedizin Berlin (Germany)

The Clinic for Dermatology, Venerology and Allergology of the Charité utilises various methods to investigate the penetration and storage of nanoparticles in the skin, with hair follicles being in the focus of attention, because they are an ideal target for drug delivery. Laser scanning microscopic investigations of nanoparticles of different size and materials showed that particles with a diameter of circa 600 nm penetrate into the hair follicles particularly efficiently and can be stored...
In this work a recently developed technique called microdialysis is very real example of improvements in clinical care. The particularly efficient penetration of the particles with a diameter of circa 600 nm is due to the surface structure of the skin. The cuticula has a mean thickness of circa 600 nm and forms a “zigzag” structure on the hair surface. Obviously, this makes the moving hair acting like some sort of a gear pump and stimulates the transport process. The investigations did not show, however, that any particles with diameters between 40 nm and 1 µm penetrated from the hair follicle into living tissue if the barrier was intact. This is plausible as the hair follicle, too, has a barrier structure of its own.

There are a number of multidisciplinary research studies using OCT-guided microdialysis to diminish in the quest for an end product. This work outlines a novel the patient and end user in a real, clinical environment can often be diminished. This is plausible as the hair follicle, too, has a barrier structure of its own.

Ultra-high-sensitive optical microangiography provides 3D visualization of microcirculations within human skin under psoriatic conditions

J. Qin, L. An, D. S. Gareau, R. K. Wang, Oregon Health & Science Univ. (United States)

Vascular abnormalities may play crucial role in several dermatologic diseases (e.g., psoriasis, port wine stain and skin cancer). To improve our understanding of vascular involvement in these skin conditions, there is a need for a non-invasive imaging modality which is capable of noninvasively assessing 3D microcirculations within skin tissue beds. Here, we demonstrate the utilization of ultra-high sensitive optical microangiography (UHS-OMAG) to visualize skin microcirculations in 3D and to quantify vessel density under normal and psoriatic conditions. The system was operating at 1310 nm wavelength with a spatial resolution of ~10 µm × 20 µm (axial × lateral). Through the UHS-OMAG algorithm, we could achieve ~4 µm/s velocity sensitivity of the system, which is high enough for imaging cutaneous capillary blood vessels. The experiments were performed on the skin of a volunteer with stable plaque-type psoriasis. The cross-sectional images, en-face (x-y plane) images and volumetric in vivo perfusion maps of lesion and normal skin areas were generated after post processing the data sets, from which we can visualize and differentiate the blood vessel networks within psoriasis lesion and normal skin. The statistic analysis of the blood vessel densities of these two types of skins shows that the blood vessel density in the randomly selected regions of psoriasis lesions was much higher than that in the normal skin. UHS-OMAG can be a valuable tool for imaging skin microcirculations non-invasively with high speed and high sensitivity, and therefore may have a useful role in future clinical diagnosis and treatment of dermatologic diseases such as psoriasis in human subjects.

Optical coherence tomography: imaging architect for dermal microdialysis in psoriasis

M. O’Connell, W. O’Connor, Univ. of Limerick (Ireland); B. Ramsay, Mid-Western Regional Hospital (Ireland); E. Guihen, Univ. of Limerick (Ireland); W. L. Ho, Mid-Western Regional Hospital (Ireland); M. J. Leahy, Univ. of Limerick (Ireland)

Frequently, the evolution of biomedical equipment will be driven by a technological push rather than a medical pull. With an ever increasing emphasis on research commercialization, the practical benefits to the patient and end user in a real, clinical environment can often be diminished in the quest for an end product. This work outlines a novel multidisciplinary research study using OCT-guided microdialysis to increase our understanding of psoriasis which represents not only a technological progression in instrumentation but also demonstrates a very real example of improvements in clinical care. In this work a recently developed technique called microdialysis is incorporated whereby a microscopic hollow tube - mimicking a tiny artificial blood vessel - is temporarily placed into a layer of the skin in a group of psoriasis sufferers and is then used to measure the levels of histamine and other important biological molecules in psoriasis. Microdialysis catheters were implanted into healthy, peri-lesional and lesional skin regions. The catheters’ entry and exit points and their precise location in the epidermal layer of the skin were confirmed using Optical Coherence Tomography (OCT). This work demonstrates the effective dialysis catheters in an in vivo environment.

Characterisation of skin layer properties using laser-generated surface acoustic waves validated by FEM method

C. Li, Oregon Health & Science Univ. (United States) and Univ. of Dundee (United Kingdom); Z. Huang, Univ. of Dundee (United Kingdom); R. K. Wang, Oregon Health & Science Univ. (United States)

Unlike traditional ultrasonic methods, laser Ultrasonics uses a very short pulsed laser beam as remote ultrasonic input source, generates ultrasonic waves in the material. The absorption of laser radiation results in a localised temperature increasing. The generated temperature further induces a localised thermal expansion and hence results in the generation and propagation Rayleigh wave. Rayleigh wave attracts great attention between these waves because its phase velocity depends strongly on the mechanical properties of materials including Young’s modulus, Poisson’s ratio and density, etc. The main advantages of using a laser ultrasonic system are due to its remote generation and detection without physical contact with the material, the low coupling constraint and no sensitivity to surface orientation. Laser ultrasonic has the potential as an access to quantifying skin mechanical properties, such as thickness and elasticity, for diagnosis and accurate assessment of skin disease. It is non-contact which enhance the speed of inspection and no-harm diagnosis. This work presents a finite element (FE) modelling technique which studies the effect laser has on the generated surface acoustic waveforms in a multilayered skin model. By the use of Finite Element Modelling techniques, it aims to obtain an understanding of the way in which the laser source interacts with tissues of different types and the resulting signals generated by the tissues. This is done in order to provide specifications for a laser ultrasonic system that is suitable for measuring skin properties such as thickness and elasticity. Analysis also involves the study of the dispersion characteristics of the generated surface waveforms in a variety of skin models.
In this work we report a novel application of Optical coherence tomography for the determination of cleavage line orientation in in-vivo human skin. The technique operates by pressing a small circular indenter onto the skin to deform the skin. This is then imaged using optical coherence tomography. Analysis of the resulting deformation can be seen to have an ellipsoidal shape which is related to the cleavage line orientation. We demonstrate that the technique can be used to map the cleavage line orientation at various locations around the body.

7898-32, Session 5

Measurements of adipose derived stem cell vitality with optical coherence phase microscopy

P. Bagnaninchi, The Univ. of Edinburgh (United Kingdom); C. Holmes, McGill Univ. (Canada); N. Drummond, The Univ. of Edinburgh (United Kingdom); J. Daoud, M. Tabrizian, McGill Univ. (Canada)

Live cells display a constant vertical motility due partly to a constant rearrangement of focal contacts and to cell shape fluctuations. This cellular micromotion has been clearly demonstrated with electric cell impedance sensing (ECIS) on 2D micro-electrodes, and correlated to cell vitality. In this study we investigated if optical coherence phase microscopy (OCPM) was able to report phase fluctuations of adult stem cells in 2D and 3D that could be correlated to cell motility.

An OCPM has been developed around a Thorlabs engine (\(\lambda=930\)nm FWHM: 90nm) and integrated in an inverted microscope with a custom scanning head. Human adipose derived stem cells (ADSCs, Invitrogen) were cultured in Mesenpro RS medium and seeded either on ECIS arrays, 2D cell culture dishes, or in 3D and highly porous microplotted polymeric scaffolds.

ADSC motility was measured by ECIS and a spectral analysis was performed to retrieve the power spectral density (PSD) of the fluctuations. Cells in standard media, high glucose media, and fixed cells were investigated.

The same conditions were then investigated for ADSCs in 2D and in 3D with optical coherence phase microscopy. Significant differences were found in phase fluctuations between the different conditions, which correlated well with the ECIS experiments and the Fourier domain analysis.

These preliminary results indicated that optical coherence phase microscopy could assess cell vitality in 2D and potentially in 3D microstructures.

7898-33, Session 5

Finger tissue model and blood perfused skin tissue phantom

V. V. Tuchin, A. N. Bashkatov, E. A. Genina, V. I. Kochubey, V. V. Lychagov, S. A. Portnov, N. A. Trunina, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); S. Cho, H. Oh, B. Shim, M. Kim, J. Oh, H. Eum, Y. Ku, D. Kim, Y. Yang, D. R. Miller, LG Electronics Inc. (Korea, Republic of)

No abstract available

7898-34, Poster Session

Monte Carlo simulation on how optical clearing technique influences predicting precision of non-invasive optical blood glucose sensing

J. Jiang, W. Chen, L. Zhang, Tianjin Univ. (China); R. K. Wang, Oregon Health & Science Univ. (United States); K. Xu, Tianjin Univ. (China)

It is necessary to get optical information within tissue in order to improve the application of non-invasive blood glucose sensing. However, the light penetration depth is seriously limited due to high scattering effects of biological tissues, which restricts the detection precision of non-invasive blood glucose sensing. Tissue optical clearing technique is one of the effective approaches to reduce the scattering effect and increase the light penetration depth into biological tissues. In this talk, it is our aim to study the preliminary application of optical clearing to non-invasive blood glucose sensing based on Monte Carlo simulation.

Firstly, optical properties of intralipid solutions mixing with different concentration of glucose were calculated within the wavelengths of 1000–1700nm. The transmittance spectra of intralipid solutions with and without glycerol as optical clearing agent were investigated through Monte Carlo simulation. Different concentrations of glycerol were taken into account. Furthermore, the root mean square error of prediction (RMSEP) was obtained by performing partial least squares (PLS) model. Simulation results showed that the transmittance increased gradually with the increase of glycerol concentration, which suggested that the optical clearing effect appeared. Meanwhile, the RMSEP decreased as the glycerol concentration increased. RMSEP has improved by 30.91% in the simulation, which showed the great potential of tissue optical clearing technique to effectively improve the predicting precision of non-invasive blood glucose sensing.

7898-35, Poster Session

Quantifying low-frequency fluctuations in the laser Doppler flow signal from human skin

G. E. Calvo Nogueira, M. Santos Folgosi-Correa, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

Low-frequency fluctuations in the laser Doppler flow signal (LDFS) from the skin are related to microvascular mechanisms of flow control. Wavelet spectral analysis has been used to correlate fluctuations in the LDFS with the metabolic, neurogenic and myogenic mechanisms of control in the frequency intervals 0.0095–0.02 Hz, 0.02–0.05 Hz and 0.05–0.15 Hz, respectively.

Generally the signal power, in each frequency interval, derived from the respective wavelet coefficients, is used as a measure of the activity of the related mechanism of microvascular control. However, the time-domain characteristics of the fluctuations in the LDFS in each frequency interval are poorly known. As a consequence, there is a lack of objective criteria to properly measure, in each frequency interval, the related hemodynamic parameters.

Here a time-domain method is proposed to analyze and quantify fluctuations in the LDFS in each frequency band. Baseline (32 degrees Celsius) and thermally stimulated (42 degrees Celsius) LDFS of solar forearms from 15 healthy volunteers were collected and analysed. The data obtained indicate that inappropriate time windows, frequently used for measurements, increase the variability of the measured signal power, diminishing the capability of the method when assessing microvascular dynamics and dysfunctions.

To overcome this limitation, an objective method to measure the LDFS power in each frequency band is proposed.
In the work the possibilities of applying the luminescent-kinetic probe revealed by phosphorescent probe Structural changes in glycated protein will support the study of neurovascular pathology and further facilitate distinguish between normal and abnormal metabolic activity, which using OMAG can be successfully applied to the mouse brain and reliably spatiotemporal tracking of cerebral macro- or micro-hemodynamic in a similar fashion. In total, our preliminary results suggest that the vascular response is different between the venules and arterioles, region of the brain to another; in some given region (e.g., parietal cortex) results show the hemodynamic response to alcohol varies from one to another, and this is necessary to take steps to decrease these effects.

The correlation relationship has been presented to reveal that it is the aforementioned blood components influence blood glucose sensing. Furthermore, PLS modelling results demonstrate that the correlation relationship has been presented to reveal that it is the aforementioned blood components influence blood glucose sensing. The correlation relationship has been presented to reveal that it is necessary to take steps to decrease these effects.

Optical hemodynamic imaging employed in pre-clinical studies with high spatial and temporal resolution is significant to unveil the functional activities of brain and the mechanism of internal or external stimulus effects in diverse pathological conditions and treatments. Most current optical systems only resolve hemodynamic changes within superficial macro-circulatory beds, such as laser speckle contrast imaging; or only provide vascular structural information within micro-circulatory beds, such as multi-photon microscopy.

In this study, we introduce a hemodynamic imaging system based on Optical Micro-angiography (OMAG) which is capable of resolving and quantifying 3D dynamic blood perfusion down to capillary level. By use of a hybrid scanning protocol, this system can measure the optical phase shifts caused by fast moving blood cells in macro-circulation and slow moving blood cells in micro-circulation. Here, the utility of OMAG was demonstrated by monitoring the hemodynamic response to alcohol administration in mouse cortex which may lead to a better understanding of the dysregulation of metabolism in the cortex underlying compulsive drug intake. For the first time, we continuously and detailedly recorded the changes of blood flow distributing over different cerebral regions and in different vascular types during drug administration. Our preliminary results show the hemodynamic response to alcohol varies from one region of the brain to another; in some given region (e.g., parietal cortex) the vascular response is different between the venules and arterioles, and in some given region (e.g., prefrontal cortex) all vascular tone is in a similar fashion. In total, our preliminary results suggest that the spatiotemporal tracking of cerebral macro- or micro-hemodynamic using OMAG can be successfully applied to the mouse brain and reliably distinguish between normal and abnormal metabolic activity, which will support the study of neurovascular pathology and further facilitate investigation of efficacy of treatments.

An increase in glycosylation time and glycosylation degree connected with it led to the decrease in fluorescence intensity and to the long-wave shift of the maximum of the probe (eosin) fluorescence in BSA solution with glucose. Decrease in intensity and long-wave shift of the eosin fluorescence maximum points to displacement of probe sorption region in the protein globule during an increase in the glycosylation degree. With an increase in the protein glycosylation degree increase in the scattering intensity of eosin bound to protein is observed. This can be explained by an increase in the number of eosin molecules bound to hydrophobic regions of glycated protein owing to increase in accessibility of these regions during conformation changes stimulated by glycosylation. We studied the processes of triplet-triplet (TTET) energy transfer between energy donor - eosin and acceptor - anthracene bound to native or glycated BSA. Glycosylation during 90 minutes led to the decrease in the rate constant of energy transfer. On our assumption the decrease in the TTET rate constant is concerned with the increase in the distance between donor and acceptor as a result of changes in the geometry of hydrophobic region in the globule itself.

On the base of obtained results it is possible to conclude that fluorescence and phosphorescence spectral characteristics and also the rate constant of triplet-triplet energy transfer between luminescent probes bound to proteins are sensitive to structural changes in bovine serum albumin during the early stages of nonenzymatic thermal glycosylation.

Optical hemodynamic imaging employed in pre-clinical studies with high spatial and temporal resolution is significant to unveil the functional activities of brain and the mechanism of internal or external stimulus effects in diverse pathological conditions and treatments. Most current optical systems only resolve hemodynamic changes within superficial macro-circulatory beds, such as laser speckle contrast imaging; or only provide vascular structural information within micro-circulatory beds, such as multi-photon microscopy.

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7898-41, Poster Session
High-sensitive volumetric imaging of renal microcirculation in vivo using ultra-high-sensitive optical micro-angiography
Z. Zhi, Y. Jung, Y. Jia, I. An, R. K. Wang, Oregon Health & Science Univ. (United States)

Studying the renal microvascular dynamics is extremely important to understand the renal function and to further advance the diagnosis, prevention and treatment of many renal pathologies. To achieve this goal, a non-invasive, contrast agent free, high-resolution and high-sensitive imaging technique capable of visualizing detailed renomicrovasculature, especially capillary networks within renal cortex would be a significant advance. In this study, we present such a kind of non-invasive, label-free imaging technique called Ultrahigh Sensitive Optical Microangiography (UHS-OMAG) for high sensitive volumetric imaging of renal microcirculation. The UHS-OMAG imaging system used here is based on spectral domain optical coherence tomography (SD-OCT), which uses a broadband light source centered at 1300nm with an imaging speed at 150 frames per second that requires only ~4 sec to complete one 3D scan of ~2.5 x 2.5 mm² area. The technique, capable of measuring slow blood flow down to 4 µm/s, is sensitive enough to image capillary networks, such as peritubular capillaries and glomerulus within renal cortex. We show superior performance of UHS-OMAG in providing depth-resolved volumetric images of rich renal microcirculation. To further prove the ability of UHS-OMAG for studying the renal microvascular dynamics, we monitored the dynamic change of renal microvasculature due to renal ischemia and reperfusion. We observed obvious reduction of renal microvascular density in response to renal ischemia and increase of capillary density after reperfusion. Quantitative analysis was done as well. This technique can be helpful for the assessment of chronic kidney disease (CKD) and acute kidney failure which are related to the abnormal microvasculature.

7898-42, Poster Session
Estimation of flow dynamics on nanopillar-structured microchannels using ODT
H. Jeong, J. Kim, K. Lee, B. Kim, Korea Univ. (Korea, Republic of)

Surface engineered biomaterials to modify surface properties are pretty important due to the effects for protein adsorption, and cellular interactions prominently applicable to scaffolds for cell culture and tissue engineering, biosensors, biological microchips, and so on. The self-cleanable and antireflection-enhanced structure was fabricated on a quartz plate by our innovative mask-free lithography method with coverage of heptadecafluoro-1,1,2,2-tetrahydrodecyl-trichlorosilane (HDFS) monolayer. The well-controlled nanopillar structured surface of quartz plate demonstrated the super-hydrophobic self-cleaning effect with a high contact angle of approximately 160°. We fabricated micro fluidic chamber with these nano structured quartz plates and estimated flow dynamics correlated with a contact angle of a chamber wall using optical doppler tomography technique. For many BioMEMS devices flow dynamics is quite dependent on the surface features of micro-fluidic channel. Optical doppler tomography is a quite promising tool for measuring and quantifying micro structures and flow dynamics simultaneously of BioMEMS devices without calibration. In this study, we used spectral domain optical doppler tomography system composed of SDL centered at 1310nm with FWHM of 160nm and InGaAs line scan camera with the maximum line rate of 46.99 kline/sec directly providing a limit of measurement velocity in a spectral domain technique. 10 % intra-lipid suspension of 60 mL is used to obtain Doppler signals from scattering particles. The distribution of scattering particles was polydisperse and the size of particles was within 2 ~ 20 µm. An The cross-sectional area of the fabricated micro-channel was 0.5 mm x 0.5 mm.

7898-43, Poster Session
3D optical imaging of skin blood perfusion at true capillary level in vivo
G. Guan, Oregon Health & Science Univ. (United States) and Univ. of Dundee (United Kingdom); Z. Huang, Univ. of Dundee (United Kingdom); R. K. Wang, Oregon Health & Science Univ. (United States)

In this study, we demonstrate for the first time that the detailed cutaneous blood flow at capillary level within dermis of human skin can be imaged by optical micro-angiography (OMAG) technique. In the system development, we employed a novel scanning protocol, i.e. fast B scan mode to achieve the capillary flow imaging. We used a 1310nm system to scan the skin tissue at an imaging rate of 300 frames per second, which requires only ~5 sec to complete one 3D imaging of capillary blood flow within skin. We experimentally demonstrate that the system has a sensitivity to blood flow from ~4 µm/sec to 22 µm/sec, which sensitivity we expect is sufficient to image depth-resolved capillary flow within the dermis. We show that the volumetric imaging results of the skin micro-vasculatures agree well with those published in the standard textbook obtained by invasive histopathology. The promising results show a great potential of OMAG's role in the diagnosis, treatment and management of human skin diseases.

7898-45, Poster Session
Propagation of circular polarized light in a scattering medium influenced by optical clearing
I. Meglinski, C. Macdonald, Univ. of Otago (New Zealand)

Previous studies explored the high potential of polarimetric tissue diagnostics by use of circular polarized light. However, strong multiple scattering of optical radiation in biological tissues leads to the loss of initial polarization, direction, phase and waveform of incident light. It has been demonstrated that the polarization of backscattered light survives more scattering events than the direction of its propagation, whereas the helicity of backscattered light depends noticeably on the size of scattering particles. We report the results obtained by experimental study of propagation of circular polarized light in a turbid scattering medium influenced by optical clearing.

7898-46, Poster Session
Determination of blood types using a chirped-photonic fiber
A. Malinin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation) and SPE LLC NGT (Russian Federation); A. Zanishevskaia, J. S. Skibina, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); I. Silohin, SPE LLC NGT (Russian Federation); V. A. Dubrovskiy, Saratov State Medical Univ. (Russian Federation); V. V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); A. Dolmaskhin, Saratov State Medical Univ. (Russian Federation)

The report presents the results of work on definition of human blood groups using chirped photonic fibers (CPF). A chirped fiber structure delivers lower transmission values than designs with identical cells. Chirping, however, also enables substantial widening of the narrow transmission bands of conventional hollow-core designs and suppresses detrimental dispersive resonances that grow concomitantly with increasing quality and number of identical subcavities. The concept of cell size chirping has already been very successfully applied in one-dimensional photonic structures such as chirped mirrors and chirped
fiber Bragg gratings. In these structures, the reflection of different spectral components is localized at different positions inside the chirped structure. The resulting spectrally dependent penetration depth gives rise to strong engineered dispersion. A transfer of the chirp concept has already been envisioned theoretically, and prototypical graded photonic crystals have even been manufactured.

Method using chirped fiber allows one to obtain a high spectral response for agglutination of blood cells after interaction with clumping serum. The perspectives of using of blood biosensor on the basis of CPFs as sensitive and small-volume blood probe are discussed.

7898-47, Poster Session

Study of optical clearing of blood by immersion method

O. S. Zhernovaya, Univ. of Limerick (Ireland) and N.G. Chernyshevsky Saratov State Univ. (Russian Federation); E. Jonathan, Univ. of Limerick (Ireland); V. V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); M. J. Leahy, Univ. of Limerick (Ireland)

Light scattering in blood caused by refractive index mismatch between erythrocyte cytoplasm and blood plasma leads to a reduction in imaging spatial resolution, imaging depth and contrast of optical imaging techniques. A possible solution to this problem is the addition of biocompatible clearing agents, such as glucose, fructose, glycerol, dextrans etc. The basic principle of the optical clearing technique is refractive index matching between erythrocyte cytoplasm and blood plasma. Optical clearing, a technique that has been successfully demonstrated with biologic tissue, represents a promising approach to increasing the imaging depth for various techniques, for example optical coherence tomography (OCT).

OCT is based on low-coherence interferometry to produce cross-sectional tomographic imaging of the internal microstructure in materials and biological tissues by measuring the echo time delay and magnitude of back scattered light. One of the main advantages of this technique is the ability to investigate of turbid and highly scattering media, such as whole blood.

To determine the optimal concentration of clearing agents required for blood optical clearing in order to improve light penetration depth for optical coherence tomography technique clearing agents, such as glucose and fructose, with various concentrations were added to blood and investigated by OCT. In addition, we controlled the shape and size of erythrocytes by blood smear microscopy. Changes in light attenuation and sedimentation and aggregation properties of blood depending on particular agent and its concentration were studied. Specifically, we determined the most appropriate agents and their optimum concentration for efficient optical clearing of blood.

7898-48, Poster Session

Speckle-correlation monitoring of the internal microvascular flow

P. Novikov, D. A. Zimnyakov, R. A. Zdrajevsky, V. V. Tuchin, M. Vilensky, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

EXPERIMENT: Speckle -correlation monitoring of the variations in the micro-hemodynamics in the surface layers of different organs of laboratory animals (rats) under the influence of different factors (short-term blocking of microcirculation via the clipping of blood vessels with the subsequent restoration of microcirculation level; the artificial provocation of peritonitis; of the injection of the vasodilating agents, which cause making more active or suppression of hemodynamics) it was produced with the aid of the laboratory model of the speckle-correlometer of the full field with the multichannel fiber-optic to transfer of speckle-modulated scattered radiation to the detector. One-mode He-Ne laser (HN5-R, - 632.8 nm, power - 5mW), used as the light source. Transfer of laser light to the area of interest and scattered radiation from the probe zone to the detector (monochromatic CMOS camera Basler a602f; the number of pixels in the matrix of 565×489, the size of pixel 9.9 m of ×9.5 m; 8 bits/pixel) was carried out via fiber-optic bundles of endoscopic system.

The diameter of analyzed area was approximately 3 mm (300 pixels) at a distance on the order of 3 mm from the distal end of endoscopic system (which corresponded to working distance from the objective of endoscope to the surface). Recording data was accomplished with a field repetition rate of 40 Hz in the regime of incomplete sequence (subframe of mode) with the sizes of 50×50 of pixels with the time of the exposure of sequence 20 ms. In our experiments on a study of the possibilities of the speckle- correlation monitoring of the micro-hemodynamics of internal organs as the subjects of studies are used white the rats (males) without pedigree by the mass 200 - 250 g. under the general anesthetization (intraperitoneal introduction of anesthetic) was satisfied abdominal section. The intensity of micro-hemodynamics in the course of experiments changed by artificially created ischemia (as a result the overcompression of the corresponding main blood vessels), simulation of peritonitis, and also as a result the application of different medicines. Peritonitis was simulated by introduction into the abdominal cavity of animal 2 ml of intestinal contents. For studying the influences of elementary medicines on the micro-hemodynamics the intraperitoneal introduction of the solutions of novocaine, lidocaine, papaverine, adrenaline was used.

EXPERIMENTAL RESULTS: As a result of experiments we have seen direct dependence of increase of contrast on reduction of linear speed of scatterers of a surface of object.

7898-49, Poster Session

Saturation thresholds of evoked neural and hemodynamic responses in awake and asleep rats


Neural activation generates a subsequent hemodynamic response, replenishing metabolites to cells which expended energy resources. Vascular expansion increases basal blood perfusion but physical limitations may circumscribe transient increases in blood delivery due to decreased vascular compliance. Previous investigations have demonstrated a linear relationship between electrical and hemodynamic responses which do not saturate except under pathological conditions. However, these experiments were performed under sedation or anesthesia. Our previous studies suggest that waking activity may decrease vessel compliance. We implanted 5 adult Sprague Dawley rats with an EEG screw electrode over the auditory cortex to measure ERPs and an LED emitting 660 nm light and a photodiode placed 3 mm away collecting back scattered light. In order to investigate saturation thresholds during wake, we simultaneously measured evoked neural and hemodynamic responses from the auditory cortex in rats following different intensity stimulation ranging from 50-85 dBA. Additionally, we sleep deprived animals for 2, 4, or 6 hours to increase basal neural activity and blood perfusion and measured ERPs and hemodynamic responses to 64 dBA auditory stimulation. ERP and hemodynamic response amplitudes increased with increasing stimulus intensities; however, the hemodynamic response amplitude reached saturation at a lower stimulus intensity than the ERP. Evoked response amplitudes decreased following longer periods of sleep deprivation. These results suggest that there may be limits to cortical blood delivery and longer periods waking activity may decrease vascular compliance, limiting blood delivery following evoked neural activity.
Photoacoustic imaging of intravascular plaques using integrin-targeted gold nanoparticles

D. E. Yeager, P. P. Joshi, B. Wang, The Univ. of Texas at Austin (United States); J. H. Amirian, N. Matthias, The Univ. of Texas Health Science Ctr. at Houston (United States); K. V. Sokolov, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); R. W. Smalling, The Univ. of Texas Health Science Ctr. at Houston (United States) and Memorial Hermann Heart and Vascular Institute (United States); S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Identification of biomarkers associated with chronic inflammation present within atherosclerotic lesions to serve as receptors for molecularly targeted contrast agents is of great interest. One such target is the integrin αvβ3, which is up-regulated during angiogenesis and highly expressed by endothelial and smooth muscle cells within atherosclerosis. Previously, in-vivo delivery of gold nanoparticle (AuNP) contrast agents to atherosclerotic lesions via non-specific uptake by macrophages followed by the subsequent ex-vivo intravascular ultrasound (IVUS) and intravascular photoacoustic (IVPA) imaging of aorta sections has been demonstrated in a rabbit model of atherosclerosis. The focus of our current research is to extend the combined IVUS/IVPA imaging modality to the imaging of αvβ3 within atherosclerotic plaques. Spherical and rod-shaped AuNPs were synthesized via seed mediated growth and surface conjugation of two separate molecules targeting αvβ3 in combination with polyethylene glycol (PEG) was performed. First, a dithiol linker was attached to anti-αvβ3 antibodies (clone 23C6) allowing conjugation to AuNPs. Second, cyclic RGD peptides mimicking αvβ3 ligands were conjugated to AuNPs using a thiol group present on cysteine residues. The conjugated AuNPs were incubated for two hours with human epithelial carcinoma cells (A431) expressing αvβ3. Following incubation, dark field microscopy revealed labeling of the cells with both spherical and rod-shaped AuNPs conjugated with either 23C6 antibodies or cyclic RGD peptides. Labeling of αvβ3 with rod-shaped AuNPs provides a means of delivering an IVPA contrast agent with a tunable absorbance spectrum. These results indicate that integrin-targeted IVPA imaging is possible using antibody or RGD peptide conjugated AuNPs.
7899-04, Session 1

Multispectral optoacoustic tomography resolves smart probe activation in vulnerable plaques

D. Razansky, N. J. Harlaar, Helmholtz Zentrum München GmbH (Germany); J. Hillebrands, Univ. Medical Ctr. Groningen (Netherlands); A. Taruttis, E. Herzog, Helmholtz Zentrum München GmbH (Germany); C. Zeebregts, G. van Dam, Univ. Medical Ctr. Groningen (Netherlands); V. Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

It is currently generally accepted that high activity levels of certain biomarkers, such as cathepsins, integrins, and matrix metalloproteinase (MMP), are associated with atherosclerotic plaque instability. However, high fidelity imaging of those markers is challenging due to intense scattering of light in biological tissues, limiting the ability to deliver accurate information on structure and molecular activity in large tissue volumes. Herein, we developed a multispectral optoacoustic tomography (MSOT) method suitable for simultaneous high-resolution visualization of morphology and molecular activity in intact tissues. Human carotid plaque sample from a symptomatic patient was incubated with a MMP-sensitive activatable fluorescent probe (MMPsense 680, VisEn, Medical, Boston, Mass) directly after endarterectomy. An intact sample was subsequently imaged in the MSOT scanner to acquire volumetric images of plaque anatomy and distribution of MMP activity with 100 micron resolution. The hot and cold spot regions, identified by MSOT of an intact specimen, had a good correspondence to planar fluorescence imaging of cryosliced plaque, and was further validated by observing macrophage and smooth muscle cells appearance in immunohistochemistry and immunofluorescence. Based on the achieved results, it is anticipated that in a future clinical setting, MSOT could provide new ways for highly specific visualization and staging of plaque vulnerability in atherosclerosis.

7899-05, Session 1

Optical-resolution photoacoustic microscopy of ischemic stroke

S. Hu, K. Maslov, Washington Univ. in St. Louis (United States); E. Gonzales, J. Lee, Washington Univ. School of Medicine (United States); L. V. Wang, Washington Univ. in St. Louis (United States)

Ischemic stroke refers to the death of brain tissue resulting from an inadequate supply of blood and oxygen due to blockage of a major artery. As a result of ischemic stroke, the affected area of the brain is unable to function properly, which may lead to body paralysis, communication difficulty, or vision impairment. A major obstacle in understanding the underlying mechanism of stroke is the lack of an imaging tool to noninvasively or minimally invasively monitor cerebral hemodynamics, covering the entire span of the stroke period. Here, we applied optical-resolution photoacoustic microscopy (OR-PAM) to longitudinally study ischemic stroke in a middle cerebral artery (MCA) occlusion mouse model. OR-PAM observed that within the core region, the average hemoglobin oxygen saturation (sO2) within arteries and arterioles drops ~20% and the average sO2 in veins drop ~45% during the MCA occlusion. After reperfusion, the vessel sO2 in the core region recovered back to normal values. Vasodilation was also observed during MCA occlusion and after MCA reperfusion.

7899-06, Session 1

Photoacoustic imaging for deep targets in the breast using a multichannel 2D array transducer

Z. Xie, P. L. Carson, Univ. of Michigan Medical School (United States); R. F. Morris, Iph LLC (United States); F. R. Padilla, G. L. Lecarpentier, X. Wang, Univ. of Michigan Medical School (United States)

A photoacoustic (PA) imaging system was developed to achieve high sensitivity for the detection and characterization of vascular anomalies in the breast in mammographic geometry. Signal detection from deep breast was achieved by a broadband 2D PVDF planar array that has a round shape with an arc removed to fit near the chest wall. This array has 572 active elements and a -6dB bandwidth of 0.6-1.7 MHz. The low frequency enhances imaging depth and increases the size of vascular collections displayed without edge enhancement. The PA signals from all the elements go through low noise preamplifiers in the probe that are very close to the array elements for optimized noise control. Driven by 20 independent on-probe signal processing channels, imaging with both high sensitivity and good speed was achieved. To evaluate the imaging depth and the spatial resolution of this system, 2.38mm I.D. artificial vessels embedded deeply in ex vivo breasts harvested from fresh cadavers and a 3mm I.D. one in breast mimicking phantoms made of pork loin and fat tissues were imaged. Using near-infrared laser light with incident energy density within the ANSI safety limit, imaging depths of up to 49 mm in human breasts and 52 mm in phantoms have been achieved. With a high power tunable laser, this system working on multiple wavelengths might contribute to 3D noninvasive imaging of morphological and physiological tissue features throughout the breast.

7899-07, Session 1

Toward in-vivo photoacoustic imaging of human ovarian tissue for cancer detection

A. Aguirre, Y. Ardeshirpour, Univ. of Connecticut (United States); M. M. Sanders, M. A. Brewer, Univ. of Connecticut Health Ctr. (United States); Q. Zhu, Univ. of Connecticut (United States)

We have investigated the potential role photoacoustic imaging in ovarian cancer detection. We studied thirty-three ex-vivo human ovaries using co-registered ultrasound and photoacoustic imaging system based on a 1.75D transducer. An assessment of the photoacoustic images revealed features in the optical absorption distribution that could differentiate normal, diseased and malignant ovaries from each other. Additionally, estimation of the light absorption levels in the ovary has indicated that, in postmenopausal ovaries, malignant ovaries showed significantly higher light absorption than normal ones (p = 0.0237). For these two groups we have obtained a sensitivity of 83% and a specificity of 83%.

Ultrasound-guided diffuse optical tomography images of the same ovaries showed similar trends as those obtained with photoacoustic imaging. The malignant postmenopausal ovaries had the highest µα (0.153 cm⁻¹ 0.037 considering whole ovaries only), while the normal postmenopausal ovaries had the lowest one (0.069 cm⁻¹ 0.006). A linear regression curve for the photoacoustic absorption levels versus measured absorption coefficient with the diffuse optical tomography system indicates a correlation coefficient of 0.59 with p< 0.0023 at a confidence level of 0.05 reflecting the goodness of fit.

In an effort to bring this technique closer to clinics, we have developed a co-registered ultrasound and photoacoustic transvaginal probe. A fiber coupling assembly has been developed to deliver the light around the transducer in reflection geometry. Co-registered ultrasound and photoacoustic images of swine ovaries through vagina muscle wall demonstrate the potential of co-registered ultrasound and photoacoustic imaging for non-invasively detection of ovarian cancer in-vivo.
Three-dimensional photoacoustic imaging with a clinical two-dimensional matrix ultrasound transducer

T. N. Erpelding, Philips Research North America (United States); Y. Wang, Washiington Univ. in St. Louis (United States); L. Jankovic, Philips Research North America (United States); Z. Guo, Washington Univ. in St. Louis (United States); J. Robert, G. David, Philips Research North America (United States); C. Kim, L. V. Wang, Washington Univ. in St. Louis (United States)

Photoacoustic tomography provides both structural and functional imaging in vivo based on optical absorption contrast. A novel imaging system that incorporates a two-dimensional matrix ultrasound probe for combined photoacoustic (PA) and ultrasonographic (US) three-dimensional (3D) volumetric imaging is presented. The system consists of a tunable dye laser pumped by a Nd:YAG laser, a commercial US imaging system (Philips iU22) with a two-dimensional matrix transducer (Philips X7-2, 2500 elements, 2-7 MHz), and a multichannel data acquisition system which allows us to acquire RF channel data. Compared with alternative 3D techniques, this system is attractive because it can generate co-registered 3D PA/US images without mechanical scanning. Moreover, the lateral and elevational resolutions are measured to be 0.6 mm at 2 cm depth on reconstructed PA images of phantoms containing individual human hairs. Finally, in vivo 3D PA sentinel lymph node mapping using methylene blue dye in a rat model is demonstrated.

High-resolution ultrasound imaging and optoacoustic monitoring of blood variables in small blood vessels

I. Y. Petrova, Y. Y. Petrova, D. S. Prough, R. O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Ultrasound imaging is being widely used in clinics to obtain diagnostic information non-invasively and in real time. A high-resolution ultrasound imaging platform, Vevo (VisualSonics, Inc.) provides in vivo, real-time images with exceptional resolution (up to 30 microns) using high-frequency transducers (up to 80 MHz). Recently, we built optoacoustic systems for probing radial artery and other superficial blood vessels that can be used for noninvasive monitoring of total hemoglobin concentration, oxyhemoglobin saturation, and concentration of important endogenous and exogenous chromophores (such as ICG). In this work we used the high-resolution ultrasound imaging system Vevo 770 for visualization of the radial artery and other superficial blood vessels and acquired optoacoustic data from them using the optoacoustic systems. Analysis of the blood vessel geometry (depth, size, shape) was performed in volunteers at multiple sites. We studied the influence of overlying tissue and the blood vessel geometry in a wide range (diameter from 1 to 8 mm and depth from 1 to 4 mm) on optoacoustic signals from the blood vessels and accuracy of optoacoustic measurement. Our results suggest that a combination of high-resolution ultrasound imaging and optoacoustic probing of the radial artery and other relatively small blood vessels can be used to accurately measure total hemoglobin concentration, oxyhemoglobin saturation, and other important blood variables.

Combination of optoacoustics and ultrasound imaging for non-invasive, rapid assessment and management of circulatory shock

Y. Y. Petrova, I. Y. Petrova, R. O. Esenaliev, M. Kinsky, D. S. Prough, The Univ. of Texas Medical Branch (United States)

We developed a noninvasive, optoacoustic diagnostic platform for monitoring of multiple physiologic variables in inpatients and outpatients. One of the most important applications of this platform is noninvasive, rapid assessment and management of circulatory shock, a common condition in critically ill patients. At present, monitoring of circulatory shock requires measurement of central venous blood oxygenation using invasive procedures such as insertion of catheters in central veins. Hemoglobin saturation below 70% in central veins indicates circulatory shock that requires immediate treatment. We built a portable optoacoustic system for noninvasive measurement of central venous oxygenation. In this study we used an optoacoustic and clinical ultrasound imaging systems for rapid optoacoustic probing of these veins. The optoacoustic system utilizes a custom-made, sensitive optoacoustic probe that was developed in our laboratory for monitoring of blood oxygenation in deep blood vessels. The studies were performed in human subjects with different geometry (depth, size) of the veins. The ultrasound imaging systems permitted rapid identification of specific blood vessels for optoacoustic probing. We developed a correction algorithm for accurate measurement of blood oxygenation in blood vessels that decreases the influence of overlying tissue properties on optoacoustic monitoring. Our results indicate that the combination of optoacoustics and ultrasound imaging systems can provide more rapid and accurate assessment and management of the circulatory shock.
Volumetric dual-wavelength photoacoustic and ultrasonic endoscopy of upper gastrointestinal tract in vivo

J. Yang, C. P. Favazza, Washington Univ. in St. Louis (United States); R. Chen, Univ. of Southern California (United States); K. Maslov, X. Cai, Washington Univ. in St. Louis (United States); Q. Zhou, K. K. Shung, Univ. of Southern California (United States); L. V. Wang, Washington Univ. in St. Louis (United States)

We have successfully acquired in-vivo esophageal images from a rabbit, using a side-scanning photoacoustic endoscopic probe. Our endoscopic system is capable of simultaneous ultrasound and multi-wavelength photoacoustic imaging by interleaving dual wavelength optical pulses with acoustic pulses at each angular step of the scanning mirror. The endoscopic probe employs a focused ultrasonic transducer with high acoustic numerical aperture (~0.25) and central frequency (~40 MHz), yielding a high spatial resolution that approaches the theoretical limit. Experimentally measured photoacoustic and ultrasound imaging resolutions at the focal zone of the transducer were respectively ~30 μm and ~55 μm in the radial direction, and ~60 μm and ~80 μm in the transverse direction. Since all the data acquisitions and peripheral systems' operation sequences were synchronized with the scanning mirror’s angular A-line steps, spatially coincident image registration of the corresponding data sets was achieved. Simultaneous multi-wavelength and dual-modality endoscopy is expected to be a useful tool for better characterization of internal tissue abnormalities, such as esophageal tumors.

Recent progress on in-vivo multimodal imaging of the retina

H. F. Zhang, Univ. of Wisconsin-Milwaukee (United States); S. Jiao, The Univ. of Southern California (United States)

Multiple high-resolution optical imaging technologies, including photoacoustic ophthalmoscopy, optical coherence tomography, and confocal scanning laser ophthalmoscopy, have been seamlessly fused to measure optical absorption, optical scattering, and autofluorescence properties in the retina in vivo. As a result, comprehensive tissue characterization can be achieved to study fundamental physiological parameters such as the retinal hemodynamics and retinal pigmentation, which will facilitate the understanding of the pathogenesis of several significant blinding diseases such as retinopathy and macular degeneration.

Application of the optoacoustic method for temperature monitoring during HIFU impact on biological tissues: preliminary study

I. M. Pelivanov, S. M. Nikitin, M.V. Lomonosov Moscow State Univ. (Russian Federation)

Preliminary study of the possibility of the optoacoustic (OA) method application in dynamic monitoring of high intensity focused ultrasound (HIFU) impact on biological tissues is performed. HIFU therapy is a new promising method for treatment of different diseases, but the absence of a temperature monitoring tool prevents it from being widely used in clinic. The proposed OA method is based on the temperature dependence of the efficiency of light-to-sound transformation in tissues and organs. Layered samples consisted of healthy and thermally coagulated tissues as well as the samples with HIFU lesions inside were studied experimentally to prove the OA method applicability in monitoring of a lesion location. The next stage of this work was focused in the experimental in-vitro study of the dependence of the OA signal amplitude on the temperature in different ex-vivo tissues, in the temperature range of 20°C - 75°C. We used chicken breast as a model of muscle, porcine liver as a model of richly perfused tissue, and lard as a model of fatty tissue. Characteristic features of the temperature dependencies in tissues under study were described in terms of the difference of its structures. Finally we performed the numerical simulation of the OA signal profile dynamics during HIFU impact. It has been shown that the amplitude of the additional transient induced by HIFU is linear proportional to the efficiency of light-to-sound transformation in the point of maximal heating. Thus, the possibility of the dynamic temperature monitoring inside the tissue was demonstrated.

Optoacoustic technique for non-invasive monitoring of endotracheal tube placement and positioning

D. S. Prough, Y. Y. Petrov, I. Y. Petrova, M. Kinsky, R. O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Improper placement or positioning of an endotracheal tube may be lethal. Correct placement and positioning of endotracheal tubes is an essential component of life support during resuscitation from cardiac arrest or severe multiple trauma, during mechanical ventilatory support and during most surgical procedures under general anesthesia. To properly ventilate the lungs, endotracheal tubes must be inserted into the trachea rather than the esophagus, must be properly positioned in the mid-trachea and must remain properly positioned. We proposed to use optoacoustic technique for noninvasive monitoring of endotracheal tube placement and positioning. In this work we developed a compact, near infrared optoacoustic system for this application and performed in vitro and in vivo tests of the system. The in vitro tests were performed in tissue phantoms (simulating overlying tissue) with endotracheal tube, while in vivo tests were implemented in a large animal model (sheep). The optoacoustic measurements were noninvasively performed from the skin surface in the in vitro and in vivo studies using custom-made optoacoustic probes. The placement and positioning of the endotracheal tubes were monitored with submillimeter axial and millimeter lateral resolution using the optoacoustic system. The obtained data indicate that optoacoustics can provide real-time, precise, cost-effective monitoring of placement and positioning of endotracheal tubes.

Photoacoustic imaging of brachytherapy seeds using a channel-domain ultrasound array system

T. Harrison, J. C. Ranasinghesagara, R. J. Zemp, Univ. of Alberta (Canada)

Brachytherapy is a technique commonly used in the treatment of cancers, most notably prostate, that relies on the precise placement of miniscule radioactive seeds at or near the tumor location. The advantage of this technique over traditional radiation therapies is that treatment can be continuous and uniform, resulting in fewer clinic visits and a shorter treatment duration. Two important phases of this treatment are guidance for implantation and post-placement verification, where the seed location needs to be precisely determined. Ultrasound is a common imaging modality used for these purposes, but it can be difficult to distinguish the seeds from surrounding tissues. Photoacoustic imaging may offer a viable alternative. Using a photoacoustic system based on an L7-4 array transducer and a realtime ultrasound array system capable of parallel channel data acquisition streamed to a multi-core computer via PCI-express, we have demonstrated imaging of these seeds at an ultrasound depth of 16 mm and a laser penetration depth of 40 mm in chicken tissue. Ultrasound and photoacoustic images are co-registered.
Beyond thermal confinement: heat-transfer modulation for enhanced photoacoustic imaging contrast

W. Frey, S. R. Aglyamov, Y. Chen, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Photoacoustic imaging has gained momentum from the use of metallic nanoparticles, because the plasmon resonance provides for a strong absorption that can be tuned to a desired wavelength. Additionally, metallic nanoparticles are relatively biocompatible, and have a simple bioconjugation strategy that allows for directed targeting on the tissue, cellular and subcellular level. The photoacoustic image signal from metal nanoparticles originates in the fluid adjacent to the nanoparticle heated by the energy absorbed in the nanoparticle. The details of the heat transfer from the plasmonic absorber to the adjacent fluid can therefore be expected to have a strong influence on the photoacoustic signal strength and frequency spectrum, as well as on the biological environment. We have developed an analytical solution for the heat and pressure profile that develops in the fluid as a consequence of an arbitrary light pulse being absorbed in a spherical plasmonic nanoparticle. We show that sphere size, interfacial heat resistance, the properties of the fluid, and the introduction of a dielectric solid coating can be used to design the temperature profile around the nanoparticle and therefore the resulting photoacoustic response. The results support and provide a deeper understanding of our recent experimental results that show a multifold enhancement of the photoacoustic signal due to silica coating and cell uptake. The analytical temperature and pressure description also builds a platform to tune the local heating of cells and tissues during diagnostic imaging, controlled drug release, and therapeutic ablation.

Improved photoacoustic imaging and image-guided photothermal therapy using gold nanoparticles with plasmon resonances tuned to 1064 nm

G. P. Luke, K. A. Homan, Y. Chen, W. Frey, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Gold nanoparticles are effective photothermal therapy agents because their tunable plasmon resonance can be coupled with lasers in the near-infrared, where tissue absorption is relatively low. The same nanoparticles can also be used as photoacoustic contrast agents for real-time imaging of the therapy. Most studies have focused on nanoparticles with resonance peaks near 800 nm. However, there are several reasons to perform image-guided photothermal therapy at 1064 nm. Relatively inexpensive diode pumped solid state lasers generate continuous wave light at 1064 nm. Tissue absorbance at 1064 nm is lower and more uniform than at 800 nm, allowing for higher fluences and deeper penetration of the light. In addition, we found that gold nanoparticles tuned to 1064 nm have a greater absorption cross section, leading to more efficient heating of the tissue. We modeled gold triangular nanoplates and nanorods with absorption peaks ranging from 760 to 1064 nm using a finite difference time domain model. We then synthesized the same nanoparticles using seed-mediated growth methods. Finally, we collected combined ultrasonic and photoacoustic images of tumors containing the nanoparticles. The results of our modeling showed that a 1064 nm rod exhibited a two-fold increase in absorption cross section over a 760 nm rod. Furthermore, the 1064 nm plate showed a ten-fold increase over the 760 nm rod. Ultraviolet-visible spectroscopy confirmed these theoretical findings. Therefore, the increased absorption allows us to use fewer nanoparticles and the associated tumor targeting agent. The increase in absorption cross section also enhances photoacoustic imaging contrast.

Photoacoustic image-guided needle biopsy of sentinel lymph nodes

C. Kim, Washington Univ. in St. Louis (United States); T. N. Erpelding, Philips Research North America (United States); K. Maslov, Washington Univ. in St. Louis (United States); L. Jankovic, Philips Research North America (United States); W. J. Akers, L. Song, S. Achilefu, J. A. Margenthaler, L. V. Wang, Washington Univ. in St. Louis (United States)

We have implemented a hand-held photoacoustic and ultrasound probe for image-guided needle biopsy using a modified clinical ultrasound array system. Pulsed laser light was delivered via bifurcated optical fiber bundles integrated with the hand-held ultrasound probe. We photoacoustically guided needle insertion into rat sentinel lymph nodes (SLNs) following accumulation of indocyanine green (ICG). Strong photoacoustic image contrast of the needle was achieved. After intradermal injection of ICG in the left forepaw, deeply positioned SLNs (beneath 2-cm thick chicken breast) were easily identified in vivo and in real time. Further, we confirmed ICG uptake in axillary lymph nodes with in vivo and ex vivo fluorescence imaging. These results demonstrate the clinical potential of this hand-held photoacoustic system for facile identification and needle biopsy of SLNs for cancer staging and metastasis detection in humans.

Subwavelength-resolution photoacoustic microscopy for label-free detection of optical absorption in vivo

C. Zhang, K. Maslov, L. V. Wang, Washington Univ. in St. Louis (United States)

Mainstream optical microscopy technologies normally detect fluorescence or scattering, which may require undesirable labeling, but cannot directly sense optical absorption, which provides essential biological functional information. Here we reported in vivo and label-free subwavelength-resolution photoacoustic microscopy (SW-PAM) by using a water-immersion optical objective with a 1.23 NA. Capable of detecting nonfluorescent endogenous pigments, SW-PAM provides exquisitely high optical-absorption contrast. And, as a result of background-free detection, the sensitivity of SW-PAM to optical absorption reaches 100%. SW-PAM was demonstrated with wide-field optical microscopy by imaging gold nanospheres, ex vivo cells, and in vivo vasculature and melanoma. We use SW-PAM to detect the ultimate diffraction-limited optical resolution-220 nm resolution at 532 nm wavelength. Subcellular organelles, such as melanosomes, can be resolved by SW-PAM. Vasculature and early-stage melanoma were imaged with 1200% and 1700% contrasts, respectively, without labeling. For all these applications, SW-PAM has contrasts orders of magnitude higher than wide-field optical microscopy. Therefore, SW-PAM is expected to join the mainstream microscopy technologies.
7899-21, Session 4

Integrated photoacoustic and fluorescence confocal microscopy

Y. Wang, K. Maslov, C. Kim, S. Hu, L. V. Wang, Washington Univ. in St. Louis (United States)

Optical-resolution photoacoustic microscopy has demonstrated utility in imaging and characterizing microvasculature networks in vivo. This work presents a novel imaging system that integrates optical-resolution photoacoustic microscopy and fluorescence confocal microscopy for simultaneous photoacoustic and fluorescence imaging. The two complementary imaging modalities share the same laser source and objective lens. Hence, intrinsically registered photoacoustic and fluorescence images can be acquired in a single scan. The micrometer resolution allows imaging of blood vessels down to the capillary level. Capable of providing microscopic imaging of both optical absorption and fluorescence contrasts, the system demonstrates in vivo imaging of oxygen saturation and oxygen partial pressure in mouse ears.

7899-22, Session 4

Non-invasive quantification of the metabolic rate of oxygen (MRO2) by photoacoustic microscopy: a hyperthermia study of the mouse ear

J. Yao, K. Maslov, L. V. Wang, Washington Univ. in St. Louis (United States)

Aerobic metabolism satisfies the primary energy demand of human body, providing 88% of all ATP molecules. The efficiency of oxygen consumption is a key to the energy usage, and thus to tissue viability and functionality. Many diseases, normal decay and physiological functions are closely related to alterations in the metabolic rate of oxygen (MRO2). An accurate and robust measure of MRO2 would provide a powerful weapon for treating diseases and studying other physiological function-metabolism couplings. In this study, for the first time, we demonstrate that all the parameters for MRO2 quantification can be simultaneously obtained by optical-resolution photoacoustic microscopy (OR-PAM). Specifically, the total hemoglobin concentration and oxygen saturation were measured using two-wavelength excitation, blood flow velocity was estimated on the basis of photoacoustic Doppler bandwidth broadening, and the vessel cross-section was quantified from the structural PA image. As an example, we studied MRO2 of the mouse ear under normothermia (31 °C skin temperature) and controlled systematic hyperthermia (42 °C skin temperature). The experimental results show that hyperthermia increased blood flow speed, vessel diameter and the total hemoglobin concentration, decreased the oxygen extraction fraction, and as the net effect, increased the MRO2 of the mouse ear by 28.3 ± 9.4% (averaged across three mice). These results show that, OR-PAM, as a single noninvasive imaging modality, is intrinsically suitable for quantitative MRO2 measurement in microenvironments.

7899-23, Session 4

Optimal light illumination for photoacoustic microscopy beyond the soft penetration limit

C. P. Favazza, Z. Guo, K. Maslov, L. V. Wang, Washington Univ. in St. Louis (United States)

To image beyond the quasi-ballistic photon regime, photoacoustic tomography systems must rely on diffuse photons; however, there still exists an optimal illumination pattern that results in the largest number of photons reaching a target at a given depth. Many photoacoustic imaging systems incorporate weak optical focusing through oblique or dark-field illumination, but these systems are not often optimized for deep light delivery. Multiple parameters and constraints, particularly for in vivo imaging, need to be considered to determine the optimal illumination scheme for a given system: beam diameter, incident angle, pulse repetition rate, laser fluence, and target depth. In a set of tissue phantom and in vivo experiments, we recorded the photoacoustic signal from different target depths while varying the relative position of the geometric optical focus with respect to the acoustic focus of the transducer. The results reveal the most efficient optical focal position to maximize the number of photons delivered to a target depth, therein maximizing the PA signal. Similarly, Monte Carlo simulations of varied beam geometries and incident angles also show the best optical illumination schemes for different imaging depths. The principles and results discussed here are not limited to the system investigated, but can be applied to other system configurations to improve the photoacoustic signal strength.

7899-24, Session 4

On the feasibility of photoacoustic microendoscopy using image guide fibers and fiber-laser technology

S. M. Kerr, W. Shi, R. J. Zemp, Univ. of Alberta (Canada)

Optical-resolution photoacoustic microscopy (OR-PAM) is an imaging technology that achieves high optically-defined lateral spatial resolution of optically-absorbing superficial structures in vivo. Using image guide fibers and a unique fiber laser system we demonstrate the feasibility of photoacoustic microendoscopy. The image guide consists of 30,000 individual fibers in a bundle 800 µm in diameter, and provides transmission of high optical resolution images in a compact, flexible fiber. To our knowledge this is the first time that an image guide has been used for photoacoustic applications. Using a diode-pumped, pulsed Ytterbium fiber laser we produced ~1 ns, 0.20 µJ pulses at 532 nm with repetition rates up to 600 kHz. The light was coupled to the 1-m long image guide using an objective lens focused on the fiber input, and the beam was scanned across the fiber tip using a high speed mirror galvanometer. Contact was maintained between the fiber output and phantom target, while photoacoustic signals were directed into an ultrasound transducer using a 45-degree glass prism. Phantom studies indicate 10-20 µm resolution. The compact, flexible nature of the image guide and the small footprint of the apparatus make it ideal for photoacoustic microendoscopy, as well as increasing the usability of OR-PAM for external clinical applications.

7899-25, Session 4

Naturally integrated confocal and photoacoustic microscopy

Q. Wei, Univ. of Wisconsin-Milwaukee (United States); S. Jiao, Univ. of Southern California (United States); H. F. Zhang, Univ. of Wisconsin-Milwaukee (United States)

Photoacoustic microscopy (PAM) is one of the fastest-growing imaging technologies due to its unique capability to achieve high-spatial resolution imaging according to optical absorption contrast in biological tissue. In this paper, we reported a multi-modal laser scanning imaging system which integrated confocal and photoacoustic microscopy naturally. The confocal microscopy was achieved by combining the reflected photons from sample and coupled them into a 2x2 single mode fiber coupler. Unlike other multi-modal imaging equipments, only one laser source was used to excite both optical absorption and back reflection imaging. The axial resolution of the confocal microscopy was measured experimentally and agrees with the theoretical estimation well. To demonstrate the complimentary contrast of multimodal imaging, a printed mesh grid was imaged first. The grid has carbon lines on a printing transparency, where the dark lines have strong optical absorption coefficient. Confocal and photoacoustic microscopy imaged opposite contrasts: the black printed lines induced strong PA signal but very weak back-reflected photons for confocal microscopy due to stronger optical
absorption compared with the transparency. Since the photoacoustic and confocal microscopy share the same optical focus, the two images are intrinsically registered. The complementary contrast was also demonstrated in small animals in vivo. The skin structure and microvasculature of a Swiss Webster mouse ear can be observed by confocal and photoacoustic microscopy simultaneously. The significance of this work is that it exhibits the feasibility of integration PAM with the well-established confocal scanning laser ophthalmoscope (cSLO) for potential multimodal ophthalmic imaging. We have demonstrated the capability to image highly dynamic processes in vivo functional, intravital microvasculature imaging applications and cellular imaging applications. For rapid, dynamic cellular imaging applications, such as in vivo flow cytometry, high-speed OR-PAM is desirable. Here we report real-time OR-PAM in inverted microscope configuration with a 100,000 Hz pulse repetition rate, a nanosecond pulsed laser, and a customized, small aperture ultrasonic transducer. The inverted microscope configuration allows both two-dimensional optical scanning and efficient PAM signal detection with a non-focused ultrasonic transducer that is very close to the sample surface. We achieved 77 frames per second with 1024 A-lines per frame. High-speed cellular imaging was demonstrated.

Development of a real-time photoacoustic microscope
L. Wang, J. Yao, L. Li, K. Maslov, L. V. Wang, Washington Univ. in St. Louis (United States)
We present the development of a photoacoustic imaging system which has real-time imaging capability with optical resolution. The system consists of a single-element focused ultrasound transducer, a fiber-based light-delivery subsystem, a voice-coil translation stage, a motion controller, and a data acquisition subsystem. A unique cube is employed to combine light and acoustic beams. The mass of the entire scanning photoacoustic head is less than 40 grams, which minimizes potential vibrations and inertial effects, therefore, makes it possible to scan in real time. The imaging system is capable of acquiring 20 photoacoustic frames per second with a scanning range of nine millimeters, and up to 40 photoacoustic frames per second with smaller scanning ranges. Focused laser beams provide a lateral resolution of less than five microns. To demonstrate real-time imaging and high resolution, micron-sized carbon particle flow was imaged with a 40 Hz frame rate. Whole blood flow in silicone tubing was imaged in real time, where flowing red blood cell (RBC) clusters were observed. Single RBC flow in a capillary vessel in a mouse ear was also imaged using this system, which demonstrated the capability to image highly dynamic processes in vivo with micron resolution. This real-time high-resolution photoacoustic imaging system provides a promising approach for various in vivo studies.

Ultrahigh resolution photoacoustic microscopy via transient absorption
R. L. Shelton, B. E. Applegate, Texas A&M Univ. (United States)
We have developed a novel, hybrid imaging modality, Transient Absorption Ultrasonic Microscopy (TAUM), which fuses photoacoustic microscopy with non-linear microscopy. Photoacoustic microscopy is well known for its ability to image chromophores deep (> 1 mm) in scattering media with spatial resolutions in the 10s of microns. Non-linear microscopy is well known for its exquisite spatial resolution in three dimensions. This superior spatial resolution is attributed to the fact that the collected signal has a non-linear dependence on the light intensity. This dependence confines the signal to a very small focal volume, producing optically resolved voxels. Transient absorption is a non-linear process often used to map the excited state lifetimes of molecules exhibiting low fluorescence quantum efficiency. This sensitivity to non-radiative transitions makes transient absorption an attractive process to combine with photoacoustic imaging. We have built a prototype transient absorption ultrasonic microscope, implementing off-axis photoacoustic detection to allow the use of a high-quality, high numerical aperture objective. This high-quality, commercial lens is required to provide the tight focusing needed to optimize non-linear effects. We have demonstrated the increased spatial resolution of TAUM by imaging capillaries in the cheek pouch of a Syrian hamster. The capillary cross-sections are fully resolved, suggesting an axial resolution between 1-5 microns. A 25 MHz transducer was used in this experiment, which results in an axial resolution of 150 microns when used in a traditional photoacoustic microscope. Boasting the superior penetration depth and absorption contrast offered by photoacoustic emission and complemented by spatial resolutions comparable to confocal microscopy, we believe that Transient Absorption Ultrasonic Microscopy has excellent potential for producing volumetric images with cellular/subcellular resolution in three dimensions deep inside living tissue.

Investigation of DOT-assisted photoacoustic tomography in reflection geometry
C. Xu, P. D. Kumavor, A. Aguirre, Q. Zhu, Univ. of Connecticut (United States)
Photoacoustic tomography (PAT) combines the high ultrasound resolution and excellent optical contrast in a single imaging modality, and is capable of providing structural and functional information of lesions embedded in a highly scattering medium. Unfortunately, conventional PAT can only provide the distribution of absorbed optical energy density which is not the intrinsic functional parameter for breast cancer diagnosis. In this paper, we report the experimental investigation of a novel fitting procedure which can detect and quantitatively characterize the optical contrasts of targets using diffuse optical tomography (DOT)-assisted photoacoustic tomography. The hybrid system combines a 64-channel photoacoustic system with a 9-source, 14-detector frequency-domain DOT system. A white probe was used to house the ultrasound transducer, the optical sources and detectors. The experiment was performed in the reflection mode which is more realistic to clinical applications. The fitting procedure included a complete photoacoustic forward model, which incorporated an analytical model of light transport and a model of acoustic propagation. Using the structural information from the PAT images and the background information from DOT measurements, the photoacoustic forward model was used to recover the target absorption coefficient quantitatively. Phantom absorbers, 1 cm in diameter, with absorption coefficients ranging from 0.07 to 0.28 cm^-1 were imaged at depths up to 3.0 cm. The fitting results were at least 80% of their true values for both high and low contrast targets. The sensitivities of this fitting procedure to DOT and PAT measurements (target location, target radius, and background optical properties) were also investigated. We found that this fitting procedure was most sensitive to the target radius accuracy. The blood sample in a thin tube (radius 0.58 mm) was used to simulate the blood vessel imaged. The reconstructed images and fitted absorption coefficients were demonstrated. These results illustrate the possible application of DOT-assisted photoacoustic system and this fitting procedure in breast cancer diagnosis.
7899-30, Session 5

**Absolute measurement of absorption coefficient by combining photoacoustics and acousto-optics**

K. Daoudi, R. Molenaa, T. G. van Leeuwen, W. Steenbergen, Univ. Twente (Netherlands)

Biomedical photoacoustic tomography can provide qualitative images of biomedical soft tissue with high resolution. However quantitative measurements of chromophore concentration in vivo are of big interest and remain a challenge question. The amplitude of the photoacoustic signal induced by optical absorption in biological tissue is proportional to optical energy deposition which is the product of absorption coefficient and local light fluence. As a result of wavelength-dependent optical attenuation and scattering, the local fluence in biological media varies with the position and the optical wavelength. This fluence heterogeneity needs to be compensated in order to recover the absolute absorption coefficient. In this work we describe a new approach based on the combination between photoacoustic and acousto-optic signals without use of computational models. Our method is based on two principles: (1) a given photon trajectory through a scattering medium can be traveled in two directions with equal probability and (2) photons which traverse a certain volume can be labelled in that volume with the use of focussed ultrasound. Using Monte Carlo simulations, we give proof of the feasibility of the technique to recover the absolute absorption coefficient. Furthermore we demonstrate experimentally, using a tissue-mimicking phantom, the possibility of the technique to correct for the fluence heterogeneity caused by the optical absorption and scattering.

7899-31, Session 5

**Quantification of optical absorption coefficients from acoustic spectra with photoacoustic tomography**

Z. Guo, S. Hu, C. P. Favazza, Washington Univ. in st. Louis (United States); T. N. Erpelding, L. Jankovic, Philips Research North America (United States); L. V. Wang, Washington Univ. in St. Louis (United States)

Optical absorption is closely associated with many physiologically important parameters, such as the concentration and oxygen saturation of hemoglobin, and it can be used to quantify the concentrations of non-fluorescent molecules. We introduce a method to quantify the absolute optical absorption based upon the acoustic spectra of photoacoustic (PA) signals. This method is self-calibrating and thus insensitive to variations in optical fluence. Factors such as the detection system bandwidth and acoustic attenuation can affect the quantification but can be canceled by measuring the acoustic spectra at two optical wavelengths. This method has been implemented on various PA systems, including optical-resolution PA microscopy, acoustic-resolution PA microscopy, and reconstruction based PA array systems. We quantified the optical absorption coefficients of copper chloride samples at various wavelengths. We also quantified the oxygen saturation of hemoglobin in live mice.

7899-32, Session 5

**Biosensing of single cells at GHz frequencies by laser-ultrasonics**

B. Audoin, M. Ducouso, T. Dehoux, Univ. Bordeaux 1 (France); C. Chollet, O. Zouani, C. Chanseau, M. Durrieu, Univ. Victor Segalen Bordeaux 2 (France)

We use femtosecond laser pulses absorbed in a metallic transducer, namely the picosecond ultrasonic technique, for the remote optical generation and detection of gigahertz acoustic frequencies in single cells by pump-probe sampling.[1]

A TiAlV alloy mimicking a bone implant is functionalized with a RGD peptide layer to induce adhesion of MC3T3 cells. A BMP2 protein is injected in the cell to stimulate the formation of adhesive spots. Both pump and probe beams are focussed at the cell/transducer interface. The pump absorption yields a temperature rise and a picosecond acoustic pulse is generated through the thermoelastic effect. The probe beam is partially reflected from the metallic interface and partially scattered by the acoustic wavefront propagating in the cell. The change of reflectivity of the cell is measured as a function of the pump-probe time delay.

Interferences arise from the two probe contributions causing the so-called Brillouin oscillations. Optical phase variations due to acoustic-induced changes in cell thickness are simultaneously measured. The result of a semi-analytical calculation is fitted to the experimental data. We thereby determine the sound velocity at ~30 GHz from the frequency of the Brillouin oscillations and the nanometer thickness of single cells from the acoustic echoes propagating back and forth through the cell. Statistical variations in the sound velocity reveal nanoscale modifications of the cytosqueletal structure of the cells upon BMP2-induced reorganization. These results should enlighten the mechanics of cell adhesion on a subcell scale.


7899-33, Session 5

**Biophotoacoustic Sonar: Principles of operation, imaging and signal-to-noise analysis in time and frequency domains**

S. A. Telenkov, A. Mandelis, Univ. of Toronto (Canada)

Sensitivity analysis of photoacoustic measurements is conducted using estimates of the signal-to-noise ratio achieved under two different modes of optical excitation. The standard pulsed time-domain photoacoustic imaging is compared to the frequency-domain counterpart with a modulated optical source. The feasibility of continuous wave depth-resolved photoacoustics with frequency-swept (chirp) modulation pattern has been demonstrated. Utilization of chirped modulation waveforms achieves dramatic SNR increase of the periodic signals dramatically and preserves axial resolution comparable to the time-domain method. Estimates of the signal-to-noise ratio were obtained using typical parameters of piezoelectric transducers and optical properties of tissue.

7899-34, Session 5

**Dynamics of laser induced thermoelastic expansion of native and coagulated ex-vivo bovine liver samples and their mechanical properties**

B. Soroushian, Ryerson Univ. (Canada); W. M. Whelan, Univ. of Prince Edward Island (Canada); M. C. Kolios, Ryerson Univ. (Canada)

It has been shown that optoacoustic imaging can be used to monitor thermal therapy. The differential contrast in optoacoustic signals from native and thermally treated tissues can be attributed to the differences in their optical and thermo-mechanical properties. To assess these differences and to better understand the mechanism by which they affect the optoacoustic signals, the laser induced thermoelastic deformation of native and coagulated ex-vivo bovine livers after their irradiation by short (5-10ns) laser pulses was studied. The measurements were carried out using an enhanced Michelson interferometer setup capable of measuring tissue surface displacements with a temporal resolution of less than 10 ns and spatial resolution of less than 10 nm. One of the key advantages of this technique is that from the same dataset both optical and thermo-mechanical properties of the samples can be obtained. A
decrease in the optical attenuation depth of coagulated tissue samples compared to the native ones was observed (a mean value ± 99% confidence level of 860 ± 197 µm for the native and 421 ± 66 µm for the coagulated samples). There was no statistically significant difference for the Gruneisen coefficient of the two groups (varying between 0.071 and 0.2 with a mean value of 0.12). This could be partially attributed to reduced sensitivity of our measurement due to mechanical vibrations and environment noise, as well as the natural variability of the biological samples. However two distinctly different dynamics for the thermoelastic deformation of native and coagulated liver tissues were observed. A finite difference method was employed to numerically solve the wave equation of the laser induced thermoelastic deformation of tissue. Results of this approach helped to relate these two different experimentally observed trends to the mechanical properties of the tissue samples. The experimental data agreed with theoretical predictions based on the numerical solutions of the wave equation when an elastic modulus of 25 kPa was used for native tissue samples, but 450 MPa for the coagulated tissue samples, which indicates large changes in this mechanical parameter. The developed method is well suited for assessment of differences that in the optical and thermomechanical properties of soft tissue cause contrast of optoacoustic signals.

7899-35, Session 5
Optoacoustic Sensor for Nanoparticle Linked Immunosorbent Assay
A. Conjusteau, A. Liopo, D. Tsyboulski, S. A. Ermilov, TomoWave Labs., Inc. (United States); W. R. Elliott III, N. Barsalou, Naval Health Research Ctr. Detachment Brooks City-Base (United States); S. M. Maswadi, R. D. Glickman, The Univ. of Texas Health Science Ctr. at San Antonio (United States); A. A. Oraevsky, TomoWave Labs., Inc. (United States)

We developed an optoacoustic biosensor for detection of blood borne microorganisms using immunoaffinity reactions of antibody-coupled gold nanorods (Ab-NR) as a contrast agent specifically targeted to the antigen of interest. The Nano-LISA (Nanoparticle Linked Immunosorbent Assay) method has been adapted to detect three very common blood-borne viral infectious agents, i.e. human T-lymphotropic virus (HTLV), human immunodeficiency virus (HIV) and hepatitis-B (Hep-B). A working laboratory prototype of a Nano-LISA microplate reader-sensor has been assembled and tested with the model panel of the infectious agents. Optoacoustic (OA) responses generated by the samples were detected using an ultrawide band ultrasonic transducer.

The sensitivity of the technique has been assessed by determining minimally detectable optical density which corresponds to the minimum, detectable concentration of the target viral surface antigens. To carry out sensitivity analysis, recombinant proteins of selected viral surface antigens were used as the targets at known dilutions. Gold nanorods served as the contrast agent generating the optoacoustic response, and were conjugated using our standard protocol to antibodies that recognized and bound the viral antigens.

The sensitivity of Nano-LISA is at least OD=10^-6 which allows reliable detection of 1 pg/ml (depending on the commercial antibodies that are used). The detection reagents do not cross-react with non-complementary antigens, i.e. there is adequate detection specificity. Thus, adequate detection sensitivity, as well as lack of non-specific cross-reaction between antigens, has been demonstrated for the OA microplate reader-sensor, and supports the use of Nano-LISA as a clinical in vitro diagnostic technique.

7899-36, Session 6
Toward small-animal whole-body imaging using a photoacoustic full-ring array system
J. Xia, Z. Guo, Washington Univ. in St Louis (United States); A. Aguirre, Q. Zhu, Univ. of Connecticut (United States); L. V. Wang, Washington Univ. in St Louis (United States)

In this report, we present a novel 3D photoacoustic computed tomography (PACT) system for small-animal whole-body imaging. The PACT system, based on a 512-element full-ring transducer array, received photoacoustic signals primarily from a slice of 2 mm thickness. The light was generated by a pulse laser, and was reshaped to illuminate the sample from the side, using a conical lens and an optical condenser. Dark-field illumination was utilized to minimize surface signals. The PACT system was capable of acquiring an in-plane image in 1.6 s; by scanning the sample in the elevational direction, a 3D tomographic image could be constructed. We tested the system by imaging a cylindrical phantom made of human hairs immersed in a scattering medium. The reconstructed image achieved an in-plane resolution of 0.2 mm; due to the arc-shaped elevational focusing, the elevational resolution was around 1.2 mm. The Richardson-Lucy deconvolution algorithm was utilized to improve that value. The deconvolved image exhibited improved elevational resolution, and the 3D image was found to match well with the phantom. Our result demonstrated that the PACT system has the potential to be used for fast small-animal whole-body tomographic imaging.

7899-37, Session 6
Photoacoustic tomography of water in biological tissue
Z. Xu, C. Li, L. V. Wang, Washington Univ. in St. Louis (United States)

As an emerging imaging technique that combines high optical contrast and ultrasonic detection, photoacoustic tomography (PAT) has been widely used to image optically absorptive objects in both human and animal tissues. PAT overcomes the depth limitation of other high-resolution optical imaging methods, and it is also free from speckle artifacts. To our knowledge, water has never been imaged by PAT. Here, for the first time, we experimentally imaged water in both tissue phantoms and biological tissues using a near infrared (NIR) light source. The differences among photoacoustic images of water with different concentrations indicate that laser-based PAT can usefully detect and image water content in tissue.

7899-38, Session 6
Imaging the small animal cardiovascular system in real-time with multispectral optoacoustic tomography
A. Taruttis, E. Herzog, D. Razansky, V. Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

Small animal molecular imaging of the cardiovascular system plays a vital role in basic research of disease and development of therapeutic strategies. Multispectral Optoacoustic Tomography (MSOT) is an emerging technique for high resolution macroscopic imaging of optical absorption and molecular contrast. Its intrinsic sensitivity to hemoglobin enables visualization of the vascular and cardiac tissues of interest in cardiovascular disease (CVD). Additionally, the multispectral detection of chromophoric agents based on their spectral absorption signatures enables molecular contrast. We present results from an MSOT system recently developed for molecular small animal imaging studies. Based on near-infrared excitation and 64-element ultrasound detection, it is capable of generating tomographic slices at a frame-rate of 10 images per second. Anatomical features relevant to CVD research, such as the carotid arteries, the aorta and the heart, have never been imaged by PAT in mice, proving the ability to resolve features in the order of hundreds of microns in a whole mouse. For imaging of the heart, the system’s fast acquisition time, in tens of microseconds, allows images virtually free of motion artifacts from heartbeat and respiration, allowing the heart wall to be distinguished from surrounding tissues. Finally, to investigate
the feasibility of molecular cardiovascular imaging, we explore, by simulations and imaging studies, the suitability of a variety of available contrast agents for MSOT detection. We present in-vivo results showing detection of agents such as indocyanine green and gold nanorods at high spatial and temporal resolution, thus paving the way for molecular imaging applications.

7899-39, Session 6

Visualization of mouse kidney perfusion with multispectral optoacoustic tomography (MSOT) at video rate

A. Buehler, E. Herzog, D. Razansky, V. Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

Vast majority of current optoacoustic systems considered for small animal imaging utilize scanning arrangements that are typically inappropriate for real-time whole body imaging. Yet, control of animal physiological parameters, motion and anesthesia during these extended measurement periods can present a significant challenge for obtaining high quality images. The use of ultrasound transducer arrays from clinical systems has also been considered for increasing the imaging speed, however commercial arrays, optimized for clinical imaging, do not have the geometrical arrangement and broadband frequency characteristics that would make them appropriate for optoacoustic small animal imaging. Herein, we developed a novel multispectral optoacoustic tomography (MSOT) scanner capable of volumetric real-time animal imaging in-vivo. It provides three dimensional imaging capability while simultaneously acquiring data at multiple wavelengths for visualization of functional and molecular contrast. The system has proven capable of cross-sectional animal imaging with video-rate data acquisition and the reconstructed two-dimensional images delivered at 10 Hz rates. Imaging performance was demonstrated by resolving mouse kidney anatomy and perfusion using Indocyanine Green (ICG), an exogenously introduced blood-pool agent. Spectral unmixing further demonstrated the ability of the system to visualize externally administered contrast based on its unique spectral signature without using background measurements made prior to the probe administration.

7899-40, Session 6

In vivo longitudinal photoacoustic imaging of subcutaneous tumours in mice

J. G. Laufer, P. Johnson, E. Z. Zhang, B. Pedley, P. C. Beard, Univ. College London (United Kingdom)

Photoacoustic tomography provides 3D images of vascular networks in superficial tissues with high spatial resolution and can be used to visualise the distribution of contrast agents. It is therefore ideally suited to studying the origin, progression and treatment of human diseases in small animal models. In this study, images of subcutaneous tumours, which had been inoculated subcutaneously in nude mice (MF1/Nu), were acquired in vivo over a period of several days (5-14 days post-inoculation) using a photoacoustic imaging system, the sensing mechanism of which is based on the interferometric detection of ultrasound. Two lines of human colorectal tumour cells (LS174T, SW1222) with highly different tumour vascularity, were studied. Photoacoustic images acquired in the same tumour over several days allowed the monitoring of its growth and the changes in the vasculature that are associated with tumour development. The differences in tumour vascularity between models were also discernable. By acquiring images at multiple excitation wavelengths and by performing a spectral analysis of the wavelength-dependent photoacoustic image intensity, differences in the spatial distribution of blood oxygenation across the tumour were measured. The results suggest that photoacoustic imaging could play a role in the development of new clinical cancer therapies. For example, it could be used in longitudinal studies into the efficacy of vascular disrupting agents, such as OXi4503, on the re-development of tumour vasculature following antiangiogenic therapy.

7899-41, Session 6

In vivo optoacoustic imaging of a transgenic murine model of prostate cancer

M. Patterson, Univ. of Prince Edward Island (Canada); C. B. Riley, The Univ. of Adelaide (Australia); M. C. Kolios, Ryerson Univ. (Canada); W. M. Whelan, Univ. of Prince Edward Island (Canada)

Prostate cancer is currently the most common cancer among Canadian men. Due to an increase in public awareness and screening, prostate cancer is being detected at earlier stages and in younger men. This is raising the need for better treatment monitoring approaches. Optoacoustic images of a tumour bearing mouse, a mouse displaying prostatic hyperplasia, and age-matched controls were acquired for a 1064 nm illumination using a reverse-mode imaging system. In our study, a murine model of prostate cancer, TRAMP (transgenic adenocarcinoma of mouse prostate), was investigated. Around the age of 18 weeks, TRAMP mice spontaneously develop prostate cancer which closely mimics the progression of the human disease. Two-dimensional images of the prostate region were generated using maximum intensity projections of the optoacoustic signal. The prostate tumour and hyperplasia regions generated greater signals than that of the surrounding tissues, with contrast ratios of 3.3 and 2.2, respectively. The increase in signal may be explained by the molecular and physical properties of the tumour/hyperplasia. The dimension of the tumour in the OA image agreed with the true, 9.0 mm dimension, to within 1.6 mm. The frequency content of the optoacoustic signals generated by the tumour, hyperplasia and the surrounding tissues were analyzed and will be presented.

In this study we show that there are detectable changes in OA signal strength that arise from the presence of both tumour and hyperplasia in the prostate, which demonstrates the potential of OA imaging for monitoring prostate cancer.

7899-42, Session 7

Ultrasound-modulated optical tomography of thick biological samples using spectral hole burning

X. Xu, H. Liu, L. V. Wang, Washington Univ. in St. Louis (United States)

We improved the efficiency of the spectral hole burning (SHB) aided detection for ultrasound-modulated optical tomography (UOT) by using a Tm3+: YAG crystal of higher doping concentration (2.0-at.%) and a double pass pumping configuration. We demonstrated that absorbing, scattering, and phase objects embedded in the middle of a 40-mm thick phantom sample can be imaged with 0.5 mm lateral resolution and 1.5 mm axial resolution. We also obtained UOT image of two absorbing objects embedded in a 32-mm thick chicken breast sample. Our results suggest the feasibility of using SHB-UOT for optical imaging in real biological tissue.

7899-44, Session 7

Ultrasound-modulated optical sensing of oxygenation enhanced by microbubbles

J. E. P. Honeysett, E. Stride, T. S. Leung, Univ. College London (United Kingdom)

Diffuse optical sensing in tissue is insensitive to oxygenation changes inside larger blood vessels (diameter > 5 mm). Measurements are dominated by changes in the surrounding tissue, which has significantly lower optical absorption. A hybrid technique of focused ultrasound (US) and diffuse optics offers an improvement to this limitation. The optical properties of the blood at the focal region are modulated,
and hence photons passing through this region are modulated at the acoustic frequency. This modulated signal can be measured by observing the time-varying component of the speckle pattern formed at an optical detector. This technique is limited by the small proportion of modulated photons which pass through the US focal region compared with unmodulated photons which do not, leading to a low signal-to-noise ratio. Intravenous microbubbles (a clinical US contrast agent) are proposed to amplify this modulated signal, making it more easily detectable. The mechanisms for microbubble-enhanced US modulation are investigated by modelling the situation in a deep blood vessel within homogeneous tissue. Microbubbles oscillate under applied US: these oscillations are modelled using the Rayleigh-Plesset equation of motion, with an analytical solution derived to second order. A Monte Carlo model of ultrasound-modulated photon transport is developed to include a blood vessel containing blood and microbubbles. The aim is to extract optical oxygenation measurements from this model and compare these with experimental results using a tissue phantom. This would have many clinical applications, including critical care monitoring. Simulations are performed on a graphical-processing unit (GPU), which is significantly faster than CPU-based alternatives.

7899-45, Session 7
Focusing light into turbid media: time reversal ultrasound encoded (TRUE) light
H. Liu, L. V. Wang, X. Xu, Washington Univ. in St. Louis (United States)

Focusing light into biological tissues is desired for imaging and therapy. However, light undergoes multiple scattering in such media, which consequently imposes a fundamental limit on focusing beyond one transport mean free path. Optical phase conjugation combining with ultrasound encoding is proposed to overcome the diffusion limit to focus light into scattering media.Focused ultrasound encodes the diffused light, serves as a virtual source in a turbid medium. Then the modulated light wave front is phase conjugated by a phase conjugate mirror. Due to reciprocity the phase conjugated wave front can trace its trajectory back to the virtual source to realize focusing into a turbid medium. A demonstration simulation and experiment is presented and the broad potential applications are discussed.

7899-47, Session 7
Signal processing in the application of Ultrasound modulated optical tomography to tissue engineering
N. T. Huynh, F. Zhang, H. Ruan, D. He, B. R. Hayes-Gill, J. A. Crowe, M. L. Mather, F. R. Rose, The Univ. of Nottingham (United Kingdom); N. G. Parker, M. J. W. Povey, Univ. of Leeds (United Kingdom); S. P. Morgan, The Univ. of Nottingham (United Kingdom)

The overall aim of this research is to develop a system that uses Ultrasound modulated optical tomography (USMOT) to monitor the growth of tissue in 3D in situ within a bioreactor. A pulsed focused ultrasound transducer is applied and detection is performed using a photomultiplier tube. A number of signal processing schemes for image reconstruction are investigated, namely, Maximum Likelihood (ML), Envelope detection (ENV) and time-frequency spectrogram (TFSP). The ML method is based on a model that a pulsed USMOT signal is the convolution of a modulated optical pulse originating from a very thin layer with a profile along the acoustic column. The profile is a function of the ultrasonic axial pressure, the scattered light, and the optical absorption profile within a sample. Inverting the signal using a ML algorithm results in a 1D image. ENV measures the envelope of a narrow-band-filtered signal while TFSP demonstrates the energy distribution along both time and frequency axes. Both ENV and TFSP are shown to produce an amplitude drop in the presence of an optical absorber. Images are generally formed with the excitation illumination wavelength but the detection of ultrasound modulated fluorescent light is also investigated. Experimental results using gel-based tissue phantoms and 3D cell culture within scaffolds are demonstrated. The axial resolution is demonstrated to be better than 100μm using a 10MHz transducer. Preliminary results from a two-ultrasound-transducer system which utilizes non-linear effects to improve the imaging resolution are also presented.

7899-86, Session 7
Photorefractive acousto-optic imaging in the therapeutic window
S. Farahi, Ecole Supérieure de Physique et de Chimie Industrielles (France); G. Montemezzani, Univ. of Metz (France) and Supélec Campus de Metz (France); A. A. Grabar, Uzhgorod National Univ. (Ukraine); J. Huignard, Thales Research & Technology (France); F. Ramaz, Ecole Supérieure de Physique et de Chimie Industrielles (France)

Acousto-optic tomography is a technique that couples ultrasound and light in order to measure local optical properties through thick and highly scattering media, e.g. human breast tissues. Thanks to the acousto-optic effect, we can get the optical contrast information given by light and get the spatial localization from the ultrasound longitudinal waves. To minimize absorption in biological tissues, it is recommended to work within the so called “Optical Therapeutic window”, which mainly corresponds to the near-infrared wavelengths. Since our aim is to image objects embedded within thick skin, it is interesting to use a wavelength where the light has its maximum penetrating depth in tissues. Using a Tellurium doped Sn2P2S6 (tin thiohypodiphosphate) photorefractive crystal enables us to perform a self-adaptive wave-front holographic detection of the ultrasound modulated photons at 790 nm which is a wavelength that is weakly absorbed by biological tissues. We image absorbing objects embedded within a thick scattering phantom by use of a microsecond pulse regime to the ultrasound in order to get a dynamic millimetric axial resolution. These results open interesting perspectives for the use of this crystal for biomedical applications and a great advantage of photorefractive holography is that the amount of data that needs to be processed is low thus increasing its convenience for real-time in vivo imaging.

7899-48, Session 8
Optoacoustic imaging system with improved collection efficiency
D. Tsyboulski, A. Conjusteau, S. A. Ermilov, H. F. Brecht, A. A. Oraevsky, TomoWave Labs., Inc. (United States)

Optoacoustic microscopy is an emerging imaging technique based on optical absorption contrast which allows imaging inside turbid media with high spatial resolution, on the order of ~ 100 um or less at depths up to a cm. Acoustic waves are generated after laser pulse with a duration of several nanoseconds is absorbed by tissues and blood vessels. Under the condition of stress confinement, when the laser pulse duration is much smaller than the rate of energy dissipation, a part of the absorbed energy is converted to pressure. The resulting ultrasound waves propagate in tissues virtually without distortion, and are detected by specialized focused transducers or transducer arrays. The magnitude of the signal is directly proportional to absorptivity of a medium and thus provides for a high contrast between blood vessels and surrounding tissues. Resolution of the imaging system is defined by its collection efficiency or numerical aperture and a transducer frequency. In this report we present an improved design of our optoacoustic imaging system with high numerical aperture and compare its performance with current strictly optical-based methods.
Polymer Bragg waveguide ultrasound detectors

V. Govindan, S. Ashkenazi, Univ. of Minnesota, Twin Cities (United States)

Bragg Grating Waveguides (BGW) are demonstrated as ultrasound detectors. The device is fabricated by direct electron beam lithography technique using SU-8 as the core material. The main motivation for this design is the linear geometry of the device, which can be used in a linear array facilitating high frequency ultrasound imaging. BGW is a periodic perturbation of refractive index in the core of the waveguide along the propagation direction. This leads to the reflection of light in a narrow range of wavelengths centered at the resonance wavelength. The resonance depends on the periodicity of the grating and the effective refractive index of the structure. Higher index contrast leads to sharper resonance. In this device, the gratings are fabricated in the sidewalls of the waveguide for higher index contrast. Ultrasound wave incident on the BGW device creates a strain field due to the applied acoustic pressure, which alters the dimensions of the waveguide modifying the effective refractive index of the waveguide. This change in the effective index results in the shift of resonance wavelength. The periodicity of the device is 530 nm. The grating length is 500 µm. The resonance wavelength at which reflection occurs is around 1578 nm. The BGW device is experimentally demonstrated for the detection of ultrasound waves emitted by a 3.5MHz transducer. Detection sensitivity depends on optimal grating design for a steep resonance. The results demonstrate the potential use of BGW devices in highly compact array of optoacoustic detectors for high sensitivity ultrasound detection and photoacoustic imaging.

A miniature all-optical photoacoustic imaging probe for endoscopic applications

E. Z. Zhang, P. C. Beard, Univ. College London (United Kingdom)

There are a number of important clinical applications of photoacoustic imaging in which the target tissue can only be accessed by introducing an endoscopic probe percutaneously or through a natural orifice. Among these are the assessment of coronary artery disease, prostate cancer and gastrointestinal pathologies. The design of a photoacoustic probe for endoscopic applications poses several challenges. These include the question of how to integrate the delivery optical fibre with the ultrasound receiver to avoid obscuring the excitation laser pulses, obtaining the necessary level of miniaturisation and achieving low unit cost for single use applications. To address these challenges a range of miniature all-optical photoacoustic probes which employ a transparent Fabry Perot ultrasound sensor at the tip of an optical fibre is envisaged. There are several advantages of this approach over conventional piezoelectric based photoacoustic probes. It offers a high level of miniaturisation, inexpensive batch fabrication using vacuum deposition techniques and can readily be combined with other imaging modalities such as OCT and pulse-echo ultrasound. The concept also offers flexibility in its configuration allowing the implementation of forward and sideways looking probes and multielement imaging devices. To demonstrate the principle, a miniature (250 m outer diameter) single element forward-looking probe has been fabricated. This was achieved by depositing a thin film multilayer structure comprising a polymer or hard dielectric spacer sandwiched between a pair of dichroic dielectric mirrors on to the tip of a single mode fibre. The probe has been evaluated in terms of its acoustic bandwidth and sensitivity and by detecting photoacoustic signals in a variety of phantoms designed to simulate vascular and other tissues.

The use of tyrosinase-catalysed melanin as a molecular imaging contrast agent for photoacoustic tomography

A. Krumholz, S. Chavez, T. Fleming, W. E. Gillanders, L. V. Wang, Washington Univ. in St. Louis (United States)

Molecular imaging is an important emerging field in which biological processes are linked with contrast agents to reveal previously undetectable cellular mechanisms in vivo. Through the use of modern cloning techniques, it is possible to directly link a gene of interest with a reporter gene whose expression generates a contrast agent. As a reporter gene product, tyrosinase catalyzes melanin production from its precursors. When the tyrosinase gene is linked to a gene of interest that is over-expressed in mammary cancer cells, the resulting melanin can be used as a contrast agent to detect the presence of tumorigenic cells and metastatic disease arising from a primary breast tumor. Photoacoustic tomography (PAT), with its background free absorption contrast, is a proven molecular imaging tool whose advantage lies in the excellent imaging penetration depth. PAT, through the use of multiple wavelengths and high spatial resolution, is well-equipped to spectrally separate the three main absorbers in tissue: oxygenated blood, deoxygenated blood, and melanin.

Photoacoustic imaging of gene expression using tyrosinase as a reporter gene

R. J. Paproski, A. Forbrich, J. C. Ranasinghesagara, Y. Jiang, M. Hitt, R. J. Zemp, Univ. of Alberta (Canada)

Optical reporter genes, such as green fluorescence protein, are powerful research tools that allow visualization of gene expression. We have successfully used tyrosinase as a novel reporter gene for photoacoustic imaging which allows visualization of optically-absorbing pigments at significant tissue depths. Tyrosinase is the key regulatory enzyme in the production of melanin which has a broad optical absorption spectrum. MCF-7 cells were transfected with plasmids encoding the tyrosinase gene using Lipofectamine 2000. Three days after transfection, MCF-7 cells appeared brown compared to non-transfected MCF-7 cells. For photoacoustic experiments, MCF-7 cells were used i) as pellets in 200 µL thin-walled PCR tubes or ii) as cell suspensions (100,000,000 cells/mL) resuspended in phosphate buffered saline which was injected in 100 µm tubes. The photoacoustic signals received from pelleted transfected cells using a 553 nm pulsed laser (10 Hz repetition rate, 10 ns pulse duration) were at least 16-fold greater than the signals from tubes containing phosphate buffered saline or non-transfected cells (21.1, 1.27, and 0.01 mV, respectively). Using a 630 nm pulsed laser produced similar results. The results were verified when the tubes were embedded at 1 cm depth in a 0.3%-Intralipid fluid simulating tissue optical properties. The current data suggests that tyrosinase can be used as a reporter gene for photoacoustic imaging in vivo.

High-speed section-illumination photoacoustic microscopy with a 30-MHz linear ultrasound array

L. Song, K. Maslov, L. V. Wang, Washington Univ. in St. Louis (United States)

With a 30-MHz linear ultrasound array, we developed a high-speed high-resolution photoacoustic microscopy system. The laser beam for photoacoustic excitation was tightly cylindrically focused, which improved the elevational resolution more than 10 times–from ~300 micron...
to ~28 micron-compared with a system without cylindrically focused illumination. In addition, with each laser pulse, the entire B-scan cross section in tissue was optically illuminated, leading to unprecedented imaging speeds for high-resolution photoacoustic imaging-249 Hz and 0.5 Hz for 2-D and 3-D scans, respectively. Finally, to demonstrate the high-speed high-resolution imaging capability, we imaged microcirculation dynamics in vivo using the system.

7899-54, Session 8

Ultrafast photoacoustic imaging with improved elevational focusing
Y. Wang, P. Li, National Taiwan Univ. (Taiwan)

Conventional photoacoustic imaging system has limited temporal resolution and hence prohibits the applications in areas such as real-time 3D imaging. In this study, an ultrafast photoacoustic imaging system with its frame rate up to 2,000Hz is demonstrated. An ultrasound transducer with plane wave excitation and a high pulse repetition rate laser are utilized to acquire the data in parallel. Additionally, the 3D data acquisition which approaches the video rate is achieved when the volume data are collected by swept scanning of a motor. Nonetheless, 3D imaging by linear array beamforming suffers from deteriorated resolution in the region out of the elevational focus. The synthetic aperture focusing technique (SAFT) based on the concept of the virtual source in the elevation plane is applied to improve the imaging quality. After focusing, the ~6dB beamwidths in the point spread function are 327.6 μm, 424.8μm, and 897.1μm in lateral, axial, and elevational direction, respectively. Finally, 3D image of a phantom with scatterers and frame sequences of a moving target are presented. The 3D imaging has a frame rate of 12Hz to cover a square region of 19.2mm × 19.2mm.

7899-55, Session 8

Pulsed photoacoustic Doppler flow measurements in blood-mimicking phantoms
J. Brunker, P. C. Beard, Univ. College London (United Kingdom)

The feasibility of making spatially resolved measurements of blood flow using pulsed photoacoustic Doppler techniques has been investigated. Doppler time shifts were quantified via cross-correlation of pairs of photoacoustic waveforms generated within various blood-simulating phantoms using pairs of laser light pulses. The photoacoustic waves were detected using an ultrasound transducer. For each flow measurement, a series of 25 waveform pairs was collected. Previous data processing methods involved rejection of poorly correlated waveform pairs; the modal velocity value and standard deviation were then extracted from the selected distribution of velocity measurements. However, the data selection criteria used in this approach is to some extent arbitrary. A new data analysis protocol, which involves averaging the 25 cross-correlation functions and thus uses all of the measured data, has been designed in order to prevent exclusion of outliers. This more rigorous approach has proved effective for quantifying the linear motion of micron-scale absorbers imprinted on an acetate sheet moving with velocities in the range 0.2 to 1.5 ms-1. The technique was subsequently applied to fluids flowing at rates less than 10 cms-1 along an optically transparent tube. Several different suspensions of carbon and phenolic resin microspheres of various micron-scale diameters were explored. Experimental parameters, such as the time separation between the laser pulses and the transducer acoustic response, were evaluated in terms of their effect on the accuracy, resolution and range of measurable velocities. The distinguishing advantage of pulsed rather than continuous-wave excitation is that spatially resolved velocity measurements can be made. This offers the prospect of mapping flow within the microcirculation and thus providing insights into the perfusion of tumours and other pathologies characterised by abnormalities in flow status.

7899-56, Session 8

Integration of high repetition rate laser with real-time ultrasound for fast photoacoustic signal acquisition
J. Xia, C. Jia, Univ. of Washington Medical Ctr. (United States); L. Huang, Univ. of Washington (United States); C. H. Seo, M. O’Donnell, Univ. of Washington Medical Ctr. (United States)

Although photoacoustic (PA) imaging has shown significant potential for biomedicine, preclinical and clinical applications requiring high frame rate, real-time imaging may be limited by the pulse repetition rate of the excitation laser. Most PA imaging systems reported in the literature use a Q-switched laser with a 20 Hz repetition rate. This is very slow for many fast biological processes, including measurements in the cardiovascular system. In this study, we used a newly available, commercial, high repetition rate compact fiber laser and a multi-channel, real-time ultrasound (US) imaging system to acquire interleaved US/PA data from all channels in an imaging array. Real-time reconstructions of simultaneous US/PA images can be produced at high frame rates. The laser in this system is a MOPA fiber laser constructed from a single-mode gain-switched seed laser diode and amplified by a dual-stage Yb-doped double cladding fiber amplifier. The maximum output pulse energy is 1mJ depending on the repetition rate. The pulse duration is in the range of 10 to 100 nanosecond. The pulse repetition rate is tunable from 1 to 500 kHz. The ultrasound scanner can acquire 64 parallel channels on each laser/ultrasound firing, where the 64 channel active aperture can be multiplexed over a 128 element array. This means that a complete 128 element PA array image can be produced from only two firings of a high repetition rate laser, and interleaved US/PA images can be reconstructed in real-time at frame rates appropriate for cardiovascular applications.

7899-43, Poster Session

Acousto-optic imaging using a powerful long pulse laser in the therapeutic window
S. Farahi, E. Bossy, F. Ramaz, Ecole Supérieure de Physique et de Chimie Industrielles (France)

Acousto-optic tomography is a technique that couples ultrasounds and light in order to measure local optical properties through thick and highly scattering media, e.g. human breast tissues. Thanks to the acousto-optic effect, we can get the optical contrast information given by light and get the spatial localization from the ultrasound longitudinal waves. The use of a powerful long pulse laser (1 ms, 200 mJ) helps to improve the sensitivity of the technique by raising the optical peak power and collect more ultrasound tagged photons. Moreover its tunable wavelength around 780nm is appropriate for biological imaging since it is where light has its maximum penetrating depth in tissues.

Detection of acousto-optic signals is done by off-axis heterodyne digital holography on a high speed CMOS camera. This enables us to make a tunable spatio-temporal filter with a high signal to noise ratio. We also demonstrate the possibility to use a conventional pulsed ultrasound scanner to generate acousto-optic signals that can at the same time record ultrasound images. We image optical absorbers embedded within a thick scattering media (few centimeters). Our technique represents an interesting approach to multimodal imaging for breast cancer detection.
Optoacoustic quantitative evaluation of temperature distribution within biological tissues induced by HIFU impact: numerical study

S. M. Nikitin, I. M. Pelivanov, Lomonosov Moscow State Univ. (Russian Federation)

The possibility of the optoacoustic (OA) quantitative evaluation of temperature distribution within biological tissues induced by high intensity focused ultrasound (HIFU) impact was studied numerically. The basic idea of the OA method is the following. A tissue or organ being under HIFU impact is irradiated by probe short laser pulses. The efficiency of light-to-sound transformation (multiplication of a local light absorption coefficient on the Gruneizen parameter) depends on the temperature. Therefore, if that temperature dependence was known for an object under study, the OA signal amplitude would be used as the parameter for temperature monitoring.

Numerical calculation of OA signal profiles during HIFU impact was performed with use of the temperature dependencies measured experimentally. First, the initial temperature field induced by HIFU within the tissue was calculated and the probe laser fluence distribution was acquired by the Monte-Carlo method (taking into account both absorption and scattering in the medium). The OA response from the entire tissue volume was then calculated using the Raleigh integral summing all the signals excited by elementary tissue volumes (voxels).

Both a single point-like transducer located at different places outside the heated volume and arrays of focused transducers were considered for OA signal detection in our simulations. We have shown that even a single transducer can be used in monitoring of the temperature in the point of maximum tissue heating. The features of temperature reconstruction with the transducer array were discussed in detail.

Adaptive and quantitative reconstruction algorithm for photoacoustic tomography

S. Bu, K. Kondo, M. Yamakawa, T. Shiiina, Kyoto Univ. (Japan); K. Fukutani, Y. Someda, Y. Asao, Canon Inc. (Japan)

Photoacoustic (PA) tomography is a rapidly developing imaging modality which can provide high contrast and spatial-resolution images of light absorption distribution in tissue. The pixels in reconstructed PA images represent the level of absorbed optical energy, which is the product of the absorption coefficient and the fluence. However, the absorption coefficient is not the imaging parameter. Quantitative photoacoustic image reconstruction has been proposed to resolve this problem, but the process is based on compensating the image with a measured photon fluence distribution after image reconstruction. Because the contrast-noise-ratio (CNR) in reconstructed images of deep tissue is low, amplification also magnifies the noise, and the quality of image is degraded. Here we propose a novel adaptive reconstruction algorithm without introducing low CNR in deeper position. In this method, the quantitative processing is integrated into iterative reconstruction, and we assume that updated values in iterative are absorption coefficients. At each iteration step, the residual is calculated from detected PA signal and the signal calculated from a forward model, in the forward model initial pressure is calculated from the production of voxel value and the photon fluence. By minimizing the residual; reconstructed values can be converged to true absorption coefficients. Compared with other methods, global optimized compensation can be achieved. Therefore, better CNR can be obtained. The results of simulations and phantom experiments indicate that the proposed method performs better than conventional quantitative reconstruction methods. We expect that the capability of increasing imaging depth will broaden clinical applications.

In-vivo characterization of acute myocardial ischemia using photoacoustic imaging with a focused transducer

Z. Li, H. Li, Fujian Normal Univ. (China); H. Chen, Fujian Medical Univ. (China)

Nowadays, cardiovascular diseases are the world's largest killers, claiming 17.1 million lives a year, and myocardial ischemic disease is the focus of cardiovascular disease. We explore the feasibility of using photoacoustic imaging based on a focused transducer to characterizing acute myocardial ischemia at different stage. In this study, we blocked rat left anterior coronary descending artery (LAD) to induce the acute myocardial ischemia. In order to improving imaging speed and quality, the photoacoustic imaging system employed the single sampling and wavelet transformation. The results show that the intensity and areas of photoacoustic images of myocardial decrease with the LAD time increasing, which suggests that photoacoustic imaging has a potential for diagnosis of acute myocardial ischemia.
scattering in biological tissues. In this study, we demonstrate a newly developed imaging system for deep tissue imaging, which is named “dual illumination mode photoacoustic tomography system”. This system can alternatively or simultaneously illuminate mildly compressed tissues from a forward and backward direction toward an array transducer. This geometry of illumination makes deep tissue imaging possible in terms of light. The rectangular shaped 345-element (15x23) transducer with an element size of 2x2mm² is selected. Its shape allows direct illumination on tissue surfaces in front of array transducer through a 10mm thick compression plate, which can maximize optical fluences in our imaging area. The transducer frequency is designed for 1MHz to receive deep PA signals with minimal ultrasonic attenuation. A Ti-Sapphire optically pumped with a Q-switched Nd:YAG laser, which can tune wavelength from 750 to 850nm, has been integrated for deep light penetration in biological tissue. The laser beam is sufficiently broadened to satisfy the maximum permissible exposure limit. The system performances were tested in phantom studies. The results of our study showed that the system could visualize all the absorbers embedded in 50mm thick tissue-mimicking phantom.

7899-92, Poster Session

Advanced model-based reconstruction algorithm for practical three-dimensional photoacoustic imaging

K. Tanji, K. Watanabe, K. Fukutani, Y. Asao, T. Yagi, Canon Inc. (Japan); M. Yamakawa, T. Shinya, Kyoto Univ. (Japan)

Photoacoustic imaging (PAI) is a promising imaging modality, which can visualize biological tissues with high optical contrast and high spatial resolution. PAI has been used to visualize blood vessels, detect breast tumors, and estimate oxygenation levels. Conventional back-projection algorithms are widely used for PAI in spite of leading streak artifacts and blurring images when incomplete projection data are applied. Meanwhile, various model-based reconstruction algorithms are proposed to solve these drawbacks. However, the usage of model-based reconstruction for three-dimensional PAI is limited due to large computational cost.

In this study, we propose an advanced model-based reconstruction algorithm for three-dimensional PAI. The algorithm is based on accurate forward photoacoustic models and optimization algorithms in which one minimizes the square error between the measured acoustic signals and the signals predicted by the forward models. The forward photoacoustic models incorporate the system configuration and detector dependent factors such as frequency response and finite size effect. Conjugate gradient-based optimization algorithms are implemented for reconstructing images. In addition, we make use of the symmetry and locality of the photoacoustic waves in the computations of the forward photoacoustic models. In this way, the memory requirements and computation time are significantly reduced in three-dimensional image reconstruction.

We investigated the performance of the algorithm on both numerical and experimental phantom data in three-dimensional PAI. The results show that the proposed algorithm provides high resolution and quality photoacoustic images, which do not suffer from back-projection-related reconstruction artifacts and detector-related blur effect.

7899-93, Poster Session

Calibration of ultrasonic sensors using optoacoustics

A. Rosenthal, Helmholtz Zentrum München GmbH (Germany) and Massachusetts General Hospital (United States); D. Razansky, V. Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

The frequency response of ultrasonic detectors is commonly calibrated by finding their sensitivity to incident plane waves. However, in optoacoustic tomography, it is the response to broadband point sources that is required. In order to induce such sources using the optoacoustic effect, the illuminated object’s dimensions must be smaller than the resolution achievable by the optoacoustic system. The main difficulty in such measurements is that the magnitude of the field emitted by such sources is proportional to their dimensions, and thus may be weak compared to parasitic sources in the setup.

In this work we experimentally demonstrate two methods for calibrating acoustic detectors. In both methods, optoacoustic sources are induced in large optically absorbing slabs by illuminating them with short laser pulses. Despite the large dimensions of the illuminated objects, the geometry used yields wideband acoustic fields, which are perceived by the detectors as originating from point sources.

In the first method, the phantom is semi-transparent, leading to the creation of separated short positive- and negative-pressure pulses from its two facets facing the acoustic detector. The impulse response of the detector is obtained by cropping the contribution of the negative pulse. In the second geometry, the phantom is highly absorbing, leading to the creation of a bipolar signal from its facet proximal to the detector. The impulse response of the detector is obtained by integrating the measured signal.

7899-94, Poster Session

Detecting abnormal vasculature from photoacoustic signals using wavelet-packet features

J. Zalev, M. C. Kolios, Ryerson Univ. (Canada)

Photoacoustic systems can produce high-resolution, high-contrast images of vascular structures; however, data-acquisition for clinical real-time in-vivo imaging remains a challenge. We have developed an approach for the classification of vascular tissue using photoacoustic ultrasound radiofrequency (RF) signals prior to image reconstruction. A 3D segmentation scheme is proposed using a classifier based on the wavelet packet transform.

To reconstruct images at very high-resolution, RF signals must be collected from many transducer locations. This can be time consuming and expensive due to limitations in transducer array technology. When limited transducer locations are sampled, the system resolution is reduced, although the bandwidth of each RF signal remains the same. By examining the RF signals prior to reconstruction, it is possible to extract useful information about signal details not available after image reconstruction.

For use in cancer detection, we have developed a unique photoacoustic simulator that produces RF signals from 3D models of vascular networks. Normal and abnormal tissues are modeled as fractal trees using published branching parameters. Each vessel is a finite-length cylinder allowing simulations from large vascular networks. We perform validation against an exact equation for cylindrical photoacoustic sources and through FEM simulations. B-mode images of the unresolved vascular networks are also produced.

Our results show that it is possible to differentiate tissue types even when it is not possible to resolve each vessel. The classifier performed with sensitivity 100% and specificity 87% on the tissue models. The performance is characterized against the number of transducers and with additive white noise.

7899-95, Poster Session

Combined acoustic-photoacoustic and fluorescence imaging catheter for the detection of the atherosclerosis plaque

M. Abran, C. Matteau-Pelletier, K. Zerouali-Boukhal, Ecole Polytechnique de Montréal (Canada); J. Tardif, Montréal Heart Institute (Canada); F. Lesage, Ecole Polytechnique de Montréal (Canada)

We investigated the performance of the algorithm on both numerical and experimental phantom data in three-dimensional PAI. The results show that the proposed algorithm provides high resolution and quality photoacoustic images, which do not suffer from back-projection-related reconstruction artifacts and detector-related blur effect.
In industrialized countries, cardiovascular diseases remain the main cause of mortality. The detection of atherosclerosis and its associated plaque is still a challenge due to the requirement of detection on the background of successful cholesterol lowering therapies. Intravascular ultrasound (IVUS) imaging has been demonstrated to be a powerful tool to uncover structural information of atherosclerosis plaques. Recently, intravascular photoacoustic (IVPA) has been combined with IVUS imaging in phantoms and ex-vivo tissues to add functional and/or molecular information. The IVPA/IVUS combination has been demonstrated to provide relevant information about the composition of the plaque, as well as its vulnerability. In this work, we extend this previous work by using a combined IVPA/IVUS system using a rotating ultrasound transducer in a catheter to which an optical fiber is attached. In addition, a third modality is included through fluorescence detection in the same fiber at a distinct wavelength from PA, opening the door to complementary information using fluorescence activatable probes. Cylindrical silicon phantoms with inclusions containing fluorophores, ink or gold nanoshells were used to validate the system. Bleaching of the fluorophore by the pulsed laser used for photoacoustic is quantified. IVUS images are obtained continuously and used to co-register photoacoustic and fluorescence signals. Since the transducer is rotating while the optical fiber is illuminating the complete phantom without rotating, light is not diffused uniformly. A Monte Carlo analysis of the propagation of light is used to correct the PA recordings.

7899-96, Poster Session

Comparison of photoacoustic imaging systems using continuous-wave lasers with a chirped intensity modulation frequency to pulsed lasers

A. Petschke, P. J. La Riviere, The Univ. of Chicago Medical Ctr. (United States)

Using a Green’s function solution to the photoacoustic wave equation, we compare imaging systems using continuous-wave (CW) lasers with a chirped intensity modulation frequency to pulsed lasers. We show that the axial resolution is the same in both cases and is determined primarily by the transducer bandwidth. We also compare the signal-to-noise ratio (SNR) of the two systems assuming the fluence is limited by the American National Standards Institute (ANSI) laser safety guidelines for skin. Although the SNR depends on several parameters such as the imaging time, the chirp duration, and the pulse repetition rate, we find that the SNR is about 10 dB to 30 dB larger for pulsed lasers for reasonable values of the parameters. However, CW diode lasers have the advantage of being compact and relatively inexpensive, which may outweigh the slightly lower SNR in many applications.

7899-97, Poster Session

Investigation of photoacoustic imaging for monitoring of wound healing under a layer of blood with different coagulation

C. Song, Chonbuk National Univ. (Korea, Republic of); D. Kim, U.S. Food and Drug Administration (United States); S. H. Ryu, J. H. Seo, Chonbuk National Univ. (Korea, Republic of)

Objective and accurate monitoring method of tissue regeneration and wound closure is required for efficient treatment of diabetic foot ulcer (DFU) or wound from burns. DFU is one of the serious complications caused by diabetes mellitus which causes more than 80% of nontraumatic amputations in the diabetic patients. Biopsy is a very accurate method, however it has a potential of second wound and additional infection. Novel optical imaging techniques such as optical coherence tomography (OCT) have been applied to monitoring of dermal wounds such as cutaneous edema and burn wounds. Photoacoustic (PA) imaging has been investigated for wound healing monitoring for its superior depth penetration. However, due to the high absorption of PA signal by hemoglobin, observation of tissue layers under tissues in hemostasis process is highly limited. We have investigated PA imaging for monitoring of wound healing under a layer of blood with different degree of coagulation. We embedded simulated blood vessel structure in tissue phantoms made with polyacrylamide or gel. A thin layer of blood with different degree of coagulation was also embedded on top of the vessel structure. Using 532 nm pulse laser, we obtained PA images of the phantoms and analyzed the image quality depending on the degree of blood coagulation. We compared the results from setups using focused and unfocused transducers. Though this method is still preliminary and needs refinement prior to taking it to clinical application, the ability to classify different degree of coagulation. We believe our investigation will provide valuable information of tissue of healing process which lies under blood coagulation layer.

7899-98, Poster Session

Regional sensitivity comparison between optical and acousto-optic (AO) sensing

S. Gunadi, T. S. Leung, Univ. College London (United Kingdom)

Typical optical sensing probes biological tissue oxygenation of a relatively large region. The AO method can tag photons by focused ultrasonic waves in a region of interest within the tissue for potential localised oxygenation sensing. This study aims to compare the regional sensitivity between the optical and AO sensing techniques. The regional sensitivity is defined as the amount of change observed in the measured signal in response to a small localised change in the optical absorption. Conventional optical systems have been shown to be more sensitive to absorption variation in the superficial region. In this work, we show that the AO method can provide a high sensitivity in the deeper region. Using the sensitivity map (photom measurement density function) approach, two types of AO detection schemes are also compared, i.e. the single element scheme (single photon counter) and the parallel scheme (charged coupled device CCD). In all cases, the optical probes are configured for reflectance measurement and the position of the ultrasound focal region within the turbid medium is kept unchanged. The baseline measurements are made on a liquid phantom by both optical (without ultrasound excitation) and AO methods (with ultrasound excitation). A small and highly absorbing perturbation is then introduced and systematically repositioned throughout the phantom. At each location of the absorber, both optical and AO measurements are made so that sensitivity maps could be computed and compared. The experimental results are further verified by graphics-processing-unit accelerated Monte Carlo simulations.

7899-99, Poster Session

Fast semi-analytical acoustic inversion for quantitative optoacoustic tomography

A. Rosenthal, Helmholtz Zentrum München GmbH (Germany) and Massachusetts General Hospital (United States); D. Razansky, V. Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

We present a fast inversion algorithm for quantitative two- and three-dimensional optoacoustic tomography. The algorithm is based on an accurate and efficient forward model, which eliminates the need for regularization in the inversion and can achieve real-time performance. Our model-based framework offers generalization of the forward solution to more comprehensive optoacoustic propagation models, such as including detector frequency response, without changing the inversion procedure. The reconstruction speed and other algorithmic performances are demonstrated using numerical simulation studies and experimentally on tissue-mimicking optically heterogeneous phantoms and small animals. The tissue-mimicking phantoms were cylindrically shaped with a diameter of 16 mm and were uniformly illuminated from their side.
animal imaging was performed for an intact adult one-year-old zebrafish. Because of the large size of the phantom, significant light attenuation and did not suffer from the artifacts which usually afflict commonly used manifested correctly the effect of light attenuation through the objects and did not suffer from the artifacts which usually afflict commonly used filtered backprojection algorithms, such as negative absorption values.

Simulating the spatially-dependent frequency response of arbitrary-shape acoustic detectors for optoacoustic imaging

A. Rosenthal, Helmholtz Zentrum München GmbH (Germany) and Massachusetts General Hospital (United States); V. Ntziaichristos, D. Razansky, Helmholtz Zentrum München GmbH (Germany)

One of the major challenges of optoacoustic imaging is that it involves relatively weak acoustic signals, which need to be detected with high signal-to-noise ratio (SNR). Because the SNR is generally proportional to the area of the detector’s face, large area detectors are commonly used. Although the use of such detectors offers the advantage of improved SNR as compared to point detectors, it may lead to significant signal distortion resulting in artifacts in the reconstructed image. In this work, we develop a general method for simulating the spatially-dependent frequency response of acoustic detectors with an arbitrary shape. The frequency response is incorporated in a forward model for optoacoustic propagation. Our method can be used for designing detectors with desired qualities and reducing reconstruction artifacts caused by the response of the finite-size detectors.

Our method is based on an analytical solution to the optoacoustic-propagation equation for a flat detector. In order to obtain the response of an arbitrary-shape detector, we approximate its surface by a series of line detectors and sum their respective responses. We demonstrate this approach for 2D flat and focused detectors. For both detectors, the calculated response exhibited a very strong spatial dependency. In order to validate our results, we measured the response of a cylindrically focused acoustic transducer to a point source in various locations. The spatial dependency of the experimental response showed very good correspondence to the theoretical prediction.

Photoacoustic endoscopy using polymer microring resonators

S. Chen, T. Ling, H. W. Baac, L. J. Guo, Univ. of Michigan (United States)

Photoacoustic (PA) imaging has potential for a variety of medical studies because of its safety, high imaging depth, and high resolution. Applications such as brain lesion detection, hemodynamics monitoring, and breast cancer diagnosis suggest PA imaging is a promising modality. PA imaging uses a laser to excite a sample into soft tissue, enabling the deep imaging ability. However, imaging signal-to-noise ratio (SNR) and resolution will degrade at large imaging depth. To address this issue, an endoscopic PA probe can be positioned close to the region of interest and thus preserve the high SNR and resolution. We have developed a miniaturized imaging probe for PA endoscopy application. The probe consists of a light-guiding optical fiber, a polymer microring resonator as an ultrasonic sensor, and a prism to guide ultrasonic waves. The waveguide-based polymer microring resonator has a small size, which renders it a good candidate for catheter-type application. Moreover, its ability of low-noise and wideband ultrasound detection could produce images with high SNR and superior resolution.

Visualization of micro-calcifications using photoacoustic imaging: feasibility study

T. Hsiao, Industrial Technology Research Institute (Taiwan) and National Tsing Hua Univ. (Taiwan); P. Wang, C. Fan, Y. Cheng, M. Li, National Tsing Hua Univ. (Taiwan)

Recently, photoacoustic imaging has been intensively studied for blood vessel imaging, and shown its capability of revealing vascular features suggestive of malignancy of breast cancer. In this study, we explore the feasibility of visualization of micro-calcifications using photoacoustic imaging. Breast micro-calcification is also known as one of the most important indicators for early breast cancer detection. The non-ionizing radiation and speckle free nature of photoacoustic imaging overcomes the drawbacks of current diagnostic tools - X-ray mammography and ultrasound imaging, respectively. We employed a 10-MHz photoacoustic imaging system to verify our idea. A sliced chicken breast phantom with granulated calcium hydroxyapatite (HA) - major chemical composition of the breast calcification associated with malignant breast cancers - embedded was imaged. With the near infrared (NIR) laser excitation, it is shown that the distribution of ~500 μm HAs can be clearly imaged. In addition, photoacoustic signals from HAs rival those of blood given an optimal NIR wavelength. In summary, photoacoustic imaging shows its promise for breast micro-calcification detection. Moreover, fusion of the photoacoustic and ultrasound images can reveal the location and distribution of micro-calcifications within anatomical landmarks of the breast tissue, which is clinically useful for biopsy and diagnosis of breast cancer staging.

Photoacoustic endoscopy using polymer microring resonators

S. Chen, T. Ling, H. W. Baac, L. J. Guo, Univ. of Michigan (United States)

Photoacoustic (PA) imaging has potential for a variety of medical studies because of its safety, high imaging depth, and high resolution. Applications such as brain lesion detection, hemodynamics monitoring, and breast cancer diagnosis suggest PA imaging is a promising modality. PA imaging uses a laser to excite a sample into soft tissue, enabling the deep imaging ability. However, imaging signal-to-noise ratio (SNR) and resolution will degrade at large imaging depth. To address this issue, an endoscopic PA probe can be positioned close to the region of interest and thus preserve the high SNR and resolution. We have developed a miniaturized imaging probe for PA endoscopy application. The probe consists of a light-guiding optical fiber, a polymer microring resonator as an ultrasonic sensor, and a prism to guide ultrasonic waves. The waveguide-based polymer microring resonator has a small size, which renders it a good candidate for catheter-type application. Moreover, its ability of low-noise and wideband ultrasound detection could produce images with high SNR and superior resolution.

Longitudinal optical-resolution photoacoustic microscopy of tumor neovascularization

S. Hu, R. Sohn, A. C. Santeford, K. Maslov, J. M. Arbeit, L. V. Wang, Washington Univ. in St. Louis (United States)

Neovascularization is essential for tumor growth and metastasis; however, existing technologies have difficulty in performing label-free chronic imaging of tumor microvascular elaboration in an individual animal. Here, we applied longitudinal optical-resolution photoacoustic microscopy (OR-PAM) for noninvasive determination of vascular elaboration and microcirculatory dynamics of different human cancer xenografts grown in mouse ears. OR-PAM determined changes in functional neovascularization during tumor growth and VEGF signaling inhibition. Ex-vivo tissue analysis co-registered tumor biological responses (tumor cell perfusion, proliferation, apoptosis, microvessel density, and hypoxia) with regional functional neovascular data acquired by OR-PAM. Combinatorial biological and OR-PAM analysis will facilitate novel drug design and scheduling for more effective antineoplastic therapies.
Effect of the illumination method on photo-acoustic image quality with array transducer based system
K. Tsujita, Y. Satou, FUJIFILM Corp. (Japan); M. Ishihara, T. Hirasawa, M. Kikuchi, National Defense Medical College (Japan)

Photo-acoustic imaging is a hybrid imaging technique which can offer a high contrast tomographic image with ultrasound like resolution in depth of centimeters. Additionally, it has been studied well as functional imaging modality using characteristics that can distinguish by absorption spectra.

Our purpose is to investigate the image quality and potential of photo-acoustic image as a preliminary study toward the medical diagnosis applications. For this purpose, firstly we focused on the difference of image quality between photo-acoustic image and ultrasound image using array transducer system. Secondly we examine the effect of illumination method on photo-acoustic image quality.

We compared both photo-acoustic image and ultrasound image of a phantom including hair or tube using original experimental production system with 192ch PZT array probe. Resolution of photo-acoustic image and ultrasound image enabled to be revealed, based on the same reconstruction method using each element data. We got higher resolution and contrast of photo-acoustic image than ultrasound image, in hair phantom experiment.

To examine the effect on photo-acoustic image quality, we analyzed phantom images, which were taken under various illumination conditions, in terms of contrast and resolution. Our analysis with experiments and Monte Carlo simulation are performed to show the necessity for illumination optimization depend on the application and probe size.

This work was partially supported by the Japan Science and Technology Agency A-STEP. (Feasibility studies stage No. 2111032)

Functional transcranial photoacoustic micro-imaging of mouse cerebrovascular cross-section and hemoglobin oxygenation changes during forepaw electrical stimulation
L. Liao, Y. Chen, C. Lin, J. Chang, National Chiao Tung Univ. (Taiwan); M. Li, National Tsing Hua Univ. (Taiwan)

In this study, we report on using a 50-MHz functional photoacoustic microscopy (PAM) to transcranially image the cross-section and hemoglobin oxygenation (SO2) changes of single mouse cortical vessels in response to left forepaw electrical stimulation. Three difference levels of the cortical vessels (i.e., with different-sized diameters of 350, 120 and 55 um) on activated regions were marked to measure their functional cross-section and SO2 changes as a function of time. Electrical stimulation of the mouse left forelimb was applied to evoke functional changes in vascular dynamics of the mouse somatosensory cortex.

The applied current pulses were with a pulse frequency of 3 Hz, pulse duration of 0.2 ms, and pulse amplitude of 2 mA. The cerebrovascular cross-section changes were probed by images acquired at 570 nm, a hemoglobin isosbestic point, while SO2 changes were monitored by the derivatives of 560-nm images normalized to 570-nm ones. The averaged vasodilatation at the three vessel levels was about 18%, 23% 34% upon stimulation, respectively. The correlation between the electrical stimulation paradigm with the cross-section and SO2 changes at the three different vessel levels was also statistically analyzed. The results show that vessel diameter and SO2 were significantly dilated and increased when compared with those in the controlled one. In conclusion, the presented methods are applied to both numerical and experimentally obtained phantom and in-vivo measurement data, and the improvements in image magnitude and resolution are discussed.

Multifunctional photo-acoustic signals detected by P(VDF/TrFE) film sensor with a wide range of frequency
M. Ishihara, T. Hirasawa, National Defense Medical College (Japan); K. Tsujita, FUJIFILM Corp. (Japan); M. Kitagaki, I. Bansaku, National Defense Medical College (Japan); M. Fujita, National Defense Medical College Research Institute (Japan); M. Kikuchi, National Defense Medical College (Japan)

Photo-acoustics has been widely studied as a combined imaging modality of both optical and acoustical methods. The merits of the photo-acoustic imaging are realizing the full potentials of pulsed laser - tissue interaction. As the photo-acoustic waves can be induced at chromophores by pulsed laser irradiation through a thermoelastic process, it covers a wide range of frequency. In order to take advantages of the wide range frequency characteristics, we employed not PZT, but piezo-electronic copolymer film, P(VDF/TrFE) film, with various thickness ranging from 20 to 100 micrometers as photo-acoustic transducers.

Because blood vessels play a key role in homeostasis, we experimentally demonstrated blood vessels phantom, ex vivo and in vivo rabbit studies using both Q-switched Nd:YAG laser and Ti:sapphire nanosecond laser pulses through optical fiber transmission. The detected photo-acoustic waveforms showed distinctive time-of-flight signals. The photo-acoustic signals were sensitive to temperature, absorption coefficients of chromophores, and diameters of the phantom vessels and rabbit actual blood vessels. Hemoglobin oxygen saturation could be easily derived from the multi wavelength photo-acoustic signals using differential optical absorption characteristics. These results proved the functional quantitative photo-acoustic imaging using the signal characteristics. A multivariate photo-acoustic imaging approach must be promising to convenient diagnosis. This study was partially supported by the Japan Science Technology Agency, A-STEP (No. 2111032).
7899-109, Poster Session

**Statistical weighting of model-based optoacoustic reconstruction for minimizing artifacts caused by strong acoustic mismatch**

L. D. B. Xosé, D. Razansky, V. Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

A modified iterative inversion algorithm is presented that minimizes the effects of internal acoustic reflections or scattering in tomographic optoacoustic images. The inversion procedure in our model-based algorithm consists in solving a linear system of equations in which each individual equation corresponds to a given position of the acoustic transducer and to a given time instant. Thus, the modification that we propose in this work consists in weighting each equation of the linear system with the probability that the measured wave is not distorted by reflection or scattering phenomena. We show that the probability that a reflected or scattered wave is detected at a given position and at a given instant is approximately proportional to the size of the area in which the original wave could have been generated, which is dependent on the position of the transducer and on the time instant, so that such probability can be used to weight each equation of the linear system. Thereby, the contribution of the waves that propagate directly to the transducer to the reconstructed images is emphasized. We experimentally tested the proposed inversion algorithm with tissue-mimicking agar phantoms in which air-gaps are included to cause reflections of the acoustic waves. The tomographic reconstructions obtained with the modification proposed herein show a clear reduction of the artefacts due to these acoustic phenomena with respect to the reconstructions yielded by the original algorithm. This performance is directly related to in-vivo small animal imaging applications involving imaging in the presence of bones, lungs, and other highly mismatched organs.

7899-110, Poster Session

**Ultrasonic attenuation of biomaterials for compensation in photoacoustic imaging**

J. Bauer-Marschallinger, T. Berer, H. Grün, H. Roitner, B. Reitinger, P. Burgholzer, RECENDT GmbH (Austria)

Attenuation of ultrasonic signals (including photoacoustic signals which are broadband ultrasonic signals excited by a short laser pulse) limits the quality and resolution of ultrasonic imaging, especially for large penetration depths. We investigated how attenuation influences image reconstruction in photoacoustic tomography (PAT) using integrating detectors and proposed possible compensation methods. For compensation it is important to know how attenuation evolves with frequency. Thus, the aim of this study is to determine the acoustic attenuation and its frequency behaviour of biological samples (subcutaneous porcine fat tissue, human blood and olive oil) in order to improve reconstruction algorithms for PAT.

For this purpose broadband high-frequency single transmission measurements were performed. The signals were frequency analysed and compared to reference measurements in distilled water. Unfocused high frequency piezoelectric transducers were used to detect and emit the ultrasound. Additionally, laser generated ultrasound, which provides more intensity and signals with higher bandwidth, was used for attenuation measurements.

We found several studies concerned with attenuation of fat tissue, but none of those used the single transmission approach, which turned out to be very reliable. Our results for olive oil show very good agreement with values found in literature. Many studies show linear frequency increase of attenuation of fat tissue. However, we observed significant non-linear frequency behaviour of fat tissue, e.g. a power-law exponent of 1.48 at room temperature. We show that image reconstruction in PAT is enhanced taking frequency dependent attenuation into account.

7899-111, Poster Session

**Analysis and verification of dominant factor to obtain the high resolution photo-acoustic imaging**

T. Hirasawa, M. Ishihara, M. Kitagaki, I. Bansaku, National Defense Medical College (Japan); M. Fujita, National Defense Medical College Research Institute (Japan); M. Kikuchi, National Defense Medical College (Japan)

Our goal is to develop the photo-acoustic imaging system which offers functional image of the living tissues and organs with high resolution. In order to obtain high resolution image, we implemented the Fourier-domain reconstruction method which determines an optical absorption distribution using an ideal sensor with infinite scanning area, however, the resolution of reconstructed image was restricted by the sensor directionality and finite scanning range. Therefore, there was an essential requirement to find the optimum condition.

In this research, we demonstrated the relationship between image accuracy and experimental condition. In our experimental system, the photo-acoustic signals are acquired by line scanning of our fabricated P(VDF-TrFE) film sensor. We simulated the effect of sensor directionality and scanning range. As a result, the sensor directionality strongly affected the lateral resolution and limited scanning range caused blurring especially in deep region. The results of the arteriolar phantom experiments using various sensors and scan ranges were coincident with the simulation.

The optimums of sensor and scanning range depend on the image region due to some trade-offs, for example, a sensor with wider directionality has less sensitivity, wider scanning range increases an acquisition time. Therefore, the comparison of experimental and simulation results could indicate the possibility of optimizing sensor directionality and scanning range for various depths and volumes of imaging region.

This work was partially supported by the Japan Science and Technology Agency A-STEP, (Feasibility studies stage No. 211032)

7899-112, Poster Session

**Generation-2 optical-resolution photoacoustic microscopy with improved sensitivity and scanning speed**

K. Maslov, S. Hu, L. V. Wang, Washington Univ. in St. Louis (United States)

We have developed a second-generation optical-resolution photoacoustic microscopy (OR-PAM) system with fiber-based optical delivery, steering-mirror based optical scanning, large acoustic and optical confocal numerical apertures, and improved ultrasonic detection. For optical delivery, the free-space configuration in our previously developed OR-PAM (first-generation OR-PAM) was replaced by single-mode fiber optics to enable mechanical scanning of the imaging head instead of experimental animals, thereby reducing possible motion artifacts and allowing higher scanning speeds (3 times faster than the 1st-generation OR-PAM). For ultrasonic detection, a rhomboid prism was paired with a right-angle prism diagonally, with a thin layer of silicone oil in between, to align the optical irradiation and the ultrasonic detection coaxially and confocally. Longitudinal photoacoustic waves are transformed to shear waves due to the reflection by the silicone oil. The inclined surface of the newly added rhomboid prism is able to transform the shear waves back to longitudinal waves, which are more detectable to the ultrasonic transducer. As a result, the sensitivity of the new system was improved by ~18 dB, in comparison with the 1st-generation OR-PAM. With optical scanning, OR-PAM can contiguously image a 100×100 m² area with video rate, holding the potential to monitor transient hemodynamics. The new system has been successfully used for in vivo microvasculature imaging of the mouse ear, mouse brain (through an intact skull), and human cuticle.
7899-113, Poster Session

**Does the efficiency of ultrasound-modulated fluorescence depend on the size of fluorophores?**

Y. Liu, B. Yuan, The Catholic Univ. of America (United States)

Studies from our group and others have shown that ultrasound-modulated fluorescence (UMF) is experimentally detectable. However, the modulation mechanisms are unclear and the modulation efficiencies from different reports are contradictory. Kobayashi et al reported UMF signals from a fluorescent microsphere solution. A quadratic relationship between the strength of UMF and the strength of the ultrasound pressure was claimed. However, our recent experimental studies showed a linear relationship between the two strengths from a tube filled with either Rhodamine B aqueous solution or Alexa Fluor 546 solution mixed with microparticles. To investigate this contradiction, we attempt to understand whether the sizes of fluorophores affect the modulation efficiency. This is motivated by the fact that the sizes of fluorophores used previously (such as Rhodamine B and Alexa Fluor 546) are much smaller than that of fluorescent microspheres used by Kobayashi's group.

In this study, we observed UMF from three types of fluorophores with different diameters: (1) Alex Fluor 647 (~1 nanometer), (2) CdSe/ZnS core-shell nanocrystals (~7 nanometers), and (3) fluorescent microsphere solutions (0.02, 0.04, 0.2, and 1 micrometers). A broadband lock-in amplifier was used to measure UMF signal strength and a narrowband amplifier was used for detecting the DC signal strength of the fluorescence emission simultaneously. After calibration, the ratio of the measured UMF strength to the DC strength is defined as the modulation efficiency of UMF signal, which is evaluated as a function of the fluorophore size and fluorophore concentration. The detailed results will be presented during the conference.

7899-114, Poster Session

**Combined ultrasonic and photoacoustic system for deep tissue imaging**

C. Kim, Washington Univ. in St. Louis (United States); T. N. Erpelding, L. Jankovic, Philips Research North America (United States); L. V. Wang, Washington Univ. in St. Louis (United States)

A combined ultrasonic and photoacoustic imaging system is presented that is capable of deep tissue imaging. The system consists of a modified clinical ultrasound array system and tunable dye laser pumped by a Nd:YAG laser. The system is designed for noninvasive detection of sentinel lymph nodes and guidance of needle biopsies for axillary lymph node staging in breast cancer patients. Using a fraction of the American National Standards Institute (ANSI) safety limit, photoacoustic imaging of methylene blue achieved penetration depths of greater than 5 cm in chicken breast tissue. Photoacoustic imaging sensitivity was measured by varying the concentration of methylene blue dye placed at a depth of 3 cm within surrounding chicken breast tissue. Signal-to-noise ratio, noise equivalent sensitivity, and axial spatial resolution have been quantified versus depth based on in vivo and chicken breast tissue experiments. The system has been demonstrated in vivo for detecting sentinel lymph nodes in rats following intradermal injection of methylene blue. These results highlight the clinical potential of photoacoustic image-guided identification and needle biopsy of sentinel lymph nodes for axillary staging in breast cancer patients.

7899-115, Poster Session

**Forward model of thermally-induced acoustic signal specific to intra-lumenal detection geometry**

S. Mukherjee, C. F. Bunting, D. Piao, Oklahoma State Univ.

This work investigates the exact forward model associated with intra-lumenal detection of acoustic signal originated from transient thermal-expansion of the tissue. The work is specific to intra-lumenal thermo-acoustic tomography (TAT) which detects the contrast in dielectric properties of tissue with ultrasonic resolution, but it can also be applied to intra-lumenal photo-acoustic tomography (PAT) which detects the contrast in light extinction of tissue with ultrasound resolution. Exact closed-form frequency-domain or time-domain forward model of thermally-induced acoustic signal have been studied rigorously for planar geometry and two other geometries, including cylindrical and spherical geometries, that are specific to external-imaging, i.e. breast or brain imaging using an externally-deployed applicator. This work extends the existing works to the specific geometry of internal or intra-lumenal imaging, i.e., prostate imaging by an endo-rectally deployed applicator. In this intra-lumenal imaging geometry, both the source that excites the transient thermal-expansion of the tissue and the acoustic transducer that acquires the thermally-induced acoustic signal are assumed on the surface of a long cylindrical lumen that is enclosed by the tissue. The Green's function of the frequency-domain thermo-acoustic equation in spherical coordinates is expanded to cylindrical coordinates associated with intra-lumenal geometry. Inverse Fourier transform is then applied to obtain a time-domain solution. By further applying the boundary condition to the “convex” applicator-tissue interface, an exact forward solution is obtained that leads toward accurate reconstruction for intra-lumenal thermally-induced acoustic imaging.

7899-116, Poster Session

**Multiscale three-dimensional photoacoustic imaging of reporter gene expression in vivo**

L. Li, A. Krumholz, Z. Guo, X. Cai, Washington Univ. in St. Louis (United States); T. N. Erpelding, L. Jankovic, Philips Research North America (United States); L. V. Wang, Washington Univ. in St. Louis (United States)

In this work, we demonstrated several important technical merits of photoacoustic molecular imaging. Following the application of a colorimetric assay, the in vivo expression of the lacZ reporter gene was imaged using an optical-resolution photoacoustic microscope, an acoustic-resolution photoacoustic microscope, and a commercial-array based photoacoustic imaging system. We showed photoacoustic imaging can 1) visualize specific molecular events in three dimensions at various depths with scalable resolution; 2) detect the expression of lacZ gene deeper than 5 cm inside biological tissue; 3) simultaneously quantify related functional information.

7899-117, Poster Session

**Photoacoustic and thermoacoustic tomography of dog prostates**

H. Ke, Z. Guo, Washington Univ. in St Louis (United States); T. N. Erpelding, L. Jankovic, Philips Research North America (United States); R. L. Grubb III, Washington Univ. School of Medicine (United States); L. V. Wang, Washington Univ. in St Louis (United States)

We developed a tri-modal system combining photoacoustic (PA) tomography, thermoacoustic (TA) tomography, and ultrasound (US) imaging. Acquired images of an excised dog prostate were compared to histology results. All three modalities can image distinct features. Features like the urethra were shown in both TA and US images, but TA gave a higher contrast-to-noise ratio. Fibrous tissue was more clearly imaged by TA, while the duct structure was better shown in PA images. These experimental results demonstrate the potential advantages of our tri-modal imaging system.
7899-118, Poster Session

**Real time optical resolution photoacoustic microscopy using fiber-laser technology**

W. Shi, S. M. Kerr, R. J. Zemp, Univ. of Alberta (Canada)

Optical-resolution photoacoustic microscopy (OR-PAM) is an emerging technology providing visualization of superficial structures in vivo with optical-absorption contrast. High resolution is possible as the lateral spatial resolution is determined by the optical spot size rather than acoustic detection. The imaging speed is dictated by both the beam scanning speed and the laser pulse repetition rate. We are developing a realtime OR-PAM system that uses a high repetition rate pulsed laser and high speed XY mirror galvanometers. We have demonstrated OR-PAM imaging by employing a diode-pumped pulsed Ytterbium fiber laser with a pulse repetition rate ranging from 20 Hz - 600 kHz, second harmonic generation at a wavelength of 532 nm and average output power up to 13 W. In our study, we utilized 0.08-μJ 1–ns pulses. A photoacoustic probe consisting of a 45-degree glass prism in an optical index-matching fluid is used to transmit the focused output of the laser to the sample and also to reflect exiting photoacoustic signals to an ultrasound transducer. Phantom studies with a ~7.5-µm carbon fiber demonstrate the ability to image with ~9-µm optical lateral spatial resolution. Combined with a fast-scanning mirror oscillating at 600 (B-scan) lines per second, we demonstrate a system capable of C-scan imaging at 5 frames per second. Realtime or near-realtime frame-rates will be possible in the near future, which should permit clinical applications.

7899-119, Poster Session

**Photoacoustic imaging to guide needle injections**

J. L. Su, A. B. Karpionuk, Y. Chen, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Metal needles are commonly used for drug delivery or biopsy collection in clinical settings. The ability to visualize both anatomical surrounding structures and the advancing needle is required. Needle deflection and deformation can occur when inserting needles into soft, non-homogeneous tissues which can affect the location accuracy of insertion. Ultrasound is commonly used for image-guidance of needles; however, specular reflections from the metal surface can deflect the acoustic beam away from the transducer when the needle is even slightly angled from the US transducer thereby rendering the needle invisible in the image. Photoacoustic imaging has been proposed for guidance of metal needles and other metal objects in-vivo. The high optical absorption coefficient of stainless steel can provide high photoacoustic imaging contrast. The photoacoustic signal is produced omni-directionally from the metal surface allowing for greater detection of needles at increasing injection angles compared to ultrasound imaging. Needles (21G size) were inserted into excised tissue and imaged using a 7.5 MHz ultrasound array transducer and a pulsed 800 nm laser. The results showed that at a shallow 10° insertion angle, the photoacoustic signal-to-background from the needle was four-times higher compared to ultrasound. The use of various needle coatings can further enhance photoacoustic imaging. For example, metal coated with gold nanorods were shown to improve photoacoustic signal intensity by 500% over bare metal photoacoustic imaging. Additional metal coatings were also explored to provide photoacoustic signal enhancement. Photoacoustic imaging provides sufficient depth penetration for this application and offers excellent image contrast.

7899-122, Poster Session

**Effects of calibration factors and intensity dependent non-linearity on functional photoacoustic microscopy**

A. Danielli, Washington Univ. in St. Louis (United States); J. Yao, A. Krumholz, L. V. Wang, Washington Univ. in St Louis (United States)

Functional photoacoustic microscopy is a valuable tool in quantifying hemoglobin oxygenation within single vessels. In several functional studies with this tool, quantitative sO2 measurements were taken both in vitro and in vivo. Although in vitro measurements of sO2 showed high agreement with expected values from premade samples, in practice, in vivo measurements were less accurate. The reported values of 70%-100% sO2 in the artery present large deviations from the expected range of 95-100%. Several factors, such as fluence wavelength dependence (Maslov et al., 2007), optical wavelength range, and transducer central frequency (Sivaramakrishnan et al., 2007) have been suggested and investigated in order to understand these discrepancies. Despite additional knowledge of systematic errors arising from such factors, measuring the absolute value of sO2 in vivo remains a challenge. All previous studies assumed linear dependence of the photoacoustic signal on absorption and used the linear least squares model. However, several factors, such as wavelength calibration errors, photodiode-wavelength dependence, data averaging methods, and intensity dependent non-linearity, all of which may have a significant effect on the final calculation, have not been investigated. Here we evaluate both in vitro and in vivo the effects on sO2 measurements of photodiode wavelength dependence, laser wavelength accuracy, and intensity dependent absorption of oxygenated and deoxygenated hemoglobin. We show that these factors may contribute significantly to the deviations in sO2 calculations in vivo.
Blind spectral unmixing identifies the molecular signatures of absorbers in multispectral optoacoustic tomography

N. C. Deliolanis, J. Glatz, A. Buehler, D. Razansky, V. Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

Deep tissue molecular imaging of contrast agents at realistic concentrations with photoacoustic (or optoacoustic) tomography remains a challenge. Detection of optical absorption contrast agents (nanoparticles, fluorochromes ...) has been demonstrated by utilizing multispectral measurements, however spectral unmixing heavily depends on the accurate knowledge of the spectral profile of the absorbers. It becomes more challenging when considering that reconstruction algorithms might not be quantitative and also that the spectral absorption profile is convolved with the unknown illumination spectrum. We propose a blind spectral unmixing method that recovers both the spatial distribution of the contrast agents and their absorption spectrum and demonstrate it in whole body mouse imaging.

We have employed various blind source separation algorithms, among them the Principal and the Independent Component Analysis (PCA and ICA). PCA produces an ordered set of orthogonal spectral absorption components, however, different absorber profiles may not be orthogonal, but rather independent. This condition is cured by ICA and the accurate distribution and absorption components can be retrieved. However, when the number of spectra is high, ICA algorithm slows down, and absorption components are forced to break down into similar ones. The combined use of PCA and ICA, helps to preselect a subset of the most important components, reduces the noise, and increases the unmixing accuracy and the sensitivity. We demonstrate the superior performance of these methods in whole body mouse imaging by reconstructing the spatial distribution and the absorption spectrum of multiple optical contrast agents at concentrations that correspond to changes in absorption coefficient bellow the average tissue absorption.

Isolation of circulating tumor cells using photoacoustic flowmetry and two phase flow

C. M. O'Brien, K. Rood, S. Gupta, J. Mosley, M. Schmidt, M. Uptegrove, N. Sharma, J. A. Viator, Univ. of Missouri-Columbia (United States)

Photoacoustic flowmetry (PAF) is a method to detect pathological analytes in fluid media. PAF is similar to conventional flow cytometry, though rather than detecting weak fluorescent signals from tagged cells, robust photoacoustic responses are generated in targeted particles. The nature of the photoacoustic response in optical absorbers, such as circulating melanoma cells in blood, allow for detection of small numbers of such analytes. This advantage makes PAF ideal for detection and quantification of circulating tumor cells (CTCs). By detecting pigmented melanoma cells, we have developed an in vitro test for detection of early metastatic disease.

While detection of these cells has diagnostic importance, isolation and capture of the metastatic cell may provide additional information about the patient's disease state as well as allow basic studies of the metastatic process. Captured CTCs can be studied for evidence of epithelial-mesenchymal transition that is postulated as responsible for cancer metastasis.

In order to isolate these cells we have induced a two phase flow in the blood samples composed of alternating slugs of blood cells suspended in saline and slugs of air. By irradiation successions of slugs containing blood cells, we detect melanoma cells by generating photoacoustic events. Slugs found to be positive for melanoma cells were directed to a collection cuvette for enrichment and further study. Our apparatus was used to detect and isolate single melanoma cells spiked in human blood samples.

On the role of passive elements in photoacoustic reconstruction

C. H. Slump, R. G. Willemink, S. Manohar, Univ. Twente (Netherlands)

Not surprising, prior to the reconstruction of a photo acoustic object of interest, a proper calibration and reference measurement has to be performed. For this purpose a specially designed calibration phantom is designed for obtaining the parameters describing the geometry and the gain and offset of the ultrasound detectors. We consider a cylindrical configuration for the experimental setup with a slowly rotating object and fixed positions for laser and ultrasound detectors. To our surprise, in the reconstruction process of the photo acoustic experiments, it was observed that adding a passive element to the experimental setup, greatly improves the quality of the reconstruction of the object. The passive elements become ultrasound point sources when being illuminated with pulsed optical energy. This proposal for a poster contribution analyzes this effect.

We start from an artificial and theoretically constructed optical absorption distribution that does not radiate when interrogated by the optical pulse. We show that the addition in the experimental setup of one or more passive elements to this example, leads to extra interference resolving the interference of the optically induced ultrasound waves. We will derive the relation between the number of passive elements and the number of required projections for the reconstruction in an analytical model study first. After that we will show results from pertinent experiments, with a variable number of passive elements and projections.

The reported investigation is a part of a larger study on the existence, uniqueness and stability of photoacoustic inverse source reconstructions for the biomedical application domain.
Optical droplet vaporization of micron-sized perfluorocarbon droplets and their photoacoustic detection

E. Strohm, Ryerson Univ. (Canada); I. Gorelikov, M. Matsuura, Sunnybrook Health Sciences Ctr. (Canada); M. C. Kolios, Ryerson Univ. (Canada)

Liquid perfluorocarbon (PFC) droplets are currently being investigated as an ultrasound contrast agent (UCA) and a vehicle for targeted drug delivery. Vaporization of PFC droplets using large acoustic pressures has been previously demonstrated. Upon vaporization, these droplets can act as UCAs or drug delivery vehicles. Our research proposes using light instead of ultrasound using a technique we term optical droplet vaporization (ODV). Our aim is to develop PFC droplets containing optical absorbing materials that can be selectively vaporized for use in ultrasound/photoacoustic imaging and cancer therapy.

PFC droplets were developed with and without optical absorbing materials (gold nanorods) as activating agents. A scanning photoacoustic/ultrasound microscope with 200, 400 and 1200 MHz transducers was used to image and characterize 200 nm diameter and micron-sized PFC droplets before and after vaporization. A 1064 nm laser with a fluence of up to 3 J/cm2 and 4 µm spot size was used to selectively vaporize individual droplets under optical guidance.

Immediately after laser irradiation of the emulsion, bubble formation was observed. The bubble expanded 10-20 times the original size within minutes. Pulse-echo ultrasound measurements after vaporization showed an increase in the acoustic backscatter, impedance and attenuation, indicating a liquid to gas phase change. The photoacoustic signal increased with increasing laser fluence, until vaporization or droplet rupture was observed. After vaporization, the photoacoustic signal dropped abruptly.

This research demonstrates a method to selectively vaporize or rupture liquid PFC droplets via laser irradiation. A comparison to ultrasound scattering and photoacoustic signal generation theory will be presented.

Identification of radiolucent foreign bodies in tissue using optoacoustic spectroscopic imaging

L. Page, R. D. Glickman, S. M. Maswadi, The Univ. of Texas Health Science Ctr. at San Antonio (United States)

One of the leading causes of medical malpractice claims in emergency medicine is the misdiagnosis of the presence of foreign bodies. Radiolucent foreign bodies are especially difficult to differentiate from surrounding soft tissue, gas, and bone using existing clinical imaging modalities. Because many radiolucent foreign bodies have sufficient contrast for imaging in the optical domain, we are exploring the use of laser-induced optoacoustic imaging for the detection of foreign bodies, especially in orbital and craniofacial injuries, in which the foreign bodies are likely to lie within the penetration depth of visible and near infrared wavelengths. In order to evaluate the performance of optoacoustic imaging for clinical detection and characterization, common foreign bodies have been scanned over a range of visible and near infrared wavelengths to obtain the spectroscopic properties of the materials commonly associated with these foreign bodies. The foreign bodies are also being embedded in realistic ex vivo tissue phantom samples to evaluate the changes that may occur in the spectroscopic absorption of the materials due to the interaction with tissue absorbers. Another potential complication for the clinical application of this technique is the effects of blood flow in imaged tissue. In order to evaluate this factor, tissue phantoms incorporating dynamic flow will be used to study the effects of blood flow and blood volume on the observed optoacoustic spectroscopic properties of the foreign bodies. Ultimately, we anticipate that spectroscopic characterization will help identify specific wavelengths to be used for imaging foreign bodies that will provide useful diagnostic data about the material properties of the object, thereby enabling the characterization, as well as the location, of the objects. This information will aid the clinician in choosing the optimal treatment course for the patient.

Label-free detection of melanoma metastasis in resected human lymph node using photoacoustic imaging

J. Jose, Univ. Twente (Netherlands); T. Ruers, The Netherlands Cancer Institute (Netherlands) and Univ. Twente (Netherlands); T. G. van Leeuwen, Univ. Twente (Netherlands) and Univ. van Amsterdam (Netherlands); S. Manohar, Univ. Twente (Netherlands)

Melanoma metastasis has become a major public health problem in many continents, especially where fair-skinned people receive excessive sun exposure. Modern staging of cutaneous melanoma includes the biopsy of one or more sentinel lymph node (SNLs) to determine if tumor cells have traveled to the adjacent lymph bed. Typically, after dissection all nodes are stained with hematoxylin and eosin as well as with additional immunohistochemical staining before histological examination. Results become available in 4-5 days and in case of a positive sentinel node, a node which shows metastatic disease, a second surgical procedure is necessary. During this lymphadenectomy, dissection of the entire adjoining lymph node basin is performed, resulting in numerous postoperative problems, such as lymphedema, pain, impaired joint mobility and limp weakness. As a result of this two step procedure additional patient discomfort, costs, morbidity and organizational distress occur.

Here we present the feasibility study of ex vivo photoacoustic lymph node scanning, which has potential to address the shortcomings of contemporary histopathological examinations. Most importantly, in the future when this approach is made available in the clinic, can provide a fast and accurate visualization of melanoma metastasis to the lymph nodes. The method relies on using multiple wavelengths to detect and reconstruct photoacoustic signals from the intrinsic absorption of melanoma cells from within the resected nodes. The imaging system is built around a 32 element curvilinear detector. The resected node is placed stationary on a custom made holder in the imaging tank filled with water. The detector is coupled to the rotary mechanism which allows a computed tomographic measurement with top illumination. A single slice scanning takes less than 1 minute and a modified acoustic backprojection algorithm is used to reconstruct the images. We present our initial results on resected human lymph node which compare with the conventional histopathological examinations.
Signal, and thus lead to degradation of the quality of the reconstructed photoacoustic image. Additionally, the acoustic properties of the tissue depend on the disease state. So simultaneous measurement of acoustic properties with photoacoustic imaging can not only improve disease diagnosis but also the image quality by incorporating position correction in the backprojected signal.

Here we present a system which allows simultaneous imaging of optical absorption properties and acoustic transmission properties of an object in a two-dimensional slice using a carefully positioned ‘Passive’ element in a photoacoustic imager. The passive element is placed at the far end of the sample and it acts as an ultrasound source. The generated ultrasound interacts with the sample and can be measured using the same ultrasound detector as used for photoacoustic measurements. Such measurements are made at various angles around the sample in a computerized tomography approach. Images of the ultrasound propagation parameters, attenuation and speed of sound, can be reconstructed by inversion of a measurement model. We will present detailed description of our system, effect of spatial distribution of SOS in photoacoustic imaging, followed by an iterative reconstruction algorithm which compensates for the SOS inhomogeneities in the imaging area. We have validated the method using appropriate phantoms and on a living mouse which has tumor implanted on the lower abdomen. The obtained images are quantitative in terms of the shape, size, location, and acoustic properties of the examined heterogeneities.

7899-131, Poster Session
Monitoring of HIFU thermal damage using integrated photoacoustic imaging and high intensity focused ultrasound technique
H. Cui, X. Yang, The Univ. of Kansas (United States)

In this study, we applied an integrated photoacoustic imaging (PAI) and high intensity focused ultrasound (HIFU) system to noninvasively monitor the thermal damage due to HIFU ablation ex vivo and in vivo. A single-element, spherically focused ultrasonic transducer, with a central frequency of 5MHz, was used to generate a HIFU area in soft tissue. Photoacoustic signals were detected by the same ultrasonic transducer before and after HIFU treatments using different wavelengths. The feasibility of combined contrast imaging and treatment of solid tumor in vivo by the integrated PAI and HIFU system was also studied. Gold nanorods were used to enhance PAI during the imaging of a CT26 tumor, which was subcutaneously inoculated on the hip of a BALB/c mouse. Subsequently, the CT26 tumor was ablated by HIFU with the guidance of photoacoustic images. Our results suggested that the tumor was clearly visible on photoacoustic images after the injection of gold nanorods and was ablated by HIFU. In conclusion, PAI may potentially be used for monitoring HIFU thermal lesions with possible diagnosis and treatment of solid tumors.

7899-132, Poster Session
Interlaced realtime channel-domain photoacoustic and ultrasound imaging
T. Harrison, J. C. Ranasinghesagara, R. J. Zemp, Univ. of Alberta (Canada)

Photoacoustic imaging offers a new and complementary contrast mechanism to the traditional structural contrast of ultrasound. While the combination of these two modes has been demonstrated in the past with single-element transducers, array transducers offer clear advantages in both modes by eliminating mechanical scanning and allowing image formation from a single excitation. Given the abundance of commercially available ultrasound systems, it is desirable to use them as much as possible. However, these systems only often allow access to beamformed RF data. We discuss the applicability of ultrasound beamformers for photoacoustic imaging, and find that with only software-defined control over the speed of sound, walking aperture reconstruction is optimally performed using a speed correction factor of 1.414. When sector-scanning is used, a different strategy is required. We also demonstrate a new photoacoustic-ultrasound imaging system based on a Verasonics ultrasound array system. The system streams raw channel data to a 6-core PC at up to 1.4GB/s via PCI-Express, allowing interfaced ultrasound and photoacoustic data to be acquired and reconstructed at realtime rates. Using an L7-4 linear array transducer, we demonstrate the performance of this system and discuss potential applications. The system should provide new opportunities for clinical and pre-clinical imaging.
Motivate development of a combined PAI-DOT system.

The optical properties of the three targets. These proof-of-principal results demonstrate that the PA image can be corrected to accurately quantify these objects have different optical properties. DOT reconstructions of optical properties. A raw PA image of the three targets suggests that xenograft and adjacent normal tissue in the murine model.

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for different types of transducers to speed up the imaging process. To further speed up the imaging process, the multi-threads technique is implemented to form previous frame data image and acquire current frame data simultaneously.

Initial in vivo experiments were performed using animal tumor models to map out tumor vasculature and tumor hypoxia, which were superimposed on co-registered US images. The real-time system allows capturing co-registered US/PAI images; which are captured almost instantaneously, and free of motion and delay alterations; and also provides dynamic information when contrast agents are used.

7899-139, Poster Session

Optimising the illumination geometry of a clinical reflection mode optoacoustic scanner

D. C. Birtill, M. Jaeger, A. G. Gertsch, J. C. Bamber, The Institute of Cancer Research (United Kingdom)

Clinical optoacoustic imaging relies on the ability to illuminate objects at depth. To achieve this most effectively it is important to optimise the illumination geometry of the optoacoustic transducer. This is a 7.5 MHz linear array ultrasound transducer with two fibre optic linear arrays mounted on either side. These are connected to a Digital Phased Array System (DPhAS Fraunhofer IBMT www.ibmt.fraunhofer.de/fgn/images/Produktblatt_DPhAS_en_tcm296-136589.pdf) and used to create maps of the optical absorbers in the imaging plane. The aim was to find the optimum angle of the fibre optics relative to the transducer. This angle influences the three-dimensional point spread function (3D PSF) peak intensity and the full width half maximum.

The 3D PSF was produced by moving the optoacoustic transducer in the elevational direction over a graphite rod tip using a motorised gantry. The rod tip, 2 cm below the transducer surface, was in 1/8 intra-lipid solution. The angle of the fibre bundles relative to the scanning plane was varied. Many datasets per angle were produced, and after image processing the dimensions of the PSF were shown to vary with the angle. The reconstructed image pixel value within the PSF was then plotted against the illumination angle.

The geometry ultimately chosen was that which maximised the peak intensity, as it is this which limits optoacoustics at depth. This illumination angle was found to be 75 degrees, whilst the shape of the 3D PSF was found to be dominated by the acoustic receive parameters, and the peak intensity was dominated by the illumination geometry.

7899-140, Poster Session

Limited data image reconstruction in optoacoustic tomography by constrained, total variation minimization

K. Wang, Illinois Institute of Technology (United States); E. Y. Sidky, The Univ. of Chicago (United States); M. A. Anastasio, Illinois Institute of Technology (United States); A. A. Oraevsky, TomoWave Labs., Inc. (United States); X. Pan, The Univ. of Chicago (United States)

Optoacoustic Tomography (OAT) is an emerging hybrid imaging technique with great potential for a wide range of biomedical imaging applications. Assuming point-like transducers, mathematically exact algorithms are available for image reconstruction, but they are applicable only when the measured data are densely sampled on an aperture that encloses the object. In many cases of practical interests, however, measurements may be limited in number and are acquired on an incomplete aperture, which may result in conspicuous image distortion and artifacts. Total variation (TV) minimization has been proved to be a powerful tool for limited-data reconstruction. However, most previous studies of limited-data OAT were based on an approximate imaging model that assumed point-like transducers, which limits the improvements on the reconstructed OAT image quality. In this work, we develop and investigate an iterative reconstruction algorithm incorporating ultrasonic transducer properties for limited-data OAT. The algorithm is based on the fact that the value of the image TV subject to the data consistency condition, and is conceptually and mathematically distinct from classic iterative reconstruction algorithms. Both computer-simulation and experimental studies are conducted to investigate the proposed algorithm. These studies reveal that the constrained, total variation minimization algorithm can yield accurate reconstructions in many limited-data applications where classic iterative algorithms do not perform well.

7899-141, Poster Session

Thermotherapy with a photoacoustic/ultrasound dual-modality agent

Y. Wang, A. Liao, National Taiwan Univ. (Taiwan); J. Chen, Y. Lee, C. C. Wang, National Chung Cheng Univ. (Taiwan); P. Li, National Taiwan Univ. (Taiwan)

A microbubble-based imaging/therapeutic agent is introduced. Specifically, gold nanoparticles (AuNRs) are encapsulated in microbubbles (MBs) for both ultrasound (US) imaging and laser-induced thermotherapy (LIT). In addition, this agent, AuNR-MBs, takes albumin microbubbles as a carrier and includes the AuNRs maintains its original absorption peak at around 760 nm. AuNR-MBs in different sizes are synthesized. Imaging is first performed to evaluate its feasibility. The enhanced PA and US signals in polyacrylamide gel for in vitro study are measured. The PA spectroscopy is then performed and the results generally agree with the measured optical absorption although its peak is slightly broadened and shifted possibly due to mixing. In phantoms, the contrast is 1.531, 2.447, 2.085, 1.994, 0.768, and 0.573 at wavelength of 720, 760, 800, 860, 900, and 940 nm respectively. Finally, the application of the new agent to LIT is presented. A continuous wave laser at 800 nm is used to heat the samples with the power at 1W. The photoacoustic (PA) intensity in the region of interest (ROI) is increased by an average of 5.2dB. The increased signal level implies that the temperature in the ROI can be increased by 44.3oC in aqueous filled setup. Furthermore, the dual-modality agent has the potential to be used in HIFU therapy, drug delivery and loading of DNA for gene transfer.

7899-142, Poster Session

Photoacoustic imaging to detect rat brain activation after cocaine hydrochloride injection

J. Jo, X. Yang, The Univ. of Kansas (United States)

Photoacoustic imaging (PAI) was employed to detect brain activation after the administration of cocaine hydrochloride. Sprague Dawley rats were injected with different concentrations (0, 2.5 and 5.0 mg per kg body) of cocaine hydrochloride in saline solution through tail veins. The brain functional response to the injection was monitored by photoacoustic tomography (PAT) system with horizontal scanning of cerebral cortex of rat brain. Photoacoustic microscopy (PAM) was also used for coronal view image. The modified PAT system used multiple ultrasonic detectors to reduce the scanning time and maintain a good signal-to-noise ratio (SNR). The measured photoacoustic signal changes confirmed that cocaine hydrochloride injection excited high blood volume in brain. This result shows PAI can be used to monitor drug abuse-induced brain activation.
Monte Carlo simulation study of light modulated by acoustic radiation force in elasto-viscous medium

R. Li, D. S. Elson, C. W. Dunsby, M. Tang, Imperial College London (United Kingdom)

A current problem with Acousto-optic (AO) Imaging technique is the weak modulation signal strength. Acoustic Radiation Forces (ARF) can generate larger particle displacements within a medium in the range of several micrometers compared with displacements in the range of nanometers for pure ultrasound. However, these displacements cause a collective movement of optical scatterers within a large volume of the medium, and whether the ARF itself can enhance the AO signal is an open question. In this study we investigated how the optical signal could be affected by an oscillating acoustic radiation force induced particle displacement in an elasto-viscous medium using a Monte Carlo simulation. We modelled the interactions of an optical signal with particle displacement induced by a low-frequency oscillating acoustic radiation force in an elasto-viscous medium. The particle displacement was calculated based on the classical Navier’s equation, while varying the oscillating acoustic radiation force frequency (ARFF), the medium’s elasticity and viscosity.

We conducted a series of simulations to investigate how changes in ARFF, elasticity and viscosity affects optical modulation. For constant elasticity and viscosity, the oscillating ARFF was changed from 250 Hz to 8 kHz. The results showed a significant decrease in optical signal with increased ARFF, followed by a saturation when ARFF increased beyond 1kHz. When the elastic modulus was varied from 0.25 kPa to 25 kPa (increasing elasticity), the optical modulation decreased because of the smaller particle displacement. Likewise, when the viscosity varied from 0.2 Pa.s to 1 Pa.s, the optical modulation decreased with higher viscosity.

Selective nanoparticle-directed ablation of the canine prostate

J. A. Schwartz, Nanospectra Biosciences, Inc. (United States); R. E. Price, Baylor College of Medicine (United States); K. L. Gill-Sharp, K. L. Sang, J. D. Khorcani, J. D. Payne, Nanospectra Biosciences, Inc. (United States); B. S. Goodwin, The Univ. of Texas Health Science Ctr. at Houston (United States)

This study adopted AuroLase® Therapy, previously reported for the treatment of brain tumors, to the treatment of prostate disease by 1) using normal canine prostate in vivo, directly injected with a solution of nanoparticles as a proxy for prostate tumor and 2) developing an appropriate laser dosimetry for prostate which is sub-ablative in native prostate while simultaneously producing photothermal coagulation in prostate tissue containing therapeutic nanoshells. Healthy, mixed-breed hound dogs were given surgical laparotomies during which nanoshells were injected directly into one or both prostate hemispheres. Laser energy was delivered percutaneously to the parenchyma of the prostate along 1-5 longitudinal tracts via a liquid-cooled optical fiber catheter terminated with a 1-cm isotropic diffuser after which the incision was closed and sutured using standard surgical techniques. The photothermal lesions were permitted to resolve for up to 8 days, after which each animal was euthanized, necropsied, and the prostate taken for histopathological analysis.

We developed a laser dosimetry which is sub-ablative in native prostate and simultaneously ablative of prostate tissue containing nanoshells which would indicate a viable means of treating tumors of the prostate which are known from other studies to accumulate nanoshells. Secondly, we determined that multiple laser treatments of nanoshell-containing prostate tissue could be accomplished while sparing the urethra and prostate capsule thermal damage. Finally, we determined that the extent of damage zone radii correlate positively with nanoshell concentration, and negatively to the length of time between nanoshell injection and laser treatment.

Evaluation of optoacoustic conversion efficiency of light-absorbing films for optoacoustic transmitter applications

H. W. Baac, T. Ling, H. Park, L. J. Guo, Univ. of Michigan (United States)

Light-absorbing films have been utilized as optoacoustic transmitters for ultrasound imaging and in all optical ultrasound transducers. Optoacoustic pressure strength from a thin metal film, as a common reference, is quite limited due to poor optoacoustic conversion efficiency (OCE). Several modified film structures, such as dye-doped polymer composites and 2-D gold nanostructures, have been proposed previously to improve pressure amplitude and frequency characteristics. To design and implement more efficient optoacoustic transmitters, we experimentally evaluate the OCEs to determine the transfer characteristics from optical input to acoustic output, which have not been quantified for these film-type transmitters. For OCE evaluation, temporal waveforms of the generated ultrasound pulses should be accurately determined by minimizing measurement loss and error. However, this has been challenging due to detector's limited bandwidth and diffraction-induced signal distortion influenced by source and detector geometry. Microring detector is very suitable for optoacoustic film characterization due to its broadband response (3-dB roll off at ~90 MHz) together with small physical size. Using such small detector (< laser beam's intensity variation scale over the film), the diffraction-induced error can be minimized because the optoacoustic pressure wave is close to a plane wave in near-field range. We successfully obtained well-defined Gaussian shape ultrasound pulses, which are close to the original laser pulse but contains characteristic attenuation of each optoacoustic film. This allowed us to quantitatively compare the OCEs of 2-D gold nanostructures and chromium films with various overlying polymer layers, which provides valuable guidelines to design more efficient optoacoustic transmitters.
Photoacoustic and vector Doppler ultrasound for oxygen consumption estimation: implementation on a clinical array system

Y. Jiang, T. Harrison, J. C. Ranasinghesagara, R. J. Zemp, Univ. of Alberta (Canada)

Recently, we have developed a combined photoacoustic and high-frequency Doppler ultrasound system with a single element transducer to estimate the metabolic rate of oxygen consumption in small animal models. However, the long scanning time due to mechanical motion may be a limitation of our swept-scan system. In this work, the single element transducer was replaced by a clinical array transducer which may provide more accurate flow velocity estimations, higher frame rates, improved penetration depth, and improved depth-of-field due to dynamic focusing capabilities. We used an array system from Verasonics Inc. which enables flexible pulse-sequence programming and parallel channel data acquisition, along with a pulsed laser and optical parametric oscillator. For flow estimation, we implemented a flash-Doppler sequence which transmits ensembles of plane-wave excitations. Echo signals are beamformed and subjected to wall-filtering and Kasai flow estimation algorithms. Transmit excitations are steered at a sequence of progressive angles, which permits estimation of not only flow magnitude but also vector direction. High frame rates over a wide region can be achieved. Combined interlaced photoacoustic and Doppler imaging on flow phantoms has been performed on this system. We demonstrate the ability to image blood mimicking fluid in chicken tissue phantoms to depths of 4 cm with high signal-to-noise with both modalities. We discuss the performance of Doppler flow estimation and photoacoustic oxygen saturation estimation and their role in future work of estimating oxygen consumption.

Oxygen consumption estimation with combined color Doppler ultrasound and photoacoustic bio-microscopy: a phantom study

Y. Jiang, T. Harrison, J. C. Ranasinghesagara, R. J. Zemp, Univ. of Alberta (Canada)

Recently, we have developed a combined photoacoustic and high-frequency Doppler ultrasound system with a single element transducer to estimate the metabolic rate of oxygen consumption in small animal models. However, the long scanning time due to mechanical motion may be a limitation of our swept-scan system. In this work, the single element transducer was replaced by a clinical array transducer which may provide more accurate flow velocity estimations, higher frame rates, improved penetration depth, and improved depth-of-field due to dynamic focusing capabilities. We used an array system from Verasonics Inc. which enables flexible pulse-sequence programming and parallel channel data acquisition, along with a pulsed laser and optical parametric oscillator. For flow estimation, we implemented a flash-Doppler sequence which transmits ensembles of plane-wave excitations. Echo signals are beamformed and subjected to wall-filtering and Kasai flow estimation algorithms. Transmit excitations are steered at a sequence of progressive angles, which permits estimation of not only flow magnitude but also vector direction. High frame rates over a wide region can be achieved. Combined interlaced photoacoustic and Doppler imaging on flow phantoms has been performed on this system. We demonstrate the ability to image blood mimicking fluid in chicken tissue phantoms to depths of 4 cm with high signal-to-noise with both modalities. We discuss the performance of Doppler flow estimation and photoacoustic oxygen saturation estimation and their role in future work of estimating oxygen consumption.

Development of a hand-held 3D photoacoustic imaging system for breast cancer detection

H. A. Al-Aabed, M. B. Roumeliotis, J. J. Carson, Lawson Health Research Institute (Canada)

Breast cancer is the most common major cancer among women in many countries and a leading cause of cancer deaths in women, second only to lung cancer. Photoacoustic (PA) imaging is a non-invasive imaging modality that employs non-ionizing near infrared (NIR) laser light to obtain optical images of tissues with depth penetration and resolution comparable to ultrasound imaging. PA images are created by illuminating tissues with a short laser pulse (~10 ns), which causes optically absorbing structures to heat up slightly, but so rapidly that conditions of thermal and stress confinement are met and the structure emits a pressure wave at ultrasonic frequencies. Detection of the pressure waves at the tissue surface with an ultrasound transducer array provides the data needed to reconstruct the distribution of light-absorbing structures within the tissue. Since it is recognized that cancerous breast lesions absorb light to a greater degree than surrounding normal tissue, PA imaging is a viable candidate for detection of lesions within the intact human breast. Therefore, we have constructed a transportable PA imaging system suitable for breast imaging. The system incorporates a hand-held transducer array with 30 detector elements arranged on a ring. Laser light is delivered coaxially in relation to the ring using a fiber optic light guide. The supporting hardware includes a NIR tunable laser, transducer cable, 30 preamplifiers, 30 independent data acquisition channels with onboard memory, and a computer with control and image reconstruction software. Initial tests with the transducer array suggest that it has sufficient sensitivity to detect optically absorbent objects on the order of 1-mm at a depth of 2 cm. It is anticipated that the small hand-held PA imaging unit will be amenable to patient work-up and would complement standard ultrasound imaging.

Wavelength agile photoacoustic microscopy with a photonic crystal fiber supercontinuum source

T. Buma, M. Liu, Univ. of Delaware (United States)

Photoacoustic microscopy (PAM) provides excellent image contrast based on optical absorption. Spectroscopic PAM requires a pulsed nanosecond laser with tunable wavelength, but such lasers are expensive and have poor wavelength switching speed. We are developing a rapidly tunable system based on a high repetition rate supercontinuum source. A Q-switched Nd:YAG microchip laser produces 0.6 ns duration pulses at 1064 nm at a 6.6 kHz repetition rate. These pulses are sent through 7 meters of photonic crystal fiber, where nonlinear propagation produces a supercontinuum spectrum. Wavelength selection is achieved with a rapidly tunable monochromator, where a galvanometer controls the position of an aperture in the Fourier plane. Ten arbitrarily separated wavelengths between 575 and 1100 nm can be accessed within one second for each image pixel during data acquisition (50 x 50 pixel scan). For any given wavelength, a spectral linewidth of 40 nm was chosen to achieve sufficient pulse energy (over 15 nJ). The PAM system employs optical focusing of the excitation beam. Detection is performed with a 25 MHz spherically focused f/2 transducer. Experiments involved phantoms with thin silicone tubing (0.3 mm inner diameter) containing four dyes (Red #3, methylene blue, indocyanine green, and India ink) in various known relative concentrations. The ten excitation wavelengths were uniformly distributed between 575 and 1100 nm. Linear regression analysis of the multiwavelength images determined the relative dye concentrations to within 6% error of actual values. These promising results suggest the potential of our wavelength agile source for functional photoacoustic microscopy.
7899-151, Poster Session

Optoacoustic generation of high frequency ultrasound using a carbon nanotube polymer composite film

H. W. Baac, J. G. Ok, S. Chen, T. Ling, A. J. Hart, L. J. Guo. Univ. of Michigan (United States)

For high resolution ultrasound imaging, optoacoustic generation of ultrasound is an attractive approach to develop high frequency transmitters. For efficient transmitters, light-absorbing structures should be able to generate strong pressure waves with minimal high frequency losses. A thermoelastic polymer film, polydimethylsiloxane (PDMS), was used as an acoustic transfer medium to interface with light-absorbers. While large thermal expansion of the PDMS is effective to enhance pressure amplitude, the polymer thickness should be reduced as thin as possible to minimize acoustic attenuation. Previously, carbon black mixed with PDMS was used for light-absorption, showing significantly boosted pressure strength by ~20 dB than a chromium film. But high frequency performance was severely limited by the thick polymer layer (10–20 µm). Recently, 2-D gold nanostructures on glass substrate were proposed and demonstrated, improving high frequency output by ~5 dB over 70–100 MHz than those of carbon black-PDMS composites. However, the overall pressure strength was compromised because the optical absorption at gold nanostructure is lower than that of carbon black composite. Here, we introduce a carbon nanotube (CNT)-PDMS composite to generate strong and high frequency ultrasound. Multi-walled CNTs, as strong light-absorbers, were uniformly grown on fused silica substrate, followed by spin-coating PDMS. We could make thin composite films (1.3–8 µm) suitable for high frequency performance, controlling optical absorption (40%–99%). Pressure amplitude >25 dB stronger at 80% light absorption level, as compared to the chromium film reference. Moreover, the 25 dB enhancement persists up to 150 MHz, demonstrating highly efficient ultrasound generation.

7899-152, Poster Session

In vivo multi-scale photoacoustic microscopy of human skin

C. P. Favaza, S. Hu, V. Huang, O. W. Jassim, L. A. Cornelius, L. V. Wang, Washington Univ. in St. Louis (United States)

Scalability is a key feature of photoacoustic microscopy (PAM). Reports have shown that PAM systems can be designed to possess sub-micron resolution at shallow depths or penetrate centimeters deep at the expense of resolution. This capability to readily tune the imaging parameters while maintaining the same inherent contrast could be extremely useful for a variety of biomedical applications. Human skin, with its layered vascular structure whose dimensions scale with depth, provides an ideal imaging target to illustrate this advantage. Here, we present results from in vivo human skin imaging experiments using two different PAM systems, an approach which enables better characterization of the cutaneous microvasculature throughout the imaging depth. Specifically, we show images from several distinct areas of skin: the palm, the forearm, and the cuticle. For each region, the same area was imaged with both an optical-resolution PAM (OR-PAM) and an acoustic-resolution PAM (AR-PAM), and the subsequent images were combined into composite images. The OR-PAM provides less than 5 µm lateral resolution, capable of imaging the smallest capillary vessels, while the AR-PAM enables imaging at depths of several millimeters. Several structures are identifiable in the OR-PAM images which cannot be differentiated in AR-PAM images, namely thin epidermal and stratum corneum layers, undulations in the dermal papillae, and capillary loops. However, the AR-PAM provides images of larger vessels, deeper than the OR-PAM can penetrate. These results demonstrate how PAM’s scalability can be utilized to more fully characterize cutaneous vasculature, potentially impacting the assessment of numerous cardiovascular related and cutaneous diseases.

7899-153, Poster Session

100-MHz photoacoustic microscopy system for imaging microscale tumor biology

T. S. DeWolf, J. C. Ranasinghesagara, T. Harrison, R. J. Zemp, Univ. of Alberta (Canada)

We present a photoacoustic microscopy system based on a highly focused 100-MHz ultrasound transducer and unique light-delivery optics with spatial resolution of ~35 microns. This system addresses a gap between optical-resolution PAM (OR-PAM) systems with ultra-high resolution but limited optical penetration, and past PAM systems with resolution defined by the ultrasonic focus. Resolution was quantified by scanning 7-micron carbon fibers in Intralipid. Mechanical scanning was accomplished using a 3-axis stepper-motor system with stepping accuracy and repeatability of ~5 microns. Free-space light delivery from a pulsed Optical Parametric Oscillator to dark-field illumination optics was implemented. The system is being used to study the earliest stages of tumor angiogenesis in animal xenograft models at depths beyond those attainable by OR-PAM. Phantom studies and in vivo results will be presented.

7899-154, Poster Session

Transrectal photoacoustic imaging of prostate phantoms using capacitive micromachined ultrasound transducer array

S. Kothapalli, T. Ma, S. Vaithilingam, E. Guleyupoglu, Ö. Oralcan, B. T. Khuri-Yakub, S. S. Gambhir, Stanford Univ. (United States)

Prostate cancer is the second most common type of cancer among American men, with more than 166,000 new cases being reported every year. Standard screening methods for prostate cancer - such as blood screening for prostate specific antigen (PSA), digital rectal examination (DRE), and transrectal ultrasound (TRUS) guided prostate biopsy - have limited ability (sensitivity and specificity) in early diagnosis of prostate cancer. Photoacoustic (PA) imaging is an emerging hybrid medical imaging modality that combines optical and ultrasound imaging modalities to achieve high optical contrast imaging at ultrasonic resolution. To improve the diagnostic accuracy of transrectal ultrasound (TRUS), we developed a transrectal photoacoustic (TRPA) probe that integrates a fiber optic light guide and capacitive micromachined ultrasound transducer (CMUT) array with a center frequency of 5.5 MHz. We also developed a transrectal prostate phantom that mimics both optical and ultrasound properties of the prostate tissue. Nano-second pulsed laser light (800 nm wavelength with a 10-Hz repetition rate) was delivered to the prostate phantom using a fiber optic light guide and PA signals were detected using a 16x16 element CMUT array. B-scan images and reconstructed 3D volumetric PA images of the phantom show light absorbing objects embedded as deep as 5 cm inside the phantom, with sub-millimeter spatial resolution. Due to its low noise floor, wide fractional bandwidth, and high sensitivity, the 2-D CMUT array with integrated front-end circuits proves to be an attractive candidate for transrectal photoacoustic volumetric imaging of prostate tissue.

7899-155, Poster Session

Gold nanorods tailored as tracers for sentinel lymph node biopsy imaged by photothermal optical coherence tomography

Y. Jung, R. K. Wang, Oregon Health & Science Univ. (United States)

Sentinel lymph node (SLN) is the first lymph node to drain wastes originated from cancerous tissue. To approach clinically for sentinel lymph node biopsy, this work investigates the use of near infrared
animals were scanned prior to GNR injection and then periodically that resulted in high contrast maps of optical absorbance in tissues.

In this study, we use a three dimensional laser optoacoustic imaging system (LOIS-3D) to analyze kinetics of gold nanorod (GNR) distribution after they are intravenously injected into live nude mice. We used LOIS-3D that was previously tested on a nude mouse model of breast cancer after they are intravenously injected into live nude mice. We used LOIS-system (LOIS-3D) to analyze kinetics of gold nanorod (GNR) distribution in mouse tissues after intravenous injection of other blood variables. In this study we performed numerical modeling and experimental tests of this array in vitro. The array has 8 PVDF piezoelements and was incorporated in an Nd:YAG laser-based optoacoustic system. First, we performed modeling of optoacoustic signals from 2-mm blood vessel in a scattering medium with optical properties similar to that of tissue. Spatial distribution of photons absorbed in blood and surrounding tissue was calculated using Monte Carlo simulations with a multiprocessor computer with optimized the purification of gold nanorods for in vivo use. We used centrifugation and filtration before pegylation of gold nanorods. To create an optimum ratio between GNR and the number of molecules of polyethylene glycol (PEG) different cell lines were analyzed by the release of lactate dehydrogenase (LDH), which is a measure of cytotoxicity, and cell proliferation by MIT assays. In parallel with optoacoustic imaging we investigated the accumulation of GNR in liver. We found that higher levels of GNR in liver macrophages were observed after 48 hours with a decreasing trend after 7 days. The GNR are first seen in the periphery of the mouse within hours before settling into certain organs/tissues after a day as seen on optoacoustic images. Intravenous administration of the purified and pegylated GNR to mice resulted in an enhanced contrast of normal tissues and blood vessels in the normal nude mouse.

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7899-156, Poster Session

Laser optoacoustic spectroscopy (LOS) of a gold nanorod solution embedded in a liquid tissue phantom

V. B. Cunningham, H. Rivera Lamela, Sr., D. C. Gallego, Univ. Carlos III de Madrid (Spain)

The increasing demand for techniques to enhance optical properties of in-vivo unhealthy tissue is at the forefront of innovations in nanomedicine. Attempts to spectrally characterize images of abnormal tissue have been performed using frequency domain photon migration, which has involved a combination of diffuse optical spectroscopy (DOS) and diffuse optical imaging (DOI), this spectroscopic analysis has provided valuable information on tissue functional changes (Tromberg, B.J. et al., Breast Cancer Research, 2005). However the main drawback to this technique is the limited penetration depth. More recent attempts at analysis based on optoacoustic spectroscopy has been carried out using gold nanorods at three different wavelengths (757, 807, and 820 nm) in an in-vivo animal model (Song, K.H., Kim, C., Maslov, K., Wang, L.H., EJR, 7, 2009).

We present real results using real-time Laser Optoacoustic Spectroscopy (LOS) and demonstrate the potential of the photon to ultrasound conversion showing that it is a valuable technique for nanostructure characterisation within a turbid media that mimics healthy soft tissue. This analysis has been motivated by the authors previous work on nanoparticle concentration characterization at a single wavelength (532 nm) (Cunningham, V. and Lamela, H., JOLT, 42, 2010) and spherical nanoparticle spectroscopic characterisation within the visible range (410 to 650 nm) (Lamela, H., Cunningham, V., Gallego, D.C., JOLT, 43, 2010).

In this paper, real-time LOS results over the complete wavelength range from 410 to 1000 nm are presented for a gold nanorod colloidal solution located within a highly scattering tissue phantom. The optical source used is a Q-switched Nd:YAG pumped Optical Parametric Oscillator (OPO) controlled by specifically designed software. A comparative analysis of results obtained from a parallel reference measurement scheme, standard collimated optical transmission, and UV-VIS spectrophotometry will be provided.

7899-157, Poster Session

Kinetics of gold nanorod distribution in mouse tissues after intravenous injection monitored with optoacoustic tomography

R. Su, A. Liopo, H. F. Brecht, S. A. Ermilov, A. A. Orava, TornoWave Labs., Inc. (United States)

In this study we use a three dimensional laser optoacoustic imaging system (LOIS-3D) to analyze kinetics of gold nanorod (GNR) distribution after they are intravenously injected into live nude mice. We used LOIS-3D that was previously tested on a nude mouse model of breast cancer that resulted in high contrast maps of optical absorbance in tissues. Animals were scanned prior to GNR injection and then periodically up to one week post-injection. Because GNR were stabilized using a toxic material such as cetyltrimethylammonium bromide (CTAB), we optimized the purification of gold nanorods for in vivo use. We used centrifugation and filtration before pegylation of gold nanorods. To create an optimum ratio between GNR and the number of molecules of polyethylene glycol (PEG) different cell lines were analyzed by the release of lactate dehydrogenase (LDH), which is a measure of cytotoxicity, and cell proliferation by MIT assays. In parallel with optoacoustic imaging we investigated the accumulation of GNR in liver. We found that higher levels of GNR in liver macrophages were observed after 48 hours with a decreasing trend after 7 days. The GNR are first seen in the periphery of the mouse within hours before settling into certain organs/tissues after a day as seen on optoacoustic images. Intravenous administration of the purified and pegylated GNR to mice resulted in an enhanced contrast of normal tissues and blood vessels in the normal nude mouse.

7899-158, Poster Session

A combined photoacoustic, pulse echo ultrasound and optical coherence tomography endoscopy

Y. Yang, T. Wang, P. D. Kumavor, Univ. of Connecticut (United States); X. Li, Q. Zhou, The Univ. of Southern California (United States); Q. Zhu, Univ. of Connecticut (United States)

Photoacoustic tomography (PAT) and optical coherence tomography (OCT) are two emerging imaging modalities which provide complementary optical absorption and scattering contrasts for cancer detection and diagnosis. While the PAT provides tissue vasculature information, the OCT offers micro-scale morphological imaging with penetration depth of 1–3 mms. Pulse-echo ultrasound is readily available from photoacoustic system and it provides tissue structure information at deeper depth with resolution scalable with the transducer frequency. We have developed a novel prototype combined photoacoustic, pulse-echo ultrasound and OCT endoscopy. The endoscopy consists of a ball lensed OCT probe, a right-angle multimode fiber for delivering the laser beam for PAT, and a high frequency ultrasound transducer of 35 MHz center frequency. The overall diameter of the endoscopy is less than 5mm. The lateral and axial resolutions of the OCT system are 19µm and 12µm respectively. The lateral and axial photoacoustic and pulse-echo ultrasound resolutions are 200µm and 80µm respectively. Mouse ear and pig ovary were imaged ex vivo to demonstrate the capability of this new combined endoscopy. Simultaneously acquired microvascular and high resolution anatomy images at subsurface and deeper tissue range demonstrate the synergy of the combined endoscopy over each modality alone.

7899-159, Poster Session

Wide-band optoacoustic array for non-invasive blood hemoglobin monitoring

T. D. Khokhlova, Univ. of Washington (United States); A. Bykov, Univ. of Oulu (Finland); V. G. Andreev, Lomonosov Moscow State Univ. (Russian Federation); Y. Y. Petrova, I. Y. Petrova, D. S. Prough, R. O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Optoacoustic arrays can provide rapid probing of blood vessels without scanning. We developed and built a novel optoacoustic array for noninvasive measurement of total hemoglobin concentration (THb) and other blood variables. In this study we performed numerical modeling and experimental tests of this array in vitro. The array has 8 PVDF piezoelements and was incorporated in an Nd:YAG laser-based optoacoustic system. First, we performed modeling of optoacoustic signals from 2-mm blood vessel in a scattering medium with optical properties similar to that of tissue. Spatial distribution of photons absorbed in blood and surrounding tissue was calculated using Monte Carlo simulations with a multiprocessor computer with
parallel architecture. Ten billion photons were used to provide accurate calculation of absorbed energy in the blood vessel with THb varied from 5 to 15 g/dL. Optoacoustic theory was used to calculate the generated optoacoustic waves, while the output signals of the piezoelements were calculated using the Rayleigh integral. The calculated profiles were compared with ones obtained in the phantom studies with tubes immersed in Intralipid and filled with sheep blood with THb from 6 to 14 g/dL. The experimentally measured optoacoustic signal peak-to-peak amplitudes and profiles were in good agreement with those obtained using with the computer modeling. Moreover, both simulated and experimental signals demonstrated linear increase of the peak-to-peak amplitude with THb concentration. The results of the study also indicated that for higher accuracy of THb measurement one can use averaging of signal amplitudes obtained from central array elements.

7899-160. Poster Session

**Focused, wide-band, polymer-based optoacoustic transducers for non-invasive monitoring of total hemoglobin concentration and other blood variables**

E. Saerchen, Y. Y. Petrov, I. Y. Petrova, D. S. Prough, The Univ. of Texas Medical Branch (United States); W. Neu, Fachhochschule Oldenburg/Osnabrueck/Wiljemshaven (Germany); R. O. Esenaliev, The Univ. of Texas Medical Branch (United States)

One of the most frequently performed blood tests, measurement of total hemoglobin concentration, requires invasive blood sampling. We developed an optoacoustic technique for noninvasive, monitoring of total hemoglobin concentration and other blood variables by probing the radial artery or other blood vessels. Recently, we designed and built a focused, wide-band, polymer optoacoustic transducer for blood vessel probing with high, submillimeter lateral resolution and incorporated it into a highly portable, laser diode-based optoacoustic system. The focused optoacoustic transducer combines a fiber-optic delivery system and a polymer acoustic lens and wide-band piezosensor. First, we experimentally measured transducer parameters (lateral resolution, sensitivity, focal length). To test the transducer capabilities in measurement of total hemoglobin concentration and other blood parameters from blood vessels, we prepared a tissue phantom simulating strongly-scattering tissues with blood vessels of different diameters, spacing, and depths. Optoacoustic signals were acquired from blood at different hemoglobin concentration and oxygenation during transducer scanning over the phantom. In vivo experiments were performed from radial arteries and peripheral veins of different size, depth, and spacing. Submillimeter lateral resolution was obtained in the in vitro and in vivo experiments. The high resolution combined with the wide-band detection of the optoacoustic waves can be used for monitoring of blood variables in blood vessels with high accuracy, sensitivity, and specificity.

7899-57, Session 9

**Multi-target photoacoustic molecular imaging of cardiovascular inflammatory biomarkers using bioconjugated gold nanorods**

S. Ha, S. Tripathy, L. L. Lavery, A. Carson, Univ. of Pittsburgh Medical Ctr. (United States) and Univ. of Pittsburgh (United States); A. Agarwal, N. A. Kotov, Univ. of Michigan (United States); F. S. Villanueva, K. Kim, Univ. of Pittsburgh Medical Ctr. (United States) and Univ. of Pittsburgh (United States)

Multiple cardiovascular inflammatory biomarkers were simultaneously imaged in vivo. The endothelial upregulation of leukocyte adhesion molecules is associated with the process of inflammation and plays a major role in cardiovascular disease development. Simultaneous detection of these biomarkers by photoacoustic molecular imaging (PMI) using target-labeled gold nanorods (GNR) could enable monitoring of the inflammation process in vivo. To create a mouse model of inflammation, 0.03 mL of Rose Bengal (RB) dye was injected through penile vein after a midline laparotomy. At 3 minute post RB injection, the interior vena cava distal to the renal junction was exposed to the green light for 10 minutes. GNRs of different aspect ratios (AR) with optical absorption centered at 715nm (AR 1:3) and 800nm (AR 1:4) were synthesized. GNR (800nm) conjugated to ICAM-1 antibody, and E-selectin antibody conjugated GNR (715nm) were injected (0.5mL each, 10^12 particles/mL). PMI was performed using a commercial ultrasound probe synchronized to a pulsed laser (10 mJ/cm^2, 5ns, 10Hz) at 715nm or 800nm. The mean PA intensities in the inflamed area of 3 mm in diameter were about 8 dB (715nm) to 10 dB (800nm) higher than the untreated area in the same vessel. Multi-targeting results were similar to single-targeting experiments using separate animals in the same conditions. Histopathology of the harvested tissues confirmed inflammation. Controlled experiments with blank GNR injected into a separate animal confirm negligible non-specific binding. The feasibility of simultaneous targeting and monitoring of inflammation responses in cardiovascular system using a commercial ultrasound has been demonstrated in vivo.

7899-58, Session 9

**In vivo photoacoustic detection, magnetic enrichment and photothermal purging of circulating cancer stem cells targeted by nanoparticles**

E. I. Galanza, Univ. of Arkansas for Medical Sciences (United States); J. Kim, Univ. of Arkansas (United States); L. Varticovski, National Cancer Institute (United States)

Current hypotheses suggest that tumor-initiating cells, cancer stem cells, are exclusively responsible for metastasis development; however, the identification of this rare cell population (expected <1-5% of total tumor mass) is challenging and represents a rapidly growing area in cancer research. We propose the use of our unique platform for in vivo ultrasensitive detection of these rare cancer stem cells among bulk circulating tumor cells (CTCs). Our approach allows detection of CTCs and cancer stem cells in the background of various blood cells (RBCs, WBCs and platelets). This platform integrates time-resolved photoacoustic (PA) flow cytometry (PAFC) with magnetic enrichment and photothermal purging of circulating cancer stem cells targeted by multiple bioconjugated gold and magnetic nanoparticles. Using tumor-bearing mouse models of breast cancer we demonstrate (1) in vivo molecular targeting and magnetic enrichment of breast cancer cells with stem-like phenotype, (2) real-time monitoring and quantitative enumeration of extremely rare cancer stem cells among CTCs; and (3) targeted photothermal eradication of cancer stem cells directly in the blood flow. This platform utilizes clinically-relevant and approved technologies (such as use of lasers and nanoparticles that have been approved for a pilot clinical study). Our studies have a potential to aid in resolving major challenges in cancer research for diagnosis, capturing and purging tumor-initiating cells in circulation that could potentially block metastatic spread of breast cancer and other tumors.

7899-59, Session 9

**Nano-LISA for in vitro diagnostic applications**

S. M. Maswadi, R. D. Glickman, The Univ. of Texas Health Science Ctr. at San Antonio (United States); N. Barsalou, W. R. Elliott III, Naval Health Research Ctr. Detachment (United States)

We previously reported the detection of bacterial antigen with immunoaffinity reactions using laser optoacoustic spectroscopy and antibody-coupled gold nanorods (Ab-NR) as a contrast agent specifically targeted to the antigen of interest. The Nano-LISA (Nanoparticle Linked Immunosorbert Assay) method has been adapted to detect
three very common blood-borne viral infectious agents, i.e. human T-lymphotropic virus (HTLV), human immunodeficiency virus (HIV) and hepatitis-B (Hep-B). These agents were used in a test panel to illustrate the performance of the Nano-LISA technique. A working laboratory prototype of a Nano-LISA microplate reader-sensor has been assembled and has been tested with the model panel of the infectious agents. Optoacoustic (OA) responses generated by the samples were detected using the probe beam deflection technique as a non-contact method well suited for use with potentially infectious biological samples. A LabView program was developed to control the prototype microplate reader. The user interface uses graphic tools for parameter input and data display, and presents acquired results in real-time.

The sensitivity of the technique has been assessed by determining the minimum, detectable concentration of the target viral surface antigens. To carry out sensitivity analysis, recombinant proteins of selected viral surface antigens were used as the targets at known dilutions. Gold nanorods served as the contrast agent generating the optoacoustic response, and were conjugated using our standard protocol to antibodies that recognized and bound the viral antigens. The sensitivity of Nano-LISA is at least 1 ng/ml (depending on the commercial antibodies that are used). The greatest sensitivity observed with the current reagents is toward the HTLV p24 viral surface antigen, with the threshold for detection at approximating 10 pg/ml of antigen. The detection reagents do not cross-react with non-complementary antigens, i.e. there is adequate detection specificity. Thus, adequate detection sensitivity, as well as lack of non-specific cross-reaction between antigens, has been demonstrated for the OA microplate reader-sensor, and supports the use of Nano-LISA as a clinical in vitro diagnostic technique.

7899-60, Session 9
Thermal stability of biodegradable nanoclusters for photoacoustic imaging
S. J. Yoon, Y. Chen, A. Murthy, K. P. Johnston, K. V. Sokolov, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Photothermal stability of plasmonic nanoparticles is critically important to perform photoacoustic imaging and photothermal therapy. Recently, biodegradable nanoclusters composed of sub-5 nm primary gold particles and a biodegradable polymer binder have been reported. If the nanoclusters are taken up by cells, these nanoclusters biodegrade to 5 nm primary particles inside endosomes and eventually are excreted from the body. In this paper, the optical properties and photothermal stability of the biodegradable nanoclusters compared to that of nanorods were investigated. Both nanosecond pulsed laser and continuous wave laser were used to investigate the photothermal stability of nanoparticles. First, aqueous solutions of nanoparticles were exposed to different fluences of light. Using a UV-Vis spectrometer, the extinction spectra were measured before and after the laser exposure. Furthermore, the morphological changes of the nanoparticles were monitored by transmission electron microscopy. Finally, using tissue mimicking phantoms and tissue models with cells containing nanoparticles, the photoacoustic response of the nanoparticles with respect to the number of laser pulses was compared. The results of our study indicate that the biodegradable nanoclusters show higher photothermal stability than the nanorods. Therefore, the biodegradable plasmonic nanoclusters can be effectively used for photoacoustic imaging and photothermal therapy.

7899-61, Session 9
In vivo photoacoustic imaging and therapy using silver nanoplates tuned to the mid-band of the near infrared spectrum
K. A. Homan, G. P. Luke, S. Kim, Y. Chen, B. Wang, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Silver nanoplates have excellent potential as a platform for multiplexed photoacoustic imaging and therapy of cancer. Immediately following nanoplate synthesis, their citrate-stabilized surface can be functionalized with chemotherapeutics and targeting moieties that allow for specific cancer cell uptake and subsequent destruction. Silver nanoplates functionalized with the anti-epidermal growth factor receptor antibody were studied in vivo in mouse models of pancreatic cancer. A comparison of different sizes of silver nanoplates in vivo revealed several benefits of contrast-enhanced photoacoustic imaging and therapy in the mid-band (MB) of the near infrared (NIR) spectral range. This MB is important since inexpensive solid state lasers that generate light at a primary wavelength of 1064 nm are readily available and accessible. Results from our in vivo studies showed that the contrast in photoacoustic images with silver nanoplates at the MB increased 40% compared to the 700-750 nm NIR range. This increase in contrast is largely due to the decrease in photoacoustic signal from native tissue in vivo in the MB-NIR range. For instance, in blood-laden tissue, such as certain tumors, the background photoacoustic signal was up to 70% less at 1064 nm. Therefore, contrast-enhanced photoacoustic imaging at the MB-NIR range is advantageous because of the low and more homogeneous signal from native tissue components. Furthermore, the large absorption cross-section of silver nanoplates makes them excellent photothermal therapy agents. Imaging contrast and therapeutic efficiency of molecularly-targeted, drug-loaded silver nanoparticles in both subcutaneous and orthotopic models of pancreatic cancer in mice was explored.

7899-62, Session 9
Dynamic manipulation of magnetic contrast agents in photoacoustic imaging
C. Jia, J. Xia, Y. Jin, Univ. of Washington Medical Ctr. (United States); X. Gao, Univ. of Washington (United States); M. O'Donnell, Univ. of Washington Medical Ctr. (United States)

Magnetic nanoparticles (MNPs) have been used extensively ex vivo for cellular and molecular separations. We recently showed that a coupled nanoparticle combining a superparamagnetic core with a thin, isolated gold shell providing strong absorption in the near infrared can be used for magnetomotive photoacoustic imaging (mmPA), a new technique in which magnetic manipulation of the particle during PA imaging greatly enhances molecular contrast specificity. This particle can also be biologically targeted for in vivo applications, where mmPA imaging provides a spatially localized readout of magnetic manipulations. As an initial test of potential in vivo molecular assays and integrated molecular therapeutics using magnetic manipulation of nanoparticles, we present experiments demonstrating PA readout of trapped MNPs in a flow field. An aqueous solution containing 0.08mg/ml 30-nM MNPs flowed in a 1.1-mm diameter polymer tube at a velocity of 3.6 mm/s. Opposed permanent magnets separated by 40 mm were positioned on both sides of the tube. By translating the magnets, a dynamic magnetic field (0.1-0.2 T) was alternately generated on the side of the tube closest to one of the magnets. As expected, the measured PA signal alternatively increases on that side of the tube closest to one of the magnets, indicating magnetic trapping with concomitant accumulation of MNPs. In general, simultaneous ultrasound/mmPA imaging of trapped particles can provide a highly accurate count of accumulated nanoparticles in the image voxel. This technology can potentially provide sensitive molecular assays of cellular targets travelling in the vasculature (e.g., metastatic tumor cells).

7899-63, Session 9
Hypoxia targeted carbon nanotubes as a sensitive contrast agent for photoacoustic imaging of tumors
S. Zanganeh, N. C. Biswal, A. Aguirre, C. Pavlik, M. B. Smith, Q. Zhu, Univ. of Connecticut (United States)

Development of new and efficient contrast agents is of fundamental importance to improve detection sensitivity of smaller lesions. Within
7899-64, Session 10

Quantitative photoacoustic imaging using the radiative transfer equation

B. T. Cox, Univ. College London (United Kingdom); T. Tarvainen, Univ. of Eastern Finland (Finland); S. R. Arridge, Univ. College London (United Kingdom)

There are two inverse problems in photoacoustics imaging: (1) recovering the initial acoustic pressure distribution from boundary measurements of acoustic pressure, and (2) estimating the underlying optical coefficients (or equivalently chromophore concentrations) from this initial acoustic pressure. The first is well understood; the second is more difficult as it is nonlinear and nonunique, but its solution would enable photoacoustic imaging to become a quantitative, molecular and genomic imaging tool with potentially very wide application in the life sciences. Most attempts to tackle problem (2) have used the diffusion approximation of light transport, which assumes the light is highly diffuse. In practice, this is not the case close to the surface or in regions of tissue where the scattering is low. In these cases it is necessary to use more accurate models of light transport in turbid media. In this paper we will describe nonlinear numerical inversions for the optical coefficients using a finite element model based on the full radiative transfer equation. Various ways to remove the nonuniqueness and regularise the problem will be discussed including the use of prior information, multiwavelength data, and data obtained using multiple optical sources.

7899-65, Session 10

Estimate of effective singular values of a photoacoustic imaging system based on varying signal-to-noise ratio

M. B. Roumeliotis, Lawson Health Research Institute (Canada); G. Chaudhary, M. A. Anastasio, Illinois Institute of Technology (Canada); J. J. L. Carson, Lawson Health Research Institute (Canada)

A photoacoustic tomography (PAT) approach, which employs an iterative reconstruction algorithm and incomplete measurement data, has been used in the reconstruction of simple objects [Ephrat, et al., Medical Physics, 37(4), pp. 1619-1628]. However, it is difficult to comment on the fundamental limitation of this system to accurately capture the information from an object of arbitrary complexity because of the staring, sparse nature of the transducer array. In a previous manuscript [Roumeliotis, et al., SPIE BIOS, 7564-113], a technique was introduced to experimentally acquire the imaging operator of a photoacoustic system, which was analyzed by singular value decomposition. While this method provided estimates of the singular values and singular vectors inherent to the photoacoustic imaging system, it did not supply an estimate of how many singular values and singular vectors were measureable by the imaging system (i.e. above system noise). In this work, we introduce a technique to estimate the effective number of singular values for a photoacoustic imaging system. The effective singular values are defined as the number of singular values that can be reliably recovered above the system noise. In accordance with this technique, a number of perturbation matrices were constructed that represented varying signal-to-noise ratios (SNRs) for the photoacoustic imaging system. This provided a methodology to estimate the number of effective singular values for the photoacoustic imaging system based on the intrinsic SNR as well as a number of manufactured SNRs.

7899-66, Session 10

The effect of excitation pulse duration on the spatial resolution of photoacoustic images

T. J. Allen, P. C. Beard, Univ. College London (United Kingdom)

In order to obtain high resolution photoacoustic or thermoacoustic images, excitation pulses of nanosecond duration are required. However, some excitation sources do not generate such short pulse durations but do provide other benefits. For example, the Alexandrite laser provides typical pulse durations of 50ns but benefits from large pulse energies (>100mJ) in 700-820nm wavelength range. It is therefore well suited to applications that require the illumination of large tissue volumes, such as breast imaging. Also high peak power pulsed laser diodes, when operating in the 60ns to 200ns pulse duration range, have been shown to generate photoacoustic signals with adequate signal to noise ratio for photoacoustic imaging. These devices have the advantages of being compact and a relatively inexpensive alternative to traditional excitation sources. Another example would be excitation sources for thermoacoustic imaging which typically emit RF pulses with a duration of several hundreds of nanoseconds. 

In this paper simulations and experimental work were undertaken to quantify the effects of using long pulse durations (>50ns) on the SNR and spatial resolution of photoacoustic images. It was shown that relatively long excitation pulse durations can be used without degrading spatial resolution if the duration is kept shorter than the width of the impulse response of the imaging system. Once the pulse duration becomes longer, the resolution of the photoacoustic images will degrade. However, providing the rise time of the excitation pulse is sufficiently short (Thus generating significantly high frequency components), this effect can be mitigated by deconvolving for the temporal shape of the pulse. The methods discussed here will allow for a wider range of excitation sources to be used for photoacoustic imaging without compromising spatial resolution.

7899-67, Session 10

Ultrasound-guided Bayesian image reconstruction in limited-view optoacoustic tomography

C. Huang, M. A. Anastasio, Illinois Institute of Technology (United States); S. A. Ermilov, V. V. Nadvoreskiy, A. A. Oraevsky, TomoWave Labs., Inc. (United States)

Optoacoustic tomography (OAT) is an emerging imaging modality that reconstructs images based on ultrasonic signals generated as a result of absorbed optical energy in tissues. This technology has exciting potential for many biomedical imaging applications. There is great interest in conducting B-mode ultrasound and OAT imaging studies for breast cancer detection using a common transducer array. When a small hand-held probe of ultrasonic transducers is used, the range of tomographic view angles is limited, which can result in distortions in the reconstructed OAT image. In this work, we investigate a novel
7899-68, Session 10

High contrast photoacoustic imaging with dual apodization with cross-correlation: ex vivo study

C. H. Seo, M. O'Donnell, Univ. of Washington Medical Ctr. (United States)

Photoacoustic (PA) images generally suffer from high clutter levels since only one-way acoustic beam forming is used to reconstruct an image. Several methods have been presented in the ultrasound (US) literature to suppress sidelobes and reduce artifacts due to phase aberrations. Notable is a class of methods using the DAX (Dual Apodization with Cross-correlation) to adapt beam forming coefficients based on instantaneous measurements of spatial coherence across the imaging aperture. Although a very powerful tool, DAX weighting can create artifacts in complex source environments, generally underestimating the strength of weak point scatterers and speckle regions while overestimating noise signals. This fact can work to our advantage, however, in visualizing microvessels or locating regions with a significant concentration of contrast agents using PA imaging. We examined the use of PA imaging combined with DAX processing to obtain high-contrast images of a black dye inclusion placed ex vivo into fresh bovine tissue. The tissue sample was imaged with an interleaved, real-time US/PA system including a pulse laser source operating at 20 Hz. A 5MHz linear array transducer was used both for conventional US imaging and to detect the PA signal at 720 nm wavelength. Results suggest that PA imaging with DAX weighting combined with ultrasound imaging can produce high-contrast and high-spatial-resolution visualization of particle inclusions.

7899-71, Session 11

Ultrasound-induced enhancement of cellular uptake of plasmonic nanoparticles

A. Hannah, K. E. Wilson, K. A. Homan, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

The geometric tunability of plasmonic nanoparticles offers many advantages for tumor imaging and therapy. The large optical absorption cross section of nanoparticles in the near infrared spectral range makes them excellent imaging contrast and therapeutic agents in various applications, such as photoacoustic imaging, photothermal therapy and gene delivery. The efficacy of any nanoparticle in imaging and therapy is largely dependent on its ability to reach the target tissue. A method is presented in which ultrasound treatment is utilized to improve the uptake of gold nanoparticles at the cellular level without the use of targeting antibodies. The primary goal of this study was to show improved cellular delivery of gold nanoparticles in vitro when assisted by ultrasound waves. High-intensity focused ultrasound (HIFU) was used to induce inertial cavitation at the surface of pancreatic cancer cells (the MPanc96 cell line). Temporary disruption of the cell membrane during ultrasound treatment provided a mechanism for gold nanorods to penetrate the membrane and become encapsulated within the cell via a non-endocytic pathway. Analysis of cellular gold content using inductively coupled plasma mass spectroscopy has indicated a 4-fold increase in gold content in cells treated with 1.5 MHz ultrasound waves over untreated cells.

Ultrasound provides a safe, inexpensive, and noninvasive mechanism to increase the localized uptake of gold nanoparticle contrast agents at the cellular level. The enhanced selective deposition of nanoparticles into tumor cells gives potential for increased contrast in photoacoustic imaging and improved targeting for photothermal therapy applications.
we propose an AuNR-based dual-modal - PA and ultrasound (US) - contrast enhanced imaging technique for monitoring FUS-induced BBB opening in a rat model. This is the first time that AuNRs are used as a dual-modal - PA/US contrast agent. From TEM images, it is found that AuNRs tend to aggregate to micro-scale clusters after extravasating at BBB opening foci. Such a phenomenon results in increase of US back-scattering cross-section and higher AuNR local concentration in the interstitial matrix at the BBB opening foci than that in the blood stream, offering both US and PA contrast enhancement consequently. In the in vivo experiments, PEGylated AuNRs with a mean aspect ratio of 40 nm over 10 nm which own an 800-nm optical absorption peak were intravenously injected into the rats after 1.5-MHz FUS was applied to the rat brains. The experimental results showed that both PA and US contrast enhancement was observed within the designated focal path of FUS while no localized changes were detected outside the sonication volume. The aggregation of AuNRs suggests that metallic nanoparticles smaller than 5 nm, which permit rapid and efficient renal clearance, may potentially be used as a PA/US extra-vascular contrast agent in such a study. It may provide a unique opportunity to solve the accumulation and toxicity problems of metallic nanoparticles in the body further enhancement of the photoacoustic signal is demonstrated by using silica coated gold nanorods as nano-contrast agents. The silica coated gold nanorods were prepared by facile wet chemical methods. Transmission electron microscopy was used to investigate the morphology of nanoparticles and the thickness of the silica coating, revealing fairly uniform and conformal silica coating on the gold nanorods with controllable thickness. The optical properties of gold nanorods before and after silica coating were monitored by ultraviolet-to-visible spectroscopy. The spectra indicated that the silica coating did not alter the optical absorption of the gold nanorods. The photoacoustic response of the silica-coated gold nanorods compared to the non-silica coated gold nanorods was measured using a combined ultrasound and photoacoustic (USPA) imaging system. The USPA imaging result shows the silica coating improves the imaging contrast up to 6 times. Since the optical absorption of nanoparticles was the same, the enhancement of photoacoustic response could be attributed to changes in the interfacial heat conduction from gold to water due to the silica. This study suggests that silica coating could potentially be a simple way to produce high efficiency contrast agent for photoacoustic imaging.

### 7899-73, Session 11

**Photoacoustic and ultrasound imaging using remotely triggered vaporization of an exogenous contrast nano-agent**

K. E. Wilson, K. A. Homan, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Photoacoustic imaging is a rapidly expanding imaging modality offering high spatial resolution and contrast images of deep tissues. Furthermore, with the introduction of exogenous contrast agents, the modality is capable of functional molecular and cellular imaging in vivo. Traditionally, photoacoustic imaging relies on light absorption, heat generation, and the resulting thermoelastic generation of acoustic pressure waves. However, the thermoelastic expansion phenomenon is the weakest of all photoacoustic affects. Vaporization is a photoacoustic phenomenon that produces significantly stronger signal amplitude, but has not been adopted due to the high energy deposition required and resulting destructive effects caused to living tissues. In this paper, we introduce a novel exogenous contrast nano-agent to produce significantly higher photoacoustic signal amplitude, up to 66 times than that from plasmonic nanoparticles alone, with low energy laser pulses and evasion of destruction of the tissue. This is accomplished by using silica coated gold nanorods. The photoacoustic and ultrasound images were collected using a custom-build combined photoacoustic and ultrasound imaging (PAUS) system. Image analysis shows significantly higher photoacoustic signal compared to nanoparticles alone due to vaporization of the described agent.

### 7899-75, Session 11

**Photoacoustic and nuclear imaging of 125I-labeled gold nanorod contrast agent**

X. S. Shao, Univ. of Michigan Medical School (United States); A. Agarwal, Univ. of Michigan (United States); J. Rajan, Univ. of Michigan Medical School (United States); N. A. Kotov, Univ. of Michigan (United States); X. Wang, Univ. of Michigan Health System (United States)

We have investigated the potential of emerging photoacoustic imaging and nuclear imaging in monitoring of drug delivery by using a newly developed dual-modality contrast agent. After the contrast agent composed of gold nanorods (GNRs) conjugated to the anti-tumor necrosis factor drug was produced, it was radio-labeled by 125I in a simple and fast manner with high yield and without disturbing the optical properties of the contrast agent. ELISA experiments designed to test tumor necrosis factor binding were performed to prove the specificity and biological activity of the radiolabeled conjugated contrast agent. Photoacoustic and nuclear imaging were conducted to visualize the distribution of GNRs in articular tissues of rat tail joints in situ. Findings from the two modalities corresponded well with each other. Using the current imaging systems, GNRs down to a concentration of 10 pM in biological tissues and with a radioactive label of 5 µCi can be imaged. Moreover, by radiolabeling the GNRs, the in vivo behaviors of the contrast agent can be monitored conveniently using γ-camera, allowing validation of the findings from emerging photoacoustic technique. Enabled by the high sensitivity of nuclear imaging, whole-body and longitudinal studies of the GNR contrast agent can be performed noninvasively and repeatedly in the same animal. The highly efficient method reported here provides an extensively useful tool for guidance of design and development of new gold nanoparticles as target-specific agents for both diagnostics and photothermal therapy.

### 7899-120, Session 11

**Ultrasound and photoacoustic imaging to monitor mesenchymal stem cells labeled with gold nanoparticles**

S. Y. Nam, L. M. Ricles, The Univ. of Texas at Austin (United States); K. V. Sokolov, The Univ. of Texas at Austin (United States) and The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); L. J. Suggs, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

Mesenchymal stem cells (MSCs) are versatile in many tissue engineering applications and have the potential to be used for cellular therapies...
because they can differentiate into many cell types. Specifically, the use of MSCs for the treatment of ischemic disease is promising because MSCs can express characteristics of vascular cells. MSCs can promote vascular growth at the site of injury after delivery using a PEGylated fibrin gel. In order to quantitatively assess in vivo delivery of MSCs, a non-invasive and high-resolution imaging technique is required. In this study, we investigated the ability of the combined ultrasound and photoacoustic imaging technique to monitor the growth of MSCs labeled with gold nanotracers in tissue engineered constructs. MSCs were loaded with citrate-stabilized and poly-L-lysine coated gold nanospheres to be used as both photoacoustic and optical contrast agents. It was observed that uptake of nanotracers did not have a significant effect on cell viability and proliferation over a two-week period. A high-frequency focused ultrasound transducer was used with a nanosecond pulsed laser operating at a wavelength of 532 nm to acquire the combined ultrasound and photoacoustic images. Furthermore, the 3D ultrasound and photoacoustic images were obtained by mechanically scanning the transducer over the region of interest in the tissue engineered construct. The results suggest that the growth of MSCs labeled with gold nanotracers can be monitored in vivo using the combined photoacoustic and ultrasound imaging.

7899-76, Session 12
Photoacoustic-guided focusing of light through optically diffusive media
F. Kong, Hunter College (United States); R. H. Silverman, Columbia Univ. Eye Institute Research (United States) and Riverside Research Institute (United States); L. Liu, Hunter College (United States); P. V. Chitnis, Riverside Research Institute (United States); Y. Chen, Hunter College (United States)

Convergence of light towards a desired location in optically diffusive and absorptive media is highly relevant to optical methods of biomedical imaging. In this study, we experimentally demonstrated the feasibility of employing photoacoustic signals originating from an optically absorptive target as feedback for shaping the incident wavefront to increase optical energy density at the absorptive target delivered through a diffusive medium. An array of 140 two-dimensional MEMS deformable mirrors shaped the wavefront of a collimated, 532-nm laser beam (1 ns, 37 nJ, at 100 Hz repetition rate). The wavefront-shaped beam was then transmitted through optically scattering paraffin whose thickness is equivalent to 5 light-scattering mean free path. A light absorbing sample, which consisted of graphite particles deposited sparsely on a glass slide, was placed 1 mm behind the paraffin. A focused, 40 MHz ultrasonic receiver was aligned with a graphite particle. The peak-to-peak voltage of the photoacoustic signals (averaged 20 times) originating from the graphite particles was recorded by a digital oscilloscope. The phase of each mirror was varied iteratively to maximize the amplitude of the photoacoustic signal by an autonomous Matlab routine. The mirror configuration that maximized the photoacoustic amplitude resulted in a re-focused beam whose optical energy density at the graphite spot was 10 times higher than when the mirror-array was used as a plane mirror. The photoacoustic signal potentially provides a non-invasive and reliable feedback for manipulating spatial distribution of light in diffusive media and may facilitate optical imaging at greater depths.

7899-77, Session 12
Chronic label-free volumetric photoacoustic microscopy of melanoma cells in scaffolds in vitro
X. Cai, Y. Zhang, C. Kim, S. Choi, Y. Xia, L. V. Wang, Washington Univ. in St. Louis (United States)

Visualizing cells in three-dimensional (3D) scaffolds has been one of the major challenges in tissue engineering. Current imaging modalities have limitations. Microscopy, including confocal microscopy, cannot penetrate deeply (> 300 µm) into the scaffolds; X-ray micro-computed tomography (micro-CT) requires staining of the structure with a toxic agent such as osmium tetroxide. Here, we demonstrate photoacoustic microscopy (PAM) of the spatial distribution and temporal proliferation of melanoma cells inside three-dimensionally porous scaffolds with thicknesses over 1 mm. Melanoma cells have a strong intrinsic contrast which is easily imaged by label-free PAM with high sensitivity. Different seeding and culture methods, both stationary and spinner flask, were evaluated by PAM. Spatial distributions of the cells in the scaffold were well-resolved in PAM images. Moreover, we chronically imaged the same cell/scaffold construct at different time points over 2 weeks. The number of cells in the scaffold was quantitatively measured from the PAM volumetric information. The cell proliferation profile obtained from PAM correlated well with that obtained using the traditional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytltetrazolium bromide (MTT) assay. We believe that PAM will become a useful imaging modality for tissue engineering applications, especially when thick scaffold constructs are involved, and that PAM modality can also be extended to image other cell types labeled with contrast agents.

7899-78, Session 12
Photoacoustic generation using coded excitation
S. Su, P. Li, National Taiwan Univ. (Taiwan)

Photoacoustic (PA) imaging has been used to image soft tissue with high contrast and high resolution. Generation of acoustic waves from the region-of-interest is based on the absorption of electromagnetic energy distinguishing regions with different absorption characteristics. Typically, a Q-switched Nd:YAG laser providing mJ pulse energy is suitable for biomedical applications. However, such a laser is relatively bulky and expensive. An alternative way is to use a diode laser, which can achieve kHz pulse repetition frequency (PRF) but the laser energy is generally too low for effective PA generation. One method to enhance the PA signal is to use coded excitation. The coded laser pulses can be transmitted by a diode laser with high PRF and the signal intensity of the receiving signal can be enhanced by compression. In this study, we proposed chirp coded excitation using a diode laser. The PRF of the laser pulse provided by our system can achieve 25-MHz (i.e., 20-ns pulse duration with a 50 % duty cycle). A 20-MHz PA transducer is used for backward mode PA detection. Chirp coded PA signal was generated by tuning the pulse duration of individual laser pulses with decreasing frequency in time domain. Result shows that the PA signal intensity can be enhanced after matched filtering. Nonetheless, high range sidelobes are also present. Compared to Golay codes seen in the literature, the proposed chirp coded excitation requires only a single transmission. The compression filter is an important investigation subject to further reduce the range sidelobes.

7899-79, Session 12
Coded photoacoustic Doppler excitation with near-optimal utilization of the time and frequency domains
A. Eyal, A. Sheinfeld, S. Gilead, Tel Aviv Univ. (Israel)

As part of the effort to develop affordable, practical and versatile photoacoustic (PA) imaging systems, coded excitation has recently emerged as tool for improving SNR when using low-peak power sources, enabling high throughput multispectral excitation and facilitating Doppler photoacoustic characterization of flow. Here we propose a new PA excitation and data analysis method which can achieve an almost complete utilization of the available time and frequency windows while enabling simultaneous spectral and spatial characterization of flow. The excitation signal comprises tens of interleaved tone-burst signals (N) at equally spaced frequencies. It achieves near continuous irradiation of the probed volume. The axial resolution of the method is
determined by the detector bandwidth and the spectral resolution by the total dwell time. Upon reception the response is sampled, digitized and processed using short-time Fourier transform. The result is a two dimensional map of the PA response as a function of axial position and frequency. Provided the frequency spacing is bigger than the maximum Doppler shift, the responses corresponding to the different tone-burst sequences are spectrally separated and can be averaged yielding roughly sqrt(N) improvement in SNR. Experimentally, 21 interleaved tone burst sequences with spacing of 500Hz around a center frequency of 1MHz were generated using an externally-modulated CW laser source and excited a suspension of carbon particles flowing in a submerged C-Flex tube. The proposed multi-tone averaging led to significant SNR improvement of ~6dB. The method can find significant applications in harnessing laser-diodes and other practical low-peak power sources for PA imaging.

7899-80, Session 12

Advances in multimodal photoacoustic ophthalmoscopy
S. Jiao, The Univ. of Southern California (United States); H. F. Zhang, Univ. of Wisconsin-Milwaukee (Turkmenistan)

We have developed a multimodal imaging technique called photoacoustic ophthalmoscopy (PAOM) for in vivo and ex vivo imaging of the retina, which is based on the scanning-laser optical-resolution photoacoustic microscopy. Since the first demonstration of the concept of PAOM we have made several advances of the technology. Our current development includes the integration of PAOM with optical coherence tomography, confocal microscopy, and fluorescence imaging. The integrated system has been applied in imaging ocular tissues in vivo and ex vivo. Experimental results demonstrated that the technology has potential significant impact on the research and diagnosis of ophthalmic diseases, e.g. diabetic retinopathy (DR) and age-related macular degeneration (AMD). DR and AMD are two major blinding diseases worldwide.

7899-81, Session 12

Stimulated Raman imaging with photoacoustic detection
V. V. Yakovlev, Univ. of Wisconsin-Milwaukee (United States)

There are increasing needs to develop optical modality capable of label-free, chemically-specific imaging of deep-tissue structures. The major obstacle of optical imaging is the strong optical scattering in tissue, which causes photons to diffusely propagate inside the tissue and, as a result, to wash out the spatial origins of the detected signals. Hence, existing optical imaging technologies cannot penetrate more than one optical transport mean free path (approximately 1 mm) in tissue while still maintain a high resolution. However, even in small animal models, the early chemical and anatomical changes typically initiate beneath the skin surface at a depth of several millimeters, which are beyond the reach of modern high-resolution optical imaging technologies, such as confocal and two-photon microscopy. To overcome the strong optical scattering in tissue, and to attain both the large imaging depth and high spatial resolution, the recently developed photoacoustic tomography [1] and photoacoustic microscopy [2,3] combine optical excitation with a high-frequency ultrasonic detection. These fast emerging imaging modalities rely on linear absorption of the incident optical energy in tissue. The chemical selectivity can be achieved by means of a spectroscopic method based on stimulated Raman photoacoustics, which was first proposed in the late 1960s [4]. In brief, when two laser pulses (pump and Stokes) excite the vibrational level of a molecule, the energy of the excited state is then transferred into heat, generating a photoacoustic wave. Recently, we proposed and experimentally demonstrated the feasibility of this spectroscopic technique for imaging application [5, 6].

In the talk, we will discuss the latest progress on achieving high-sensitivity, background-free stimulated Raman photoacoustic imaging in turbid media.

REFERENCES

7899-82, Session 12

Enhanced photoacoustic detection through multiple picosecond pulse excitation
T. Liu, V. V. Yakovlev, H. F. Zhang, Univ. of Wisconsin-Milwaukee (United States)

The photoacoustic (PA) imaging has experience a noticeable growth in the past few years benefits from its optical absorption based contrast mechanism. To expect PA microscopy (PAM) distinguishes signatures from different molecular, nonlinear absorption instead of linear absorption could be a solution. However, the nonlinear PAM imaging need to overcome the potential damage induced by ultrahigh laser density. In this report, we present a technique which improves the signal-to-noise ratio (SNR) by introducing multiple energy-minimized laser pulses to excite the PA waves instead of single higher energy laser pulse, when the pulse train lasts less than the minimum of the medium’s stress relaxation time and thermal relaxation time. The Michelson interferometer with which can split a laser pulse into two identical energy pulses with controllable time delay is the key component of our experimental setup. By employing three interferometers’ cascade, a pulse train consisting eight picoseconds pulses was created. The measured mean pulse energy was 11 nJ, the whole pulse train temporal duration was less than 1 ns. The multiple-pulse induced photoacoustic signals has peak-to-peak amplitude which proportional to the number of pulses in the pulse series and such linearity relationship worked for different optical absorption coefficients. The SNR increased when the number of pulse rose. Neither photoacoustic saturation effect nor nonlinear effect was detected during the experiment. Hence, we demonstrate that with an accumulated energy deposition effect, the multiple-pulse excitation can improve the SNR in ultrashort laser pulse induced photoacoustic generation. This method is inestimable in nonlinear PAM.

7899-83, Session 12

Broad-band, high-efficiency optoacoustic generation using a novel photonic crystal-metallic structure
Y. Guo, H. W. Baac, S. Chen, T. B. Norris, L. J. Guo, Univ. of Michigan (United States)

Various optical structures have been investigated for high-frequency optoacoustic generation via thermoelastic effect, such as metal films, mixture of polydimethylsiloxane (PDMS) and carbon black, two-dimensional (2-D) gold nanostructure with PDMS film, etc. However, they suffer from either low light absorption efficiency which affects the amplitude of generated ultrasound, or thick films that attenuate the amplitude and restrict its bandwidth.

Here we propose a novel one-dimensional photonic crystal-metallic (PCM) structure, which can be designed to absorb 100% optical energy...
Development and validation of a combined photoacoustic micro-ultrasound system for in-vivo oxygen saturation estimation

A. Needles, A. Heimiller, P. Ephrat, D. Bates, C. Bilan-Tracey, C. Theodoropoulos, D. Hirson, Visualsonics Inc. (Canada); F. S. Foster, Sunnybrook Health Sciences Ctr. (Canada)

Photoacoustic (PA) imaging can estimate the spatial distribution of oxygen saturation (SO2) in blood, and be co-registered with B-Mode images of the surrounding anatomy. This talk will focus on the development of a PA imaging mode on a commercially available array based micro-ultrasound (µUS) system that is capable of creating such images. Beamforming techniques, mode-interleaving, digital sampling and signal processing will be described, along with a new technique for in vivo validation of PA-SO2 estimation.

A modified µUS system (Vevo 2100, VisualSonics) was operated with a linear array transducer (MS-250, fc = 21 MHz). The array was retrofitted with a housing that held rectangular fiber optic bundles (25.4 x 1.25 mm) to either side, at an angle of 30° relative to the imaging plane. The rectangular bundles were bifurcated ends of a single bundle that was coupled to a tunable laser (Rainbow NIR, OPOTEK Inc., Carlsbad CA, 680-950 nm, fluence < 20 mJ/cm2). 64 channels of PA data were acquired in parallel and synthetic apertures of up to 256 channels were formed. A delay-and-sum beamforming algorithm was implemented in software and verified with a tungsten wire phantom. B-Mode images were interleaved with PA images and displayed in either a side-by-side or overlaid configuration. In vivo B-Mode imaging was used to guide the placement of a 26 gauge needle into the jugular vein of an adult mouse. The needle guided the insertion of a fluorescence-based oximetry probe (OxyLab, Oxford Optronix Ltd., Oxford, UK). This probe measured the partial pressure of oxygen (PPO2) within the jugular vein, while PA signals were simultaneously collected, first at 750 nm and then 850 nm. To begin, the animal inhaled isoflurane mixed with room air and subsequently with 100% oxygen. After 10 minutes, the 100% oxygen was replaced with 5% oxygen for 15 minutes, and then the process was repeated. Readings were made every 5 minutes throughout this process. Parametric maps of SO2 were processed offline with a two-wavelength approach, and compared with the PPO2 readings made with the oximeter.

The software beamforming approach allowed for single wavelength imaging frame rates of 5 Hz (256 channels) up to 20 Hz (64 channels). The wire phantom images of PA compared well with B-Mode and gave a similar -6 dB lateral resolution (B-Mode = 165 µm, PA-Mode = 180 µm). PPO2 in the jugular ranged from 40 mmHg at baseline (room air), to a peak of 110 mmHg (100% O2), and a minimum of 20 mmHg (5% O2). This compared to PA SO2 estimates of 55%, 95%, and 45% at the same time points respectively. Overall, there was a strong correlation between relative changes in PPO2 and SO2 (R2 > 0.9). In absolute terms, the relation between PPO2 and SO2 was compared to the dissociation curve for small animals found in the literature. Looking at the linear regime of the measured curves the correlation between PPO2 and SO2 was high (R2 > 0.9) however the slope of the linear regression was approximately half of what was expected from the literature. In the plateau region of the curve (> 70 mmHg) the estimates of SO2 compared well with expected values (within 10%).

A new method for correlating in vivo PPO2 readings with SO2 estimates from a combined PA/µUS system has been proposed and demonstrated in the jugular vein of a mouse. Errors in the absolute measures of SO2 were observed and may be due to a number of factors, both physiological (pH, body temp, species) and physical (attenuation, diffraction). In practice, relative in vivo measures of SO2 with PA may ultimately prove to be more robust. The method described here has been extended to 3D studies of subcutaneous murine tumours and preliminary results will be reported. With larger sample sizes, the levels of confidence in the estimates should be improved, leading to fully independent measures of relative SO2 with PA for monitoring the hypoxic state of the tumors and their responses to treatment.
Systemic induction of heat shock protein 70 following tumor treatment by photodynamic therapy

M. Korbelik, S. Merchant, The BC Cancer Agency Research Ctr. (Canada)

Oxidative stress inflicted by photodynamic therapy (PDT) is known to induce a strong upregulation in the expression of heat shock protein 70 (Hsp70) and other heat shock proteins in cells of treated tumor. It has also become evident that there is a translocation of Hsp70 to the surface of PDT-treated cells and its release from these cells. Our recent discovery that Hsp70 gene in untreated liver cells cultured in vitro becomes upregulated upon co-incubation with PDT-treated tumor cells prompted us to investigate whether such phenomenon exists in vivo. Lewis lung carcinoma (LLC) tumors growing in C57BL/6 mice were treated by Photofrin-based PDT and the tumors, livers and spleens from the mice were collected at various post-therapy intervals for quantitative PCR-based analysis of Hsp70 gene expression. Increased expression of Hsp70 gene was detected in both the tumor and distant tissues (more pronounced in the liver than in spleen) and appeared the most prominent at 4 hours post PDT. Serum ELISA measurement suggested that Hsp70 produced in the liver after PDT is released into the circulation. This effect appeared at least partly influenced by glucocorticoid hormones known to be mobilized after PDT. Flow cytometry analysis revealed that PDT-treated tumor cells (particularly those undergoing apoptotic or necrotic death) bind extracellular Hsp70. Recombinant Hsp70 protein administered to tumor-bearing mice was found to accumulate in both the liver and tumor but the tumor:liver ratio was found to increase when the tumors were treated by PDT. We conclude that, following PDT, Hsp70 is upregulated not only in the treated tumor but also at distant systemic sites including liver, from where it can be rapidly sequestered to damaged tumor tissue in order to facilitate the disposal of dying cells and influence the development of antitumor immune response elicited by this therapy.

Anti-transforming growth factor beta antibody combined with photodynamic therapy for renal cell carcinoma in mice

M. R. Hamblin, P. A. Mroz, Massachusetts General Hospital (United States)

Photodynamic therapy (PDT) has been shown to be an effective locally ablative anti-cancer treatment that has the additional advantage of inducing tumor-directed immune responses. However immunosuppressive mechanisms that can affect the outcome of PDT do exist and they can lead to decreased number of cures and less than expected activation of the immune system. Among the key immunosuppressive factors that affect anti-tumor immune responses are regulatory T cells (Treg). CD25+ Foxp3+ Treg comprise 5–10% of CD4+ T cells in naive mice and have been shown in several studies to prevent the induction or to inhibit the generation of the immune responses to tumors. Depletion or inhibition of these cells using monoclonal antibodies or cytotoxic agents has been shown to promote rejection of several tumors in mouse models and clinical trials. One of the major cytokines responsible for the immunosuppressive effects of Treg is transforming growth factor beta (TGF beta) that blunts immune surveillance, favoring tumor escape.

Our laboratory was the first to clearly demonstrate that low-dose cyclophosphamide (CY) treatment could significantly deplete Treg population and when combined with PDT treatment could lead to cures and long-lasting memory immunity. However, even non-chemotherapeutic levels of CY did significantly reduce the overall numbers of splenocytes, suggesting that, although potent, the observed immune response in the combination group was far from optimal and could be improved. We hypothesize that an even more successful approach would be to combine PDT with anti-TGF beta antibody that does not significantly affect the population of cytotoxic T lymphocytes (CTL) and, at the same time, has the potency to decrease the immunosuppressive effects of Treg mediated by TGF beta. This hypothesis was tested with aTGF-beta antibody combined with BPD-mediated PDT in a BALB/c renal cell carcinoma model.
Mechanism study of tumor-specific immune responses induced by laser immunotherapy: in situ autologous whole-cells cancer vaccine  
X. Li, Chinese PLA General Hospital (China); F. Zhou, South China Normal Univ. (China); R. E. Nordquist, Wound Healing of Oklahoma, Inc. (United States); H. Liu, The Univ. of Oklahoma (United States); T. L. M. Hode, Immunophotonics, Inc. (United States); W. R. Chen, Univ. of Central Oklahoma (United States) 

Although the efficacy of laser immunotherapy has been demonstrated in pre-clinical studies and clinical pilot trials, the possible mechanism of LIT is still not fully understood. In the current study, we conducted experiments to investigate the mechanism of LIT. The roles of glycated chitosan (GC) and laser in the anti-tumor immune response were studied separately using flow cytometry. The results showed that GC could directly activate dendritic cells (DCs). Combining GC stimulation and selective photothermal tumor cell destruction resulted in an enhanced tumor cell-DCs interaction. Thermal treatment alone and GC alone can both enhance antigen presentation to DCs, causing proliferation of T cells. The combined thermal treatment and GC stimulation can achieve the highest level of T cell proliferation. The preliminary results obtained in this study pave the way for an in-depth mechanistic study that will contribute to the development of LIT as an effective modality for the treatment of late stage cancer patients who are facing severely limited options.

Immunohistochemical analysis of immune response in breast cancer and melanoma patients after laser immunotherapy  
R. E. Nordquist, S. Bishop, Wound Healing of Oklahoma, Inc. (United States); X. Li, Chinese PLA General Hospital (China); H. Ferguson, M. B. Vaughan, W. R. Chen, Univ. of Central Oklahoma (United States) 

Laser immunotherapy (LIT) has shown great promise in pre-clinical studies and preliminary clinical trials. It could not only eradicate treated local tumors but also regress and eliminate untreated metastases at distant sites. Combining a selective photothermal therapy with an active immunological stimulation, LIT can induce systemic anti-tumor immune responses. To observe the immunological changes before and after LIT treatment, the pathological tissues of 5 melanoma and 1 breast cancer patients were processed for immunohistochemical analysis and evaluated by a new recognition and analysis program. The results suggested the expression of CD68 as the end stage cell type, i.e., this was the most numerous immune cell type in late stage resolving lesions. These dendritic cells are functionally different from the Langerhan-type cell that is the antigen presenting the surveillance system. These results may help understand the anti-tumor immune responses induced by LIT. Further study will be conducted to identify immunologic biomarkers associated with LIT-induced clinical response.

Molecular mechanism of PDT-induced apoptotic cells stimulation NO production in macrophages  
S. Song, F. Zhou, D. Xing, South China Normal Univ. (China); W. R. Chen, Univ. of Central Oklahoma (United States) and South China Normal Univ. (China) 

It is well known that apoptotic cells (AC) participate in immune response. The immune response induced by AC, either immunostimulatory or immunosuppressive, have been extensively studied. However, the molecular mechanisms of the immunostimulatory effects induced by PDT-treated AC remain unclear. Nitric oxide (NO) is an important signal transduction molecule and has been implicated in a variety of functions. It has also been found to play an important role not only as a cytotoxic effector but an immune regulatory mediator. In this study, we demonstrate that the PDT-induced apoptotic tumor cells stimulate the production of NO in macrophages by up-regulating expression of inducible nitric oxide synthase (iNOS). In addition, we show that AC, through toll-like receptors (TLRs), can activate myeloid differentiation factor-88 (MyD88) and phosphatidylinositol 3-kinase (PI3K), indicating that AC serves as an intercellular signal to induce iNOS expression in immune cells after PDT treatment. This study provided more details for understanding the molecular mechanism of the immune response induced by PDT-treated AC.

Laser immunotherapy for the treatment of human breast cancer: 1-year follow-up results  
T. L. M. Hode, Immunophotonics, Inc. (United States); O. Adalsteinsson, International Strategic Cancer Alliance (United States); G. L. Ferrel, Hospital Nacional Edgardo Rebagliati Martins (Peru); J. A. Lunn, Consultant (Bahamas); M. Guerra, Immunophotonics, Inc. (United States); X. Li, Chinese PLA General Hospital (China); R. E. Nordquist, Wound Healing of Oklahoma, Inc. (United States); W. R. Chen, Univ. of Central Oklahoma (United States) 

Laser immunotherapy (LIT) is an experimental treatment modality for late-stage, metastatic tumors, targeting solid primary and/or secondary tumors and utilizing an autologous vaccine-like approach to stimulate immune responses. Specifically, LIT combines laser-induced in situ tumor devitalization with an immunoadjuvant for local immunostimulation. Here we report the initial results and one-year survival data from a human breast cancer pilot trial with laser immunotherapy. Ten stage III and IV cancer patients were treated, all of which were considered to be out of all other options. The immediate goal of the trial was to determine the patient tolerance and the toxicity of the therapy, the optimal dose for the alteration of the course of the disease, and the reduction of the tumor burden. Each patient was individually evaluated for toxicity tolerance through physical exams and by appropriate supplemental and routine laboratory tests. No toxicity or significant adverse reactions were observed, and the treatment was very well tolerated by all patients. Almost all the treated patients have had positive responses: A majority of patients experienced large-scale reduction of primary breast tumors, and all the stage IV patients experienced either complete or significant reductions in distant metastases in the lungs, liver, bone, and the brain, indicating a strong systemic effect of the treatment. We also report two cases of triple negative breast cancer patients that showed limited or no response to LIT.
7900-09, Session 3
Interstitial laser immunotherapy in the treatment of DMBA-4 metastatic tumors in rats
W. R. Chen, D. Figueroa, Univ. of Central Oklahoma (United States); T. L. M. Hode, Immunophotonics, Inc. (United States); R. E. Nordquist, Wound Healing of Oklahoma, Inc. (United States); J. Walla, Univ. of Central Oklahoma (United States); R. F. Wolf, The Univ. of Oklahoma Health Sciences Ctr. (United States); H. Liu, The Univ. of Oklahoma (United States); X. Li, Chinese PLA General Hospital (China)

Metastases are the major cause of treatment failure and cancer deaths. Current available therapies, such as surgery, radiation, and chemotherapy, only have limited curative effects in patients with late-stage, metastatic cancer. Immunotherapy has been considered as the ultimate approach for cancer treatment since a systemic, anti-tumor immunological response is induced. Laser immunotherapy (LIT), a novel immunotherapy modality for late-stage cancer treatment, has shown great promise in clinical breast cancer and melanoma pilot trials. However, the skin color and the tumor size (often larger than 5 cm) has been a challenge for effective treatment with LIT. To induce a thermal destruction zone of appropriate size without causing thermal damage on the skin, we have developed interstitial laser immunotherapy (ILIT) using a cylindrical diffuser. To determine the effectiveness of ILIT, we treated the primary tumors of rats bearing DMBA-4 metastatic tumors and observed the progression of both treated primary tumors and untreated metastases. Our results demonstrated that the ILIT could impact a much large tumor area, and it significantly reduced the surface damage compared with traditional LIT. The survival data also indicate that ILIT can be developed into an effective tool for treating patients with deeper, larger tumors, with reduced side effects. The survival results of ILIT were also compared with LIT of dye-enhanced non-invasive laser irradiation.

7900-10, Session 3
Thermal effect induced by interstitial irradiation of near-infrared laser with cylindrical diffuser
W. R. Chen, D. Figueroa, K. Le, Univ. of Central Oklahoma (United States); X. Li, Chinese PLA General Hospital (China); J. Walla, Univ. of Central Oklahoma (United States); R. F. Wolf, The Univ. of Oklahoma Health Sciences Ctr. (United States); H. Liu, The Univ. of Oklahoma (United States); R. E. Nordquist, Wound Healing of Oklahoma, Inc. (United States); T. L. M. Hode, Immunophotonics, Inc. (United States)

Laser immunotherapy (LIT), using non-invasive laser irradiation, has resulted in promising outcomes in the treatment of late stage cancer patients. However, the penetration of laser light limits the clinical applications of LIT in patients with dark skin, or whose tumor is too large or too deep. The present study is designed to investigate the thermal effect of interstitial irradiation using an 805-nm laser with a cylindrical diffuser, in order to overcome the limitations of the non-invasive mode of treatment. Gel phantom, chicken breast tissue, bovine liver and pig kidney, as well as in vivo rat tumors were irradiated using this system. The temperature increase was monitored by thermocouples that were inserted into the tissue at different sites around the cylinder fiber. Three-dimensional temperature distributions in target tissues during and after interstitial laser irradiation were also determined by magnetic resonance thermometry. The preliminary results showed that the output power of laser and the optical parameters of the target tissue determined the light distribution in tissue. The temperature in the tissue close to the cylindrical fiber tip varied according to the distance between the tissue and the surface of the cylindrical diffuser, under different laser power densities. The tissues at different times after LIT were also collected to prepare for Hematoxylin and Eosin (H&E) stain for histological studies. Severe cell damages were observed. Twenty-four hours after laser irradiation, the tissue temperature increased markedly in rat tumors, and the damage range can reach up to 2cm with appropriate laser power density. The light distribution and temperature increase in tissue was also investigated by mathematical simulation studies. The model established by phantoms and test tissue was confirmed by the data collected from animal experiments. These results will help us understand and control the thermal effect induced by interstitial laser irradiation.

7900-11, Session 4
Studying depletion kinetics of circulating prostate cancer cells by in-vivo flow cytometer
X. Wei, J. Guo, Fudan Univ. (China)

Prostate cancer is the most common malignancy in American men and the second leading cause of deaths from cancer, after lung cancer. The tumor usually grows slowly and remains confined to the gland for many years. During this time, the tumor produces little or no symptoms or outward signs. As the cancer advances, however, it can metastasize throughout other areas of the body, such as the bones, lungs, and liver. Surgical resection, hormonal therapy, chemotherapy and radiation therapy are the foundation of current prostate cancer therapies. Treatments for prostate cancer cause both short- and long-term side effects that may be difficult to accept. Molecular mechanisms of prostate cancer metastasis need to be understood better and new therapies must be developed to selectively target to unique characteristics of cancer cell growth and metastasis. We have developed the “in vivo microscopy” to study the mechanisms that govern prostate cancer cell spread through the microenvironment in vivo in real-time confocal near-infrared fluorescence imaging. A recently developed “in vivo flow cytometer” and optical imaging are used to assess prostate cancer cell spreading and the circulation kinetics of prostate cancer cells. A real-time quantitative monitoring of circulating prostate cancer cells by the in vivo flow cytometer will be useful to assess the effectiveness of the potential therapeutic interventions.

7900-12, Session 4
The impact of the depth-of-field to image quality in high-magnification microscopic scanning
Y. Qiu, X. Chen, Y. Li, Z. Li, The Univ. of Oklahoma (United States); B. Zheng, Univ. of Pittsburgh (United States); S. Li, Univ. of Oklahoma Health Sciences Ctr. (United States); W. R. Chen, Univ. of Central Oklahoma (United States); H. Liu, The Univ. of Oklahoma (United States)

The purpose of this paper is to investigate the impact of the Depth of Field (DOF) of microscopic systems to cytogenetic image qualities under high throughput scanning. Due to the narrow DOF of high magnification objective lenses, random vibrations of even high precision scanning stages may result large amount of off-focused images. In this study, the DOF of microscopic systems with various objective magnifications / numerical apertures (N.A.) is first analyzed theoretically, and measured using standard resolution targets. The impact of the DOF to cytogenetic image qualities is then subjectively evaluated with clinical samples, by comparing the band shape and sharpness of analyzable chromosomes. For a specific digital microscopic system with 100X objective, 1.26 N.A., the results of observational studies revealed that chromosomal bands are still recognizable when the images are obtained 0.001mm away from the focusing position. The chromosomal bands become fuzzy and unrecognizable when the system is 0.002mm out of focus. Wider DOF is observed when objective lenses with lower magnifications / N.A. are
used, but at the cost of reduced resolving power for cytogenetic features. The results of this study provided useful design trade-off parameters for developing high throughput scanning microscopic systems for cytogenetic applications.

7900-13, Session 4

Direct imaging the subcellular localization of single-walled carbon nanotubes

F. Zhou, D. Xing, South China Normal Univ. (China); W. R. Chen, Univ. of Central Oklahoma (United States)

The development of single-walled carbon nanotubes (SWNTs) for various biomedical applications is an area of great promise. Here, we use confocal microscopy to image the translocation of single-walled carbon nanotubes into cells and localization on the subcellular organelle. We also observe that single-walled carbon nanotubes do not affect the cellular condition and mitochondrial membrane potential. One intrinsic property of single-walled carbon nanotubes is their strong optical absorbance in the near-infrared (NIR) region. It could be used to selectively increase the thermal destructions in the target tumors. A specific type of SWNT by the CoMoCAT method has an intense absorption band at 980 nm. When irradiated with a 980-nm laser, the single-walled carbon nanotubes affect the cellular oxidation and destroy the mitochondrial membrane potential, and induce cell apoptosis.

7900-14, Session 4

Study on the modulation of cellular activity through the integration of gold nanostructured and laser therapy

E. de Oliveira Barreto, F. de Almeida Brito, R. V. Santos, E. J. S. Fonseca, J. M. Hickmann, Univ. Federal de Alagoas (Brazil)

Gold nanoparticles (NPAu) have been attracting growing interest over the last decade because of their unique optical properties and biocompatibility for use in diagnosis, treatment, and as delivery vectors for biologic or pharmacologic agents. The low power laser irradiation (LPLI) is a non-invasive procedure that promotes a beneficial effect in several biological events. However, the effects of LPLI on cells exposed to gold nanoparticle (NPAu) are poorly understood. This study was undertaken to evaluate the effects of LPLI on proliferative response of the cells loaded with gold nanoparticles in vitro. For this, lymphocytes isolated of the lymph from naive mice were incubated overnight with NPAu and exposed to LPLI (10 mw) with variable treatment time. After 4 h the cell morphology and viability were evaluated by optical microscopy and MTT assay, respectively. We will present these evaluations for the lymphocytes incubated with and without (control group) NPAu.

7900-15, Poster Session

Roles of dynamin-related protein 1 in the regulation of mitochondrial fission and apoptosis in response to UV stimuli

Z. Zhang, S. Wu, D. Xing, South China Normal Univ. (China)

Mitochondria are dynamic structures that frequently divide and fuse with one another to form interconnecting network. This network disintegrates into punctiform organelles during apoptosis. However, it remains unclear whether this event has a significant impact on the rate of cell death or only accompanies apoptosis as an epiphenomenon. In this study, we investigate the role of dynamin-related protein 1 (Drp1), a large GTPase that mediates outer mitochondrial membrane fission, in mitochondrial morphology and apoptosis in response to UV irradiation in human lung adenocarcinoma cells (ASTC-a-1) and HeLa cells. Using time-lapse fluorescent imaging, we find that Drp1 primarily distributes in cytosol under physiological conditions. After UV treatment, Drp1 translocates from cytosol to mitochondria, indicating the enhancement of Drp1 mitochondrial accumulation. Down-regulation of Drp1 by shRNA inhibits UV-induced apoptosis. Our results suggest that Drp1 is involved in the regulation of transition from a reticulo-tubular to a punctiform mitochondrial phenotype and mitochondrial fission plays an important role in UV-induced apoptosis.

7900-16, Poster Session

Effect of low-power laser irradiation on macrophage phagocytic capacity

W. Chen, F. Zhou, D. Xing, S. Song, South China Normal Univ. (China); W. R. Chen, Univ. of Central Oklahoma (United States) and South China Normal Univ. (China)

Phagocytosis and subsequent degradation of pathogens by macrophages play a pivotal role in host innate immunity in mammals. Low-power laser irradiation (LPLI) has been found to produce photobiological effects with evidence of interference with immunological functions. However, the effects of LPLI on the immune response have not been extensively characterized. In this study, we focused our attention on the effects of LPLI on the phagocytic activity of macrophages by using flow cytometry (FCM). After irradiating at fluence of 0, 1, 2, 4 J/cm2 with He-Ne laser (632.8 nm, 3mw), the cells were incubated with microsphere and then subjected to FACS analysis. The results showed that LPLI led to an increase in phagocytosis on both mouse peritoneal macrophages and the murine macrophage-like cell line RAW264.7. In addition, we demonstrated that LPLI increased phagocytosis of microsphere in a dose-dependent manner, reaching a maximum at fluence of 2 J/cm2. Taken together, our results indicated that LPLI with appropriate dosage can enhance the phagocytosis of macrophage, and provided an immunological basis for the clinical use of the He-Ne laser.

7900-17, Poster Session

Activation of JNK/Bim/Bax pathway in UV-induced apoptosis

L. Liu, H. Li, Z. Zhang, South China Normal Univ. (China)

Cell apoptosis induced by UV irradiation is a highly complex process in which different molecular signaling pathways are involved. JNK has been proposed as an important regulator in UV radiation-induced apoptosis. However, the molecular mechanism through which JNK regulates apoptosis, especially how JNK activates Bax in response to UV irradiation is still controversial. In this study, using real-time single-cell analysis, we studied the machinery of Bax activation during UV-induced apoptosis. UV treatment resulted in a series of events: phosphorylation of JNK, mitochondrial translocation of Bim, and subsequent activation of Bax. The activation of Bim and Bax could be inhibited in the presence of SP600125 (a specific inhibitor of JNK), suggesting that UV irradiation activated the JNK/Bim/Bax pathway.

7900-18, Poster Session

Bad is not involved in DHA-induced apoptosis in human lung adenocarcinoma ASTC-a-1 cells

H. Yu, Y. Lu, T. Chen, South China Normal Univ. (China)

The Bcl-xL/Bcl-2-associated death promoter (BAD) is a proapoptotic protein in the mitochondrial mediated apoptosis pathway that involves mitochondrial membrane depolarization and the release of cytochrome c, caspase-9 and caspase-3 activation, and more attention was paid on its role in the process of apoptosis. Many researches have shown
that Bad is evenly distributed in cytoplasm in health cells, however, after activation, it translocates to mitochondria and is able to form a heterodimer with anti-apoptotic proteins Bcl-2 and Bcl-xL, and then promote apoptosis. Dihydroartemisinin (DHA), a semi-synthetic derivative of artemisinin, isolated from the traditional Chinese herb Artemisia annua, is recommended as the first-line anti-malarial drug with low toxicity. DHA has been shown to possess promising anticancer activities and induce cancer cell death through apoptotic pathways, however, the molecular mechanisms are not well understood. In this paper, we focus on whether Bad is involved in apoptotic cell death in DHA-treated ASTC-a-1 cells. We conduct our experiments under laser scanning confocal microscope and obtain quantitative results about this question. Our results indicate that Bad is still located in cytoplasm and does not translocate to mitochondria in ASTC-a-1 cells treated with 20 µg/ml of DHA for 48 h while only a small proportion of Bad located in cytoplasm in the 1µM STS-treated cells for 6 h. These results show for the first time that Bad is not involved in DHA-induced apoptosis in human lung adenocarcinoma ASTC-a-1 cells. We hope that our work could give more evidence for the molecular mechanisms of apoptosis induced by DHA.

7900-19, Poster Session

**Taxol-induced paraptosis-like A549 cell death is not senescence**

C. Wang, T. Chen, South China Normal Univ. (China)

We have found that taxol, a potent anticancer agent, induces caspase-independent cell death and cytoplasmic vacuolization. However, the mechanisms of taxol-induced cytoplasmic vacuolization are poorly understood. Cytoplasmic vacuolization have been reported to be involved in vacuolar degeneration and senescence. Here, we employed confocal fluorescence microscopy imaging to study the recovery of taxol-induced cytoplasmic vacuolization and whether taxol trigger senescence in A549 cells. We observed obvious cytoplasmic vacuolization at 6 and 9 h after treatment with 70µM taxol in A549 cells transfected with GFP plasmids, and then we refreshed culture medium to incubate cells for 24 and 72 h respectively. Interestingly, the percentage of vacuolization did not decrease but increase (from 33% to 50%) in the cells treated with taxol for 6 h and subsequently incubated for 24h with fresh medium. But the majority of the cells treated with taxol for 9 h and subsequently incubated for 72 h with fresh medium emerged apoptotic body. These data showed that removal of taxol did not rescue A549 cells from cell death. Positive SA-β-Gal (senescence-associated β-galactosidase) is the typical characteristics of senescence, and hydrolysate of X-Gal, the substrate of SA-β-Gal, can make the nucleus blue. We used cell senescence testing kit to explore whether the cells treated by taxol for 24 h underwent senescence. The nuclei of the cells treated by taxol did not change blue. These data showed that taxol did not induce senescence. Take together, our results showed that taxol-induced cytoplasmic vacuolization is neither vacuolar degeneration nor senescence.

7900-20, Poster Session

**Mathematical modeling of anti-cancer immune response with photodynamic therapy taken into account**

O. G. Isaeva, V. A. Osipov, Joint Institute for Nuclear Research (Russian Federation)

In this study, the tumor induced vascular growth is incorporated into our previous model of tumor immune dynamics. The system of six partial differential equations is formulated for tumour cells, cytotoxic T cells (CD8+ T cells), interleukin-2, endothelial and normal cells as well as for tumour angiogenic factor (TAF), for example, vascular endothelial growth factor (VEGF). The steady state analysis of underlying homogenous system shows that with increasing TAF production by tumor cells the immune system becomes unable to handle tumor growth. The influence of single photodynamic therapy (PDT) on the system dynamics is simulated. In order to include the effects of PDT we add into equations for cellular populations both the equation for the fraction of non-oxidized cellular substratum and corresponding terms describing the loss rate. The value of TAF production is chosen in the range for which there exist two dynamical regimes depending on initial conditions in the system: regression of tumor to the small size and uncontrolled growth. Two different initial densities of endothelial cells are considered in order to examine the influence of vascularization level to the anti-tumor immunity. The low and high levels of vascularization are considered as strong and weak immune response, correspondingly. In the case of strong immune response the PDT is found to give rise to the substantial reduction of tumor size while for weak immune response the tumor continues growing after treatment. These findings indicate the important role of anti-tumor immune response in the long-term tumor control after PDT.

7900-21, Poster Session

**An orange fluorescent protein mKOk for bimolecular fluorescence complementation**

T. Su, Z. Zhang, S. Zeng, Q. Luo, Britton Chance Ctr. for Biomedical Photonics (China)

The bimolecular fluorescence complementation (BiFC) imaging technology based on fluorescent protein (FP) has been successfully applied in visualizing protein-protein interactions (PPI) in many cell types and organisms. To data, several fluorescent proteins with distinct spectra characteristic and from different origin have been developed to be used in BiFC technology. Now, blue (cerulean), green (EGFP), yellow (Citrine and Venus), red (mCherry) and far-red FP (mLumin) are available for BiFC application. Recently, a newly developed orange fluorescent protein named mKO is reported here. Here, we identify mKO can be used in BiFC application below 30°C. To demonstrate the use of mKO based BiFC, we successfully visualize protein-protein interaction in low organism c.elegan. Our mKO based BiFC system testify the feasibility of orange fluorescent protein in BiFC use and fill the color gap between yellow and red spectrum.
An alternative to the classical surgical techniques of modifying the shape of facial cartilages. The method is based on exposure of mechanically deformed cartilaginous tissue to a low level electric field. Electrochemical reactions within the tissue lead to reduction of internal stress, and establishment of a new equilibrium shape. The same reactions offset the electric charge balance between collagen and proteoglycan matrix and interstitial fluid responsible for maintenance of cartilage mechanical properties. The objective of this study was to investigate correlation between the electric charge transferred during EMR and equilibrium elastic modulus.

We used a finite element model based on the modified triphasic theory to study how electric charges generated in the electro-chemical reactions in cartilage can modulate its mechanical responses to step displacements in unconfined compression and tension. The concentrations of the ions, the strain field and the fluid and ion velocities within the specimen subject to an applied mechanical deformation were estimated and apparent elastic modulus (the ratio of the equilibrium axial stress to the axial strain) was calculated as a function of generated charge. The results from finite element calculations showed that the apparent elastic modulus decreases with increase in electric charge. To compare numerical model with experimental observation we measured elastic modulus of cartilage as a function of electric charge transferred in electric circuit during EMR. Good correlation between experimental and theoretical data (R<0.89) suggests that electric charge disbalance is responsible for alteration of cartilage mechanical properties.

The mechanisms of clinical efficacy for many low energy treatments are not well understood. Some treatment mechanisms may include stimulation or inhibition of signaling pathways, growth factor production or release in wound healing, nerve conduction modulation in pain relief, cytokine or hormone release in tissue injury, or protein synthesis in wound healing. Host immune response modulation, a frequently cited treatment effect of low energy irradiation, involves most if not all of these mechanisms and can be difficult to quantitatively document. In addition, placebo effects and biologic ambiguities are ever present problems that can influence clinical and experimental study outcomes related to the medical effects of low energy applications. This two part presentation will first review our current understanding of these applications (Dr. Thomsen). In the second part, the timing and design of pathology studies for low energy applications will be presented and contrasted with those used in high energy pathology investigation (Dr. Coad).

Non-ablative hyperthermic mesenchymal regeneration: a proposed mechanism of action based on the Viveve model

J. Vos, N. Chil, J. E. Coad, West Virginia Univ. (United States)

Minimally invasive interventional technologies have significantly advanced over the past decade. Many of these devices have used supraphysiologic temperatures to ablate or directly necrose the targeted tissues. Newer non-ablative hyperthermic devices are being developed with cryogen surface cooling to rejuvenate/restore tissues without collagen scarring. Recent histology studies, using the Viveve sheep vaginal model, have revealed changes that propose both a mechanism of action and healing timeline for such non-ablative tissue restoring devices. It is proposed that subtle connective tissue changes lead to initial tissue tightening, fibroblast stimulation and subsequent old collagen replacement. In the absence of tissue necrosis, these processes renew the tissue without dense collagen scarring over the subsequent 6 weeks.

The influence of electric charge generated during EMR on mechanical behavior of cartilage

D. E. Protsenko, B. J. Wong, Beckman Laser Institute and Medical Clinic (United States)

Electromechanical reshaping (EMR) of cartilage has been suggested as an alternative to the classical surgical techniques of modifying the shape
Conformal needle-based ultrasound ablation using EM-tracked conebeam CT image guidance

E. C. Burdette, Acoustic Medsystems, Inc. (United States); F. Banovac, Georgetown Univ. (United States); C. J. Diederich, Univ. of California, San Francisco (United States); P. Cheng, E. Wilson, Georgetown Univ. (United States); K. R. Cleary, Georgetown Univ. Medical Ctr. (United States)

This study demonstrated feasibility of spatially tracked image-guided conformal ultrasound (US) ablation for percutaneous directional ablation of diseased tissue. Tissue was prepared by suturing the liver within a pig belly and 1mm BBs placed to serve as needle targets. The image-guided system used integrated electromagnetic tracking and cone-beam CT (CBCT) with conformable needle-based high-intensity US ablation in the interventional suite. Tomographic images from cone beam CT were transferred electronically to the image-guided tracking system (iGSTK). Paired-point registration was used to register the target specimen to CT images and enable navigation. Path planning is done by selecting the target BB on the GUI of the real-time tracking system and determining skin entry location until an optimal path is selected. Power was applied to create the desired ablation extent within 7-10 minutes at a thermal dose (>300eqm43). The system was successfully used to place the US ablator in planned target locations within ex vivo kidney and liver through percutaneous access. Targeting accuracy was 3-4 mm. Sectioned specimens demonstrated uniform ablation within the planned target zone. Subsequent experiments were conducted for multiple ablator positions based upon treatment planning simulations. Ablation zones in liver were 73cc, 84cc, and 140cc for 3, 4, and 5 placements, respectively. These experiments demonstrate the feasibility of combining real-time spatially-tracked image guidance with directional interstitial ultrasound ablation. Interstitial ultrasound ablation delivered on multiple needles permit the size and shape of the ablation zone to be “sculpted” by modifying the angle and intensity of the active US elements in the array. (Supported by NIH R43CA121740,R44CA134169)

Preliminary evaluation of robotic needle distal-tip repositioning in ballistics gelatin

C. J. Walsh, Harvard Medical School (United States); A. H. Slocum, Massachusetts Institute of Technology (United States); R. Gupta, Massachusetts General Hospital (United States)

Advances in medical imaging now provide detailed images of solid tumors inside the body and miniaturized energy delivery systems enable tumor destruction through local heating powered by a thin electrode. We have developed a robot for accurately repositioning the distal tip of a medical instrument such an ablation probe to adjacent points within tissue. The distal tip steering mechanism is based on the concept of substantially straightening a pre-curved Nitinol stylet by retracting it into a concentric outer cannula, and re-deploying it at different axial and rotational cannula positions. The position accuracy in ballistics gelatin was evaluated in a 2D experimental setup with a digital SLR camera that was fixed to a rig that also contained the gelatin. The robot was mounted to the rig in such a way that the stylet was deployed in a plane parallel the camera's lens. A grid paper attached to the back of the box containing the gelatin provided a stationary reference point for each of the pictures taken and also served as a coordinate system for making measurements. The measurement repeatability error was found to be the stylus tip position measurement five times for two different pictures and found to be 0.26 mm. For a stylet with a radius of curvature of 31.5 mm and a diameter of 0.838 mm, the targeting accuracy was found to be 2.5 ± 1.4 mm at points that were approximately 38 mm lateral from the cannula axis.

Patient-specific thermal treatment planning for SonoKnife therapy of head and neck tumors

X. Chen, D. Chen, R. Xia, G. Shafirstein, P. Corry, E. G. Moros, Univ. of Arkansas for Medical Sciences (United States)

The purpose is to develop a 3D thermal treatment planning platform to facilitate the application of a high intensity line-focused ultrasound ablation device (SonoKnife) for treating locally-advanced head and neck tumors. The patient specific heating configurations such as placement, power levels, and heating durations were determined to conform and maximize therapeutic heating and thermal dose coverage to the target region while minimizing the thermal damage to the non-targeted tissues. 3D head and neck tumors as well as the target volumes (e.g. PTV, CTV, GTV) were segmented and reconstructed based on multi-slice MRI/CT scans. The placement (i.e. the scanning pattern, position and orientation) of the line-focus of SonoKnife were designed so that the reconstructed desired target volume can be appropriately covered. Thermal dose-based forward simulations were solved using anatomy-based finite-element thermal solver so that the suitable power level and heating times can be determined. To evaluate the utility of this planning approach and demonstrate heating performance afforded by SonoKnife, representative cases of head and neck tumors were studied in simulations. This patient-specific treatment planning platform with SonoKnife applicators is a useful tool to accurately plan and deliver thermal ablation to superficial head and neck tumors.

Nanoparticle-based hyperthermia cancer treatment: can delivered dose and biological effect be reliably modeled and quantified?

P. J. Hoopes, Dartmouth Medical School (United States); J. A. Pearce, The Univ. of Texas at Austin (United States); R. Ikvov, The Johns Hopkins Univ. (United States); C. R. Sullivan, Dartmouth College (United States); J. C. Bischof, Univ. of Minnesota, Twin Cities (United States); A. A. Petryk, Dartmouth College (United States); A. J. Giustini, Dartmouth Hitchcock Medical Ctr. (United States); J. A. Tate, S. M. Cassim, Dartmouth College (United States); T. P. Ryan, Freefall Consulting (United States)

The reliable and effective use of heat in medicine began about 40 years ago. Essential developments included: the development of sophisticated microwave, radiofrequency and ultrasound delivery systems, the ability to model energy deposition and thermal distribution (Arrhenius models) over time in specific tissue geometries (as defined by 3-D imaging – CT/ MRI), the development of minimally and non-invasive thermometry and finally the development of clinical algorithms for the reproducible and safe use of hyperthermia in human disease. The distinct advantage of magnetic nanoparticle-based hyperthermia, in comparison to other heat therapy techniques, is the ability to target the heat to individual cells (e.g. cancer cells), thus potentially sparing associated normal cells and greatly improving the therapeutic ratio. As such, this modality has great potential as a primary and adjuvant cancer therapy. Although yet unproven, it is assumed that nanoparticle-based clinical hyperthermia therapy will require all of the above techniques. Additionally, nanoparticle hyperthermia will require an accurate determination of specific nanoparticle heating capability, the total nanoparticle content and biodistribution in the target cells/tissue and an effective and matching alternating magnetic field (AMF) for excitation of the nanoparticles. Our initial studies have shown that appropriately delivered and targeted nanoparticles are capable of achieving effective cytotoxicity at global thermal doses significantly less than the thermal doses necessary to achieve equitable tissue/tumor treatment effects using standard thermal therapy techniques. Therefore the accepted
7901-10, Session 3

FEM numerical model study of heating in ferromagnetic nanoparticles

J. A. Pearce, J. R. Cook, The Univ. of Texas at Austin (United States); P. J. Hoopes, Dartmouth Medical School (United States); A. J. Giustini, Dartmouth College (United States)

Electromagnetic heating of ferromagnetic nanoparticles is complicated by the extremely short thermal relaxation time constants and difficulty of coupling sufficient power into the particles to achieve desired temperatures. Magnetic field heating by the hysteresis loop mechanism at frequencies between about 150 and 160 kHz has proven to be an effective mechanism in γ-hematite Fe2O3 and magnetite Fe3O4 nanoparticles. Experiments at 2.45 GHz show that γ-hematite nanoparticle dispersions in the range of 10^12 to 10^13 NP/mL also heat substantially at this frequency - temperatures of 58 °C were observed after 10 minutes under a standard diathermy "corner director" at 100 W of generator power. Microwave heating in these particles is most likely due to the observed nearby relaxation maximum in the imaginary permeability, μ′′, at 1.5 GHz.

An FEM numerical model study was undertaken to estimate the order of magnitude of volume power density, Qgen (W/m^3) required to achieve significant heating in evenly dispersed and aggregated clusters of nanoparticles. The FEM models were computed using Comsol Multiphysics; consequently the models were confined to continuum formulations and did not include film nano-dimension heat transfer effects at the nanoparticle surface. As an example, the models indicate that for a single 36 nm diameter particle a volume power density in the neighborhood of 10^17 (W/m^3) will result in a steady state particle temperature of 52 °C - the total power coupled to the particle is 2.44 µW.

7901-11, Session 3

Nanoparticle heating: nanoscale to bulk effects of electromagnetically heated iron oxide and gold for biomedical applications

Z. Qin, M. Etheridge, J. C. Bischof, Univ. of Minnesota, Twin Cities (United States)

Biomedical applications of nanoparticle heating range in scale from molecular activation (i.e. molecular beacons, protein denaturation, lipid melting and drug release), cellular heating (i.e. nanophotolysis and membrane permeability control and rupture) to whole tumor heating (deep and superficial). This work will present a review of available literature on heating of two classes of biologically compatible metallic nanoparticles: iron oxide and gold with particular focus on spatial and temporal scales of the heating event. The size range of nanoparticles under discussion will focus predominantly in the 10 - 200 nm diameter size range. Mechanisms of heating range from Neelian and Brownian relaxation due to magnetic susceptibility at 100s of kHz, optical absorption due to VIS and NIR lasers and “Joule” heating at higher frequency RF (13.56 MHz). While classical approaches allow heating at the individual nanoparticle to be calculated, recent experimental efforts to measure the nanoscale temperature increase near the nanoparticle and hence the heat generation will be discussed. This review will also discuss how to create a specific absorption rate (W/g) based on individual nanoparticles heating in bulk samples. Non-idealities important to this process such as nanoparticle aggregation and inhomogenous distribution will also be discussed.

7901-12, Session 3

Modelling and characterization of photothermal effects assisted with gold nanorods in ex-vivo samples and in a murine model

F. Rodriguez, Sr., H. Rivera Lamela, Sr., V. B. Cunningham, Univ. Carlos III de Madrid (Spain)

Photothermal Therapy is a recent optical non-invasive ablation technique for tumor treatment. This method uses optical energy to induce a temperature increment within the tissue area where the tumor is located. Among the advantages of using light energy sources are the high selectivity of the energy deposition and the non-ionizing character of the optical energy. The deepest light penetration is reached when using near infrared wavelengths because this region of the spectra is within the biomedical optical window.

In order to improve the efficiency of using compact diode lasers, we have selected Gold Nanorods as an absorbing contrast agent because of their high opto-thermal conversion efficiency in comparison with other types of gold nanoparticles available (Geoffrey et al, Cancer Res, 69, 2009) as these nanoparticles, made on gold, are demonstrated to have low toxicity and they present tunable optical resonance (Dakron Pissuwan et al, Genetic Engineering Reviews, 25, 2008). We have selected Gold Nanorods with long surface Plasmon resonance (LSPR) at NIR wavelengths, concretely at 808 nm and 850 to improve the efficiency of the photothermal therapy technique.

In this context, we discuss in this paper the implementation of a laser-tissue interaction and bioheat-transfer finite-element model (FEM) for Photothermal Therapy assisted with Gold Nanorods. The goal is to model the distribution of the optical energy among the tissue including the skin absorption effects and the tissue thermal response, with and without the presence of Gold Nanorods. An optical absorption analysis has been done previously, motivated by the authors work on gold nanospheres particle concentration measurements within a turbid media that mimics healthy soft tissue at a single wavelength of 532 nm (Cunningham and Lamela, JOLT, 42, 2010) and spherical nanoparticle spectroscopic analysis characterisation within the visible range from 410 to 650 nm (Lamela, Cunningham and Gallego, JOLT, 43, 2010). Based on these absorption studies, the heat generation due to the maximum optical energy absorption and the thermal propagation will be as well optimized. The model has been evaluated and compared with experimental ex-vivo data in fresh chicken muscle samples and in-vivo BALB/c mice animal model. Finally, we will study the photothermal therapy in tumors by using the CT-26 human cancer cell-line to induce the corresponding xenografts in the animal’s back of these BALB/c mice.

7901-13, Session 3

Comparison of microwave and iron-oxide nanoparticle hyperthermia radiosensitization in murine breast tumors

A. J. Giustini, Dartmouth Medical School (United States) and Dartmouth College (United States); A. A. Petryk, Dartmouth College (United States); P. J. Hoopes, Dartmouth Medical School (United States)

Hyperthermia has been shown to be an effective radiosensitizer. Its utility as a clinical modality has been limited by a minimally selective tumor sensitivity and the inability to be delivered in a tumor-specific manner. Recent in vivo studies (rodent and human) have shown that cancer cell-specifc cytotoxicity can be effectively and safely delivered via iron oxide
magnetic nanoparticles (mNP) and an appropriately matched noninvasive alternating magnetic field (AMF). To explore the tumor radiosensitization potential of mNP hyperthermia we used a syngeneic mouse breast cancer model, dextran-coated 110 nm hydrodynamic diameter mNP and a 163 kHz / 450 Oe (35.8 kA/m) AMF. Intradermally implanted (flank) tumors (150 ± 40 mm3) were treated by injection of 0.04 ml mNP (7.5 mg Fe) / cm3 into the tumor and an AMF (35.8 kA/m and 163 kHz) exposure necessary to achieve a CEM (cumulative equivalent minute) thermal dose of 60 (CEM 60). Tumors were treated with mNP heat alone (CEM 60), radiation alone (15 Gy, single dose) and in combination. Compared to the radiation and heat alone treatments, the combined treatment resulted in a greater than two-fold increase in tumor regrowth delay (tumor treatment efficacy). None of the treatments resulted in significant normal tissue toxicity or morbidity. Studies were also conducted to compare the radiosensitization effect of mNP hyperthermia with that of microwave-induced hyperthermia. The effects of incubation of nanoparticles within tumors (to allow nanoparticles to be endocytosed) before application of AMF and radiation were determined. This information suggests cancer cell specific hyperthermia (i.e. antibody-directed or anatomically-directed mNP) is capable of providing significantly greater radiosensitization / therapeutic ratio enhancement than other forms of hyperthermia delivery.

7901-14, Session 4
Computational modeling of high-intensity focused ultrasound mediated drug delivery

D. Haemmerich, A. Gasseihuber, Medical Univ. of South Carolina (United States)

Background: Low-Temperature Sensitive Liposomes (LTSL) are drug delivery vehicles with long plasma half-life, which release the drug upon heating above ~40 °C. The combination of LTSL with local heat generated by focused ultrasound may thus allow non-invasively targeted drug delivery. The complex interplay between heat-based cancer therapy and drug delivery requires computational models to identify the relationship between heat exposure and pharmacokinetics in order to optimize drug delivery.

Methods: We created Finite Element Method computer models where SAR calculated from phantom experiments data were used as input data. Microvascular perfusion was modeled according to Pennes’ Bioheat Equation, and was varied with temperature. A spatio-temporal multi-compartment pharmacokinetic model was created to describe the release of doxorubicin (DOX) from LTSL into the tumor plasma space, and subsequent transport into the extracellular space, and the cells. Tissue was heated for up to 10 min at target temperatures between 42 °C and 60 °C at the ultrasound focal spot, and spatio-temporal drug concentration profiles were calculated.

Results: At target temperatures in the hyperthermic range, maximum drug concentrations of ~ 9 ug/g were observed in the central heating area, with very localized drug deposition. At ablative temperatures similar maximum drug concentrations were observed, with a ring-like spatial concentration profile due to progressive shut-down of perfusion at temperatures above 45 °C. Concentration increased approximately linearly with time during the examined time periods.

Conclusion: Multi-physics mathematical models may allow for optimization of heat-mediated targeted drug delivery from LTSL via high-intensity focused ultrasound.

7901-15, Session 4
Determination of cellular injury and death thresholds following exposure to high-voltage 10-ns electrical pulses

C. C. Roth, Air Force Research Lab. (United States); O. P akhomova, A. G. Pakhomov, Old Dominion Univ. (United States); G. J. Wilmink, B. L. Ibye, Air Force Research Lab. (United States)

Intense, nanosecond-duration electric pulses (nsEP) have been introduced recently as a novel modality to alter cellular function, with a mechanism of action qualitatively different from micro- and millisecond pulses (electroporation). In this study, we determined the thresholds for plasma membrane injury (acute) and cell death (at 24 hours) for 5 different cell types (ChoK1, HeLa, GH3, Jurkat and U937). Plasma membrane injury was measured by flow cytometry using two fluorescent dyes, namely Annexin V-FITC, which binds to phosphatidyserine(PS) upon its externalization (subtle membrane injury), and propidium iodide, which is typically impermeable to the cell, but enters when large pores are formed in the plasma membrane. In all cell types, 10-ns pulses caused only phosphatidyserine (PS) externalization at mild doses (<150kV/cm and 100 pulses for each cell type), but at strong doses (>150kV/cm and >100 pulses) immediate propidium iodide (PI) uptake and PS externalization was observed. Jurkat, U937, and GH3 cells lines showed substantial cell death without acute uptake of PI (15 minutes post exposure) suggesting either delayed permeabilization due to swelling, or damage to intracellular components. In CHO and HeLa cell lines, externalization of PS and PI uptake occurred at low doses relative to that necessary to cause cell death suggesting a necrotic death similar to longer pulse exposures. These findings suggest that nanosecond pulses may be beneficial in applications that require selective elimination of specific cell types.

7901-16, Session 4
How does temperature affect the function of tissue macrophages?

C. Lee, Roswell Park Cancer Institute (United States)

Macrophages sound a major danger signal following injury or infection and become activated to release pro-inflammatory cytokines. Usually, tissue macrophages are exposed to an elevated temperature during inflammation. However, whether macrophages sense and respond to temperature change to modulate their function is still not clear. Here we studied thermal effects on macrophage functions from LPS-challenged mice at early or late activation stages which are representative of the initiation and resolution phases of inflammation. Our data suggest that in the initiation phase, thermal stress acts as a stimulus to enhance macrophage functions. However, for previously activated macrophages, thermal stress provides a negative signal that suppresses their cytokine production. These results increase our understanding of the role of elevated tissue temperature on modulation specific functions of macrophages. They also provide additional rationale for the use of hyperthermia in the treatment of chronic inflammation.

7901-17, Session 4
Correction of tissue perfusion by terahertz waves

A. N. Ivanov, V. F. Kirichuk, T. S. Kiriyazi, Saratov State Medical Univ. (Russian Federation)

Changes in regional blood flow and systemic hemodynamics associated primarily with violations of microcirculation. The aim of the study was to study the effect of exposure to electromagnetic waves at frequencies of molecular spectrum of emission and absorption of nitric oxide 150.176 - 150.664 GHz in peripheral perfusion in white rats in a state of acute stress.

The studies were conducted on 45 albino male rats (weigh 180-220 g.) Acute stress caused by 3-hour immobilization of animals in the supine position. Irradiation of animals was carried out with small-sized medical device “UHF-NO-Orbita” (Saratov, Russia). A single 30 minutes exposure of animals in a state of acute stress, was carried by electromagnetic waves at frequencies of molecular spectrum of radiation and absorption of nitric oxide 150.176 - 150.664 GHz.

The study was conducted in 3 groups of animals to 15 animals each:
Femtosecond laser radiation was revealed. The effect occurred to be dose-dependent. High undulating dependence of changes of morphofunctional state and loss of Er under irradiation of femtosecond laser with corresponding parameters was revealed.

7901-20, Session 4

Removal of brain tissue by Tm-Fiber laser
B. Tunç, M. Gülsoy, Bogaziçi Univ. (Turkey)

The aim of the study was to investigate the thermal effects of the 1940-nm Tm-fiber laser on the dead brain tissue. 4-5 mm coronal sections were taken from lamb brains. Tm-fiber laser was applied at the back (cortical) and below the cortex (subcortical) of this slices with 0.5 mm distance. At the beginning of the research in order to find appropriate laser parameter to be compared for 1940-nm Tm-fiber laser, the carbonization and coagulation times of the brain slices were recorded for each power value, both for cortical and subcortical tissue. The appropriate laser parameters for lamb brain tissue were selected according to this study. Lasers were applied in both continuous and pulsed modes. In continuous mode doses were changed with fixed application time. In pulsed mode doses were modified with the change in pulse width. The lesions were determined with microscope. The radius of ablation and coagulation for each laser application was recorded. By calculating ablation efficiency (100 x ablation / calculation radius) the appropriate laser doses were determined for both cortical and subcortical tissue. The maximum ablation efficiency for cortical tissue in continuous mode was 200 mW and 600 mW and in pulsed mode was 600 mW and for subcortical tissue maximum ablation efficiency was found 600 mW in both continuous mode and pulsed mode.

7901-19, Session 4

Effects of femtosecond laser radiation on blood cell suspensions
T. Genning, Ulyanovsk State Univ. (Russian Federation); A. A. Sysoliatin, A. M. Prokhorov General Physics Institute (Russian Federation); T. Abakumova, D. Arslanova, O. Voronova, I. Zolotovsky, V. Ostatchnkov, M. Yavtushenko, Ulyanovsk State Univ. (Russian Federation)

Femtosecond laser, created in FORC RAS with the help of specialists from UlSU Univ. (Russian Federation) with the pulse repetition frequency up to 100 MHz, operating wavelength of 1560 nm, pulse duration of 400-50 fs was used in this work. The energy of separate laser pulse was up to 10 pJ. Er and Hf of human being and rat was used in the experiment in which after subpicosecond laser irradiation (duration of exposure was 1.3, 3.5, 7 and 9 min) of impulses with peak intensity 0.75 kW/sm2 and 2.0 kW/sm2 was investigated morphofunctional state regarding the level of peroxidation of lipids and the activity of enzymatic link of antioxidant system, oxygen dependence and anaerobic bactericidal action and phagocytic activity, regarding changes of topology and rigidity of cell’s membrane. The great change of morphofunctional state of blood cells in vitro under influence of femtosecond laser radiation was revealed. The effect occurred to dose-dependent. High undulating dependence of changes of morphofunctional state and loss of Er under irradiation of femtosecond laser with corresponding parameters was revealed.
Microwave thermal imaging with HIFU treatment: in-vivo and ex-vivo animal experiments

T. Zhou, P. M. Meaney, S. D. Geimer, K. D. Paulsen, Dartmouth College (United States)

High intensity focused ultrasound (HIFU) uses focused ultrasound beams to ablate localized tumors noninvasively. Multiple clinical trials using HIFU treatment of liver, kidney, breast, pancreas and brain tumors have been conducted, while monitoring the temperature distribution with various imaging modalities such as MRI, CT and ultrasound. HIFU has achieved only minimal acceptance partially due to insufficient guidance from the limited temperature monitoring capability and availability. MR proton resonance frequency (PRF) shift thermometry is currently the most effective monitoring method; however, it is insensitive in temperature changes in fat, susceptible to motion artifacts, and is high cost. Exploiting the relationship between dielectric properties (i.e. permittivity and conductivity) and tissue temperature, in vivo dielectric properties of tissue during heating were reconstructed with our microwave tomographic imaging technology. Previous phantom studies have demonstrated sub-Celsius temperature accuracy and sub-centimeter spatial resolution in microwave thermal imaging. However, these experiments were limited to geometries where the transducer was positioned within the microwave antenna array which has limited clinical utility. Experiments in this paper demonstrate the effectiveness of the combined HIFU/microwave imaging capability with the transducer heated outside of the array for a more flexible clinical configuration. Initial ex vivo and in vivo animal experiments have been conducted to further investigate its potential.

Catheter-based ultrasound hyperthermia in conjunction with HDR brachytherapy for treatment of locally advanced cancer of the prostate and cervix

C. J. Diederich, J. Wootton, P. Prakash, V. A. Salgaonkar, T. Juang, X. Chen, A. M. Cunha, J. Pouliot, I. Hsu, Univ. of California, San Francisco (United States); C. J. Diederich, Univ. of California, San Francisco (United States); E. C. Burdette, Acoustic Medsystems, Inc. (United States); C. J. Diederich, Univ. of California, San Francisco (United States)

Interstitial and endocavity ultrasound devices have been developed specifically for applying hyperthermia within tandem HDR brachytherapy implants during radiation therapy. Catheter-based ultrasound applicators are capable of 3D spatial control of heating in both angle and length of the devices, with enhanced radial penetration of heating compared to other hyperthermia technologies. A pilot study of the combination of catheter based ultrasound with HDR brachytherapy for locally advanced prostate and cervical cancer has been initiated, and preliminary results of the performance and heating distributions are reported herein. The treatment delivery platform consists of a 32 channel RF amplifier and a 48 channel thermocouple monitoring system. Controlling software can monitor and regulate frequency and power to each transducer section as required during the procedure. Interstitial applicators consist of multiple transducer sections of 2-4 cm length x 180 deg and 3-4 cm x 360 deg, heating patterns to be inserted in specific placed 13g implant catheters. The endocavity device, designed to be inserted within a 6 mm OD plastic tandem catheter within the cervix, consists of 2-3 transducers x dual 180 or 360 deg sectors. 3D temperature based treatment planning and optimization is dovetailed to the HDR optimization based planning to best configure and position the applicators within the catheters, and to determine optimal base power levels to each transducer section. To date we have treated ten cervix implants and six prostate implants. 100% of treatments achieved a goal of >60 min duration, with therapeutic temperatures achieved in all cases. Thermal dosimetry within the hyperthermia target volume (HTV) and clinical target volume (CTV) are reported. Peak temperatures at each spatial position within the HTV are reported and compared to treatment planning simulations. Catheter-based ultrasound hyperthermia with HDR appears feasible with therapeutic temperature coverage of the target volume while sparing surrounding more sensitive regions. (Acknowledgement: NIH-R01CA122276)

Experimental characterization of the acoustic edge of a SonoKnife applicator

R. Xia, D. Chen, X. Chen, G. Shafirstein, P. Corry, E. G. Moros, Univ. of Arkansas for Medical Sciences (United States)

SonoKnife is a scan-able line-focused transducer to deliver thermal ablation (52-60oC) to superficially located tumors or malignant nodes. Based on preliminary simulation results, a prototype cylindrical section transducer operating at 3.54 MHz, with a 60 mm radius of curvature, an altitude of 30 mm was constructed for laboratory testing. The three-dimensional distribution of the acoustic field was measured and compared with preliminary numerical results. Acoustic parameters such as instantaneous maximum acoustic pressure, maximum acoustic power, acoustic beam size (FWHM), and mechanical and electrical coupling efficiency of the transducer, were derived or measured. The experimental results agreed well with the theoretical simulations, and showed that the SonoKnife transducer has a very narrow acoustic edge and can generate sufficient power to ablate biological tissue. [Work supported by: NCI RC1 CA147697 and the Central Arkansas Radiation Therapy Institute (CARTI).]

Hepatic ablation with multiple interstitial ultrasound applicators: initial ex vivo and computational studies

P. Prakash, V. A. Salgaonkar, Univ. of California, San Francisco (United States); E. C. Burdette, Acoustic Medsystems, Inc. (United States); C. J. Diederich, Univ. of California, San Francisco (United States)

Radiofrequency (RF) ablation has emerged as an effective method for treating liver tumors under 3 cm in diameter. Multiple applicator devices and techniques - using RF, microwave and other modalities - are under development for thermal ablation of large and irregularly-shaped liver tumors. Interstitial ultrasound (IOUS) applicators, comprised of linear arrays of independently powered tubular transducers, enable 3D control of the spatial power deposition profile and simultaneous ablation with multiple applicators. We evaluated IUS applicator configurations (parallel, converging and diverging implants) suitable for percutaneous and laparoscopic placement with experiments in ex vivo bovine tissue and computational models. Ex vivo ablation zones measured 4.6±0.5 x 4.2±0.5 x 3.3±0.5 cm3 and 5.6±0.5 x 4.9±0.5 x 2.8±0.3 cm3 using three parallel applicators spaced 2 and 3 cm apart, respectively, and 4.0±0.3 x 3.1±0.4 x 2.9±0.2 cm3 using two parallel applicators spaced 2 cm apart. Computational models indicate in vivo ablation zones up to 4.2 x 4.0 x 5.2 cm3 and 5.5 x 5.1 x 5.3 cm3, using three applicators spaced 2 and 3 cm apart, respectively. Converging and diverging implant patterns can also be employed for conformal ablation of irregularly-shaped tumor margins by tailoring power levels along each device. Simultaneously powered interstitial ultrasound devices can create tailored ablation zones comparable to currently available RF devices and similarly sized microwave antennas.

We acknowledge support by NIH grants R01CA122276, R43CA121740, R44CA134169.
Fast optimization and planning of clinical hyperthermia using superposition and surrogate models of temperature distributions

V. A. Salgaonkar, P. Prakash, C. J. Diederich, Univ. of California, San Francisco (United States)

Hyperthermia improves the response of tumor cells to radiation treatment. Conformal hyperthermia can be administered in the prostate, immediately following radiation, using multiple (2-6) directional ultrasound transducer arrays through previously implanted HDR brachytherapy catheters. These ultrasound applicators are designed with 4 independently powered sectored tubular transducer elements to induce localized heating with spatial control in angle and length.

To plan a hyperthermia treatment, the patient anatomy and catheter geometry were reconstructed from CT images. Transducer powers and aiming directions were estimated to maximize the heated tumor volume, while sparing the surrounding organs. Fast computation of temperature elevations was performed by approximating the temperature rise induced at a point as the superposition of temperature increases resulting from individual transducers. Steady state temperature increases due to individual transducer elements (90-360° sector angle, 0-3 W power) were computed and fitted to spline models. Superposition and surrogate models were used instead of computationally expensive 3D finite element (FE) methods during treatment planning.

The approximate models were included in a global search framework reducing the time required to evaluate the objective function by a factor of 9 (~0.8 s), compared to the FE solution (~7 s). For 10 patient cases with dominant intraprostatic lesions, the optimized treatment plans were furnished in less than 12 minutes and yielded T90 > 40.0 °C, T50 > 42.8 °C, and T10 > 44.2 °C. The maximum temperatures in the bladder and rectum were below 40.2 °C, and the maximum temperature in the urethra was below 43.6 °C. (NIHRO1CA122276)

Investigation of the electrical conductivity in perfused liver using micro-electrical probe

M. Yi, IBM China Co. Ltd. (China); R. J. Podhajsky, Covidiem (United States); R. L. Mahajan, Virginia Polytechnic Institute and State Univ. (United States) and Virginia Tech (United States)

In this paper, we present the design, fabrication and calibration of a novel micro-machined electrical probe and experimental studies on liver tissues using this probe. The probe was fabricated by photolithography and mounted in a catheter, 1.5mm in diameter. First, the micro probe was calibrated against the standard four-electrode probe. The micro electrical probe was then used to investigate the effect of temperature elevation on the electrical conductivity of liver tissues by different heating methods. Also, the electrical conductivity change caused by directional placement and perfusion rate was investigated on a perfused pig liver model. The experimental results show that the local electrical conductivity varies location to location and that the electrical conductivity has a strong directional dependence. Also by varying the perfusion rate, the probe shows that the local electrical conductivity varies linearly with the square root of perfusion rate.

Ultra-miniature wireless temperature sensor for thermal medicine applications

A. B. Khairi, S. Hung, J. Paramesh, G. K. Fedder, Y. Rabin, Carnegie Mellon Univ. (United States)

This study presents a prototype design of an ultra-miniature, wireless temperature-sensor implant, with applications to thermal medicine such as cryosurgery, hyperthermia, and thermal ablation. The design aims at an implant smaller than 1.5 mm in diameter and 3 mm in length, to enable minimally invasive deployment through a hypodermic needle. While the implant may be used for local temperature monitoring, simultaneous data collection from an array of such implants can be used to reconstruct the 3D temperature field in the treated area, offering a unique capability in thermal medicine.

The implant consists of three major subsystems: a temperature-sensing core, a wireless data-communication unit, and a wireless power reception and management unit. Power is delivered wirelessly to the implant from an external source using an inductive link. To meet size requirements while enhancing reliability and minimizing cost, the implant is fully integrated in a regular foundry CMOS technology (~0.15 µm in the current study), including the implant-side inductor of the power link.

A temperature-sensing core that consists of a proportional-to-absolute-temperature (PTAT) circuit has been designed and characterized. It employs a microwatt chopper stabilized op-amp and dynamic element-matched current sources to achieve high absolute accuracy. A second order sigma-delta modulator analog-to-digital converter is designed to convert the temperature readings to a digital code, which is transmitted by backscatter through the same antenna used for receiving power. A high-efficiency multi-stage differential CMOS rectifier has been designed to provide a DC supply to the sensing and communication subsystems.

Electrical property based biopsy for prostate cancer detection and assessment

R. J. Halter, Dartmouth College (United States); J. Heaney, A. Schned, Dartmouth Hitchcock Medical Ctr. (United States)

Prostate cancer diagnosis is based solely on biopsy-based findings. Unfortunately, routine biopsy protocols only sample ~0.95% of the entire gland limiting the technique’s sensitivity to cancer detection. Previous studies have demonstrated significant electrical property differences between malignant and benign prostate tissues due to their dissimilar morphological architectures. We have taken the important step of translating these findings to the clinic by integrating an electrical property sensor into the tip of a standard biopsy needle. This novel device allows clinicians to simultaneously extract a tissue core and assess the electrical properties around the needle tip in real-time. The expected volume of tissue sensed with this device was estimated using finite-element (FEM) based simulations to model the potential fields and current distributions. Prototype devices have been constructed and evaluated in a series of saline baths in order to validate the FEM-based findings. Simulations suggest that the electrical property sensor is able to interrogate a tissue volume of ~62.1 mm3 and experimental results demonstrated a volume of sensitivity of ~68.7 mm3. This coupled device is being used to assess the increased sensitivity and specificity to cancer detection when electrical properties are sensed in concert with tissue core extraction in a series of 50 ex vivo prostates. Typical 12-core prostate biopsy protocols extract a total tissue volume of 228 mm3 for histological assessment. Employing this electrical property sensor to gauge electrical properties at both the beginning and end of the needle trajectory will provide pathological assessment of an additional 1648 mm3 of tissue.

Hyperthermia and microwave radiometry for non-invasive detection of vesicoureteral reflux (VUR)

P. R. Stauffer, P. F. Maccarini, K. Arunachalam, S. Salahi, V. De Luca, Duke Univ. (United States); O. Klemtesen, Y. Birkeland, S. K. Jacobsen, Univ. of Tromsø (Norway); F. Bardati, Univ. degli
Lateral area, thus making this a practical approach for treatment of skin of maximum structural collagen content in skin tissues. Using an which concentrate in a depth profile that coincides with the location versus 8-15% for focused ultrasound. Therapeutic ultrasound applied at 2-5mm depth. Localized collagen changes ranged from 1-3% for RF each experimental series, therapeutic dose levels (60°C) were attained were made between RF and focused ultrasound for five energy ranges. In Tissue shrinkage was measured using fiducials and video image pigs. These were treated with ultrasound (9-11MHz) focused in the deep of young porcine underbelly skin tissue and 61 in-vivo areas in 60-80 kg cancer. Acute studies were performed in 20 freshly-excised samples of various types of tissue were placed between the opposing cooling cells in contact with the windows, and irrigated from one and both directions simultaneously. Surface cooling temperatures were varied, along with irradiation conditions. Temperatures were measured at defined points within the tissue during and after irradiation. For comparison, the irradiations were repeated using a standard 808 nm diode laser. In a second set of experiments, ex vivo human tonsil specimens were irrigated and the results compared to model calculations using derived optical constants. Results show the ability of 1125 nm radiation to heat thick tissue sections and whole tonsils homogeneously, with minimal interference by tissue blood content.

7901-31, Session 7

Ultrasound therapy applicators for controlled thermal modification of tissue

E. C. Burdette, Acoustic Medsystems, Inc. (United States); C. J. Diederich, Univ. of California, San Francisco (United States)

The purpose of this study was to determine the feasibility for using acoustic energy for controlled dose delivery sufficient to produce collagen modification for the treatment of skin tissue in the dermal and sub-dermal layers. We designed and evaluated a curvilinear focused ultrasound device for treating skin disorders such as psoriasis, stimulation of wound healing, tightening of skin through shrinkage of existing collagen and stimulation of new collagen formation, and skin cancer. Acute studies were performed in 201 freshly-excised specimens of young porcine underbelly skin tissue and 61 in-vivo areas in 60-80 kg pigs. These were treated with ultrasound (9-11MHz) focused in the deep dermis. Dose distribution was analysed and gross pathology assessed. Tissue shrinkage was measured using fiducials and video image registration and analysed using NIH Image-J software. Comparisons were made between RF and focused ultrasound for five energy ranges. In each experimental series, therapeutic dose levels (60degC) were attained at 2-5mm depth. Localized collagen changes ranged from 1-3% for RF versus 8-15% for focused ultrasound. Therapeutic ultrasound applied at high frequencies can achieve temperatures and dose distributions which concentrate in a depth profile that coincides with the location of maximum structural collagen content in skin tissues. Using an appropriate transducer configuration produces coverage of significant lateral area, thus making this a practical approach for treatment of skin disorders.

7901-32, Session 7

1125-nm quantum dot laser for tonsil thermal therapy

K. McMillan, gRadiant Research, LLC (United States)

Tonsillecmy is the most common major surgical procedure in children, and its use is increasing due to the benefits it provides in the treatment of hypertrophic tonsils. However, the procedure is associated with considerable postoperative pain and risk of bleeding. Thermal therapy has the potential to provide a nonexcisional alternative. The wavelength of 1125 nm is evaluated here for its ability to produce consistent and homogeneous heating of soft tissue, including tonsils, due to its deep penetration and minimal absorption by blood. Tissue heating produced by this new wavelength was characterized using a prototype quantum dot diode laser. The output of the 30 W laser was split into two components and delivered with even energy distribution to window surfaces of two cooling cells. In a first set of experiments, specimens of various types of tissue were placed between the opposing cooling cells in contact with the windows, and irradiated from one and both directions simultaneously. Surface cooling temperatures were varied, along with irradiation conditions. Temperatures were measured at defined points within the tissue during and after irradiation. For comparison, the irradiations were repeated using a standard 808 nm diode laser. In a second set of experiments, ex vivo human tonsil specimens were irrigated and the results compared to model calculations using derived optical constants. Results show the ability of 1125 nm radiation to heat thick tissue sections and whole tonsils homogeneously, with minimal interference by tissue blood content.

7901-33, Session 7

Hyperthermic tissue sealing devices: a proposed histopathologic protocol for standardizing sealed vessel evaluation

R. Livengood, M. Jessop, J. E. Coad, West Virginia Univ. (United States)

Hyperthermic tissue sealing devices have modified laparoscopic surgical procedures. A number of studies have histologically assessed vascular seals made with these devices. However, a standardized histopathology approach has not been used, which limits the comparison of results between the devices. This paper will propose a unified approach to the dissection, processing, cutting, staining and interpretation of Day 0-3 thermally sealed a vessel that is independent of the device used. This protocol proposes a set of standardized definitions for the different thermal seal zones (seal, thermal/heat fixed zone, coagulative necrosis zone, transition zone, and viable region), the seal's architectural construct (vessel components comprising the seal), seal width, radial thermal spread, thrombosis, and others. In addition, the proposal outlines an approach to tissue handling, staining (viability stains vs. H&E), processing and sectioning that should enhance documentation of the seal's properties.

7901-34, Session 7

Dual thermal ablation modality of solid tumors in a mouse model

G. Shafirstein, E. G. Moros, K. Barnes, L. Hennings, J. Weber, B. Przybyla, R. Griffin, Univ. of Arkansas for Medical Sciences (United States)

Purpose: Develop a new combination therapy consisting of cryoablation and conductive high-temperature ablation for enhanced thermal ablation of solid tumors.
Methods: We have constructed an invasive probe that can be used for consecutive cryoablation and high-temperature ablation (C/HTA), with a single insertion. The C/HTA probe was tested, in Balb/c mice bearing solid 4T1 tumors, in comparison to cryoablation and high temperature ablation, only. Three days after ablation, the diameter of the ablated zone was evaluated with pathological examination.

Results: The C/HTA device can be used to induce larger ablation zones, in comparison to high temperature or cryoablation, at lower thermal doses and temperature than either modality alone.

Conclusions: The relatively high thermal conductivity of ice, in comparison to water and native tissue, enables rapid heating of the ice-ball that result in improved conductive high temperature ablation. We hypothesized that the initial cryoablation induces vascular thrombosis that reduced blood flow to the ablated region and sensitized the margins to the hyperthermic temperatures at the outskirts of the high temperature ablated region by reducing the intracellular pH of the treated margins and thus improve ablation outcomes at lower thermal doses in comparison to a single ablation modality.

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7901-35, Session 7

Arrhenius parameters for primary thermal injury in human tonsillar tissue

K. McMillan, gRadiant Research, LLC (United States); R. A. Radabaugh, J. E. Coad, West Virginia Univ. (United States)

Clinical implementation of thermal therapy requires an ability to predict tissue response to specific thermal histories. However, parameters characterizing this response are unknown for many tissues of potential therapeutic importance. In this work, as part of an effort to develop a nonexcisional thermal therapy alternative to traditional tonsillectomy, the response of human tonsillar tissue has been characterized and rate parameters for thermal injury have been derived. Thirty hypertrophic tonsils were obtained from 15 pediatric patients undergoing tonsillectomy. Freshly excised tissues were sectioned and placed in a water bath at temperatures of 40 to 70°C for hold times of one to seven minutes. Tissue sections were stained with nitroblue tetrazolium to assess for thermal respiratory enzyme inactivation as a marker for primary thermal cell death. Results yielded thermal matrices corresponding to a wide range thermal dose. Assessments were made by quantification of staining using image analysis, and yielded Arrhenius parameters for lymphoid tissue of the tonsil parenchyma. Viability of the lymphoid tissue was compared to squamous epithelium and perivascular smooth muscle in the tonsil. The significance of using NBT staining for guiding thermal therapy is discussed, along with the implications of the findings on the development of tonsillar thermal therapy.

New visualization strategies to study the dynamics of surgical coagulation devices in biological tissue using high-speed and absolute subsurface thermal imaging

R. M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); S. Been, J. H. Klaessens, Univ. Medical Ctr. Utrecht (Netherlands)

Visualisation of the thermo dynamics of surgical coagulation devices like diathermia, RFA and lasers in tissue are essential to get better understanding about the principles of operation. Thermal cameras have the ability to visualize absolute temperatures but are limited to the surface of a tissue at typical video rates (25 f/s). We have developed new strategies to overcome these limitations: (A) by sandwiching biological tissue between ZincSelenide windows, a thermo camera, enhanced with close-up optics, can image the temperature distribution below the tissue surface through the window when exposed from above with a diathermia pencil. High speed imaging up to 10,000 frames is used (B) to visualize the dynamic effects at the surface of biological tissue and (C) to visualize temperature gradients below the surface in a transparent tissue model. Using these imaging techniques, the (thermo) dynamics during tissue exposure with various electro surgery modalities (e.g. coag, cut, spray) were studied. Simultaneously with thermal imaging, normal close-up video footage was obtained to support the interpretation of the thermal imaging. The imaging techniques showed to be both compatible and complementary showing the characteristics each modality.

High speed and subsurface thermal imaging techniques give a better understanding of the tissue effects of electro surgery modalities and contribute to the safety and the optimal settings during clinical application.

7901-37, Session 8

Indocyanine green enhanced near-infrared laser treatment of SCK tumors in a mouse model

G. Shafirstein, Univ. of Arkansas for Medical Sciences (United States); W. Bäumler, Univ. Clinics Regensburg (Germany); R. Friedman, K. Barnes, L. Hennings, J. Weber, R. Griffin, Univ. of Arkansas for Medical Sciences (United States)

Background and Purpose. Determine the efficacy of indocyanine green (ICG) dye in enhancing near infrared (NIR) laser ablation of tumors in a mouse model.

Methods. Mammary carcinoma cells of A/J mice were injected subcutaneously in the lower back of female A/J mice (n=21). Five to seven days post inoculation the tumors (7-9 mm) were treated with 808-nm laser using 86 J/cm² radiant exposures. Epidermal cooling was accomplished by applying ultrasonic gel at 2 °C to the skin, 1 minute prior to laser irradiation. Two minutes prior to irradiation one group of mice was injected, intravenously, with 4 mg/kg body weight of ICG solution and another group was injected with sterile water at same volume as the ICG solution. Thermal camera was used to measure the temperature changes during treatment. Animals were euthanized 1 day and 7 days post treatment for histopathological evaluation.

Results. Temperature increase of 20 and 40 °C were recorded for mice that were treated with laser/water and laser/ICG, respectively. No major skin damage was observed post treatment. Minor thermal damage and necrosis was observed histologically in the tumor at 1 and 7 days post laser/water treatment and substantial damage (up to 100% coagulation necrosis) was observed in tissue collected from tumors that were treated with laser/ICG. The tumor growth was delayed by a factor of 2.5 in laser/ICG in comparison to laser/water treatment at 7 days post treatment.

Conclusions. Intravenous administration of 4mg/kg ICG significantly enhanced thermal ablation of tumors during NIR laser irradiation.

7901-39, Session 8

Thermal ablation and/or spatially fractionated radiation (GRID) therapy for down-staging locally advanced breast cancer

G. Shafirstein, R. Griffin, K. Barnes, J. Weber, S. Sharma, E. G. Moros, Univ. of Arkansas for Medical Sciences (United States)

Purpose: To test our hypothesis that thermal ablation and/or spatially fractionated radiation (GRID) therapy can be used for significant tumor cytoreduction.

Methods: Conductive interstitial thermal therapy (CITT) and/or GRID radiotherapy were used to treat locally advanced tumors in Balb/c mice bearing a 4T1 tumor. CITT was accomplished by inserting a 2 mm probe into the center of the tumor and increasing the probe temperature to a maximum of 80°C for 10 minutes. GRID therapy was conducted by delivering one radiation dose through a specially made grid collimator with an array of twelve 2 mm, in diameter, openings with center to center...
distance of 3 mm. Tumors growth delay were evaluated by following the treated and untreated animals for 8 days, post treatment day.

Results: In GRID therapy the gross tumor volume receives one, highly-non uniform, radiation dose fraction with peak doses of 20 Gy and valley doses of 3 Gy or less. During CITT, a peak temperature of 80°C was achieved within the tumor tissue that was in contact with the CITT probe. A 2 to 4-fold reduction in tumor’s growth was observed in the treated animals in comparison to the control animals.

Conclusions: Our preliminary results showed that both GRID and CITT could be effective for significant tumor cytoreduction in 4T1 tumors implanted in Balb/c mice. The additive effect of the two modalities is being evaluated in a larger group of animals.

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7901-45, Session 8

Computed effects of sweat gland ducts on the propagation of 94 GHz waves in skin
E. G. Moros, G. Shafirstein, Univ. of Arkansas for Medical Sciences (United States)

The effects of sweat gland ducts (SGD) on specific absorption rate and temperatures during millimeter wave irradiation of skin were investigated with a high resolution finite differences time domain model consisting of a 30 µm stratum corneum, a 350 µm epidermis, 1000 µm dermis and five SGD (60 µm radius, 300 µm height, 370 µm separation). The source was a WR-10 waveguide irradiating at 94 GHz. Without SGD, SAR and temperature maximum were in the dermis near epidermis. With SGD, a higher SAR maximum was inside SGD in the epidermis while temperature maximum moved to the epidermis/stratum-corneum junction. SGD significantly affected how GHz waves were absorbed in the skin. Implications of these finding in nociceptive research will be discussed as well as other potential medical applications.

7901-40, Session 9

Development of novel magnetic nanoparticles for hyperthermia cancer therapy
S. M. Cassim, Dartmouth Hitchcock Medical Ctr. (United States); P. J. Hoopes, Dartmouth Medical School (United States); I. Baker, Dartmouth College (United States)

Hyperthermia therapy has been and continues to be the subject of much research in cancer medicine. The potential for hyperthermia as a standalone or adjuvant therapy to augment clinical outcomes has been shown time and time again, however creating a standard of practice has proven difficult and this therapy has yet to achieve mainstream acceptance. One remaining challenge to the field of nanoparticle hyperthermia is one of particle quality. The current state of the art involves two types of nanoparticles; gold and iron oxide, specifically magnetite (Fe₃O₄-). With either particle technology factors such size, toxicity, colloidal stability, and heating ability must be controlled and optimized. While gold nanoparticles are excited via Plasmon resonance, magnetite particles are heated via an alternating magnetic field (AMF) the latter method showing superior tissue penetration. This ensures that once particles are localized to malignant tissue, that tissue can be treated even if it lies deep within the body. Unfortunately intense AMF fields can also cause unsafe global heating in the patient through the creation of eddy currents; this may limit the ability to safely apply therapeutic heat doses via the nanoparticles. Consequently, the nanoparticles chosen for AMF hyperthermia must have requisite magnetic properties in order that they heat to therapeutically relevant levels at low to moderate AMF intensities, where eddy currents are a non-issue. Novel nanoparticles using zero-valent iron cores and magnetite shell structure offer improved magnetic qualities, such as increased saturation magnetization and coercivity, over the pure magnetite nanoparticles; however these particles are more difficult to stabilize colloidaly and chemically. Our composite nanoparticles, produced via water in oil nanoemulsion methods, are for the first time, coated with silane coupled dextran to form a non-toxic stable dispersion. Characterization of these particles is accomplished via transmission electron microscopy, vibrating sample magnetometry, specific absorption rate (SAR), and FT-IR. Measurements of SAR and preliminary cell culture experiments with these particles show the promise of improved heating capability over standard iron oxide nanoparticles.

7901-41, Session 9

Selective nanoparticle-directed photothermal ablation of the canine prostate
J. A. Schwartz, Nanospectra Biosciences, Inc. (United States); R. E. Price, Baylor College of Medicine (United States); K. L. Gill-Sharp, K. L. Sang, J. D. Khorchani, J. D. Payne, Nanospectra Biosciences, Inc. (United States); B. S. Goodwin, The Univ. of Texas Health Science Ctr. at Houston (United States)

This study adapted AuroLase® Therapy, previously reported for the treatment of brain tumors, to the treatment of prostate disease by 1) using normal canine prostate in vivo, directly injected with a solution of nanoparticles as a proxy for prostate tumor and, 2) developing an appropriate laser dosimetry for prostate which is which is sub-ablative in native prostate while simultaneously producing photothermal coagulation in prostate tissue containing therapeutic nanoshells. Healthy, mixed-breed hound dogs were given surgical laparotomies during which nanoshells were injected directly into one or both prostate hemispheres. Laser energy was delivered percutaneously to the parenchyma of the prostate along 1-5 longitudinal tracts via a liquid-cooled optical fiber catheter terminated with a 1-cm isotropic diffuser after which the incision was closed and sutured using standard surgical techniques. The photothermal lesions were permitted to resolve for up to 8 days, after which each animal was euthanized, necropsied, and the prostate taken for histopathological analysis.

We developed a laser dosimetry which is sub-ablative in native prostate and simultaneously ablative of prostate tissue containing nanoshells which would indicate a viable means of treating tumors of the prostate which are known from other studies to accumulate nanoshells. Secondly, we determined that multiple laser treatments of nanoshell-containing prostate tissue could be accomplished while sparing the urethra and prostate capsule thermal damage. Finally, we determined that the extent of damage zone radii correlate positively with nanoshell concentration, and negatively to the length of time between nanoshell injection and laser treatment.

7901-42, Session 9

Targeted magnetic nanoparticle hyperthermia for cancer therapy
J. A. Tate, B. Gong, T. U. Gerngross, K. E. Griswold, Dartmouth College (United States); P. J. Hoopes, Dartmouth Medical School (United States)

Magnetic nanoparticle (mNP) hyperthermia shows promise as a novel cancer therapy, potentially succeeding where conventional hyperthermia has failed via delivery of an individual cell-based localized cytotoxic heat dose. To achieve this level of cytotoxic selectivity, nanoparticles must have access to and preferentially localize in tumor cells. The enhanced permeability and retention effect (EPR) present in many tumors theoretically allows for the passive targeting of appropriately sized therapeutics to tumor tissue due to poorly-formed vasculature. It is yet unclear how beneficial the EPR effect will be in specific mNP delivery to many solid tumors not to mention naive metastatic lesions or migrating metastatic cells. Active targeting of nanoparticles via anti-cancer antibodies is poised to overcome this lack of specificity. We have
developed fluorescently-labeled single-chain fragment (scFv)-conjugated PEG-coated iron oxide core mNP with four therapeutically-relevant anti-HER2 and HER3 scFvs. Increased avidity of bispecific nanoparticles was characterized by combining two scFv types on a single nanoparticle. The selected targeting moieties have a range of cellular internalization profiles from high rates of internalization to surface localization. In vitro assays demonstrate higher affinity of these targeted particles to antigen-expressing cancer cell lines over non-targeted particles. Assays also demonstrated a specificity of targeted particles towards antigen-expressing over non-antigen expressing cancer cells. Additional in vitro experimentation determined the localization difference between surface-localized targeting and internalized targeting over time, as well as bispecific vs. monospecific targeting with breast cancer cell lines having different (high vs. low) HER2 and HER3 expression profiles, at various time endpoints.

7901-43, Session 9

Kinetics and pathogenesis of intracellular iron-oxide nanoparticle hyperthermia

A. J. Giustini, Dartmouth Hitchcock Medical Ctr. (United States) and Dartmouth College (United States); R. E. Gottesman, Dartmouth College (United States); K. M. Rauwerdink, Dartmouth Medical School (United States); A. M. Rauwerdink, A. A. Petryk, Dartmouth College (United States); J. B. Weaver, Dartmouth Hitchcock Medical Ctr. (United States); P. J. Hoopes, Dartmouth Medical School (United States)

Magnetic nanoparticles excited by alternating magnetic fields (AMF) have demonstrated effective tumor-specific hyperthermia. This treatment is effective as a monotherapy as well as a therapeutic adjuvant to chemotherapy and radiation. Iron oxide nanoparticles have been shown, so far, to be non-toxic, as are the exciting AMF fields when used at moderate levels. Although higher levels of AMF can be more effective, depending on the type of iron oxide nanoparticles use, these higher field strengths and/or frequencies can induce normal tissue heating and toxicity. Thus, the use of nanoparticles which will exhibit significant heating at low AMF strengths and frequencies is desirable. Our experiments have shown that the aggregation of magnetic nanoparticles within tumor cells improves their heating effect and cytotoxicity per nanoparticle. We have used magnetic particle imaging (MPI) and transmission electron microscopy to track the endocytosis of nanoparticles into tumor cells (both breast adenocarcinoma and acute monocytic leukemia cells). We then demonstrated that nanoparticles internalized into tumor cells demonstrate greater cytotoxicity when excited with AMF than an equivalent heat dose from excited external nanoparticles or cells exposed to a hot water bath. We have also demonstrated that this increase in SAR caused by aggregation improves the cytotoxicity of nanoparticle hyperthermia therapy in vitro in.

7901-44, Session 9

Comparison of iron-oxide nanoparticle and microwave hyperthermia alone or combined with cisplatinum in murine breast tumors

A. A. Petryk, A. J. Giustini, Dartmouth College (United States); P. J. Hoopes, Dartmouth Medical School (United States) and Dartmouth College (United States)

Surgery, radiation and chemotherapy are currently the most commonly used cancer therapies. Hyperthermia has been shown to work effectively with radiation and chemotherapy cancer treatments. The major obstacle faced by previous hyperthermia techniques has been the inability to deliver heat to the tumor in a precise manner. The ability to deliver cytotoxic hyperthermia to tumors (individual cells) via iron oxide nanoparticles (IONP) is a promising new technology that has the ability to greatly improve the therapeutic ratio of hyperthermia as an individual modality and as adjuvant therapy in combination with other modalities. Although the parameters have yet to be conclusively defined, preliminary data suggests IONP hyperthermia can achieve greater tumor cytotoxicity (in vitro and in vivo), alone and combined with cisplatinum (CDDP), than conventional microwave and water bath hyperthermia methods. At this time, our theory is that intracellular nanoparticle heating is significantly more effective in achieving the combined effect than extracellular heating techniques. Our data suggests that the IONP heating technique resulted in intracellular temperatures that exceed those delivered by the microwave and water bath techniques. Ongoing in vivo experiments are designed to make direct (same thermal dose) comparisons of localized microwave hyperthermia and CDDP with localized IONP nanoparticle hyperthermia and CDDP in a subcutaneous murine breast cancer model. Cumulative equivalent minutes (CEM) is being used as a method of normalizing detected heat dose in tumor and normal tissues.
Manipulating intracellular refractive index for contrast-enhanced digital holographic imaging of subcellular structures

C. E. Rommel, C. Dierker, L. Schmidt, S. Przbiilla, G. von Bally, B. Kemper, J. Schnekenburger, Westfälische Wilhelms-Univ. Münster (Germany)

The online analysis of rapid cellular processes by morphological alterations strongly depends on the ability to rapidly visualize and to quantify cell shape and intracellular substructures. Digital holographic microscopy (DHM) enables quantitative phase contrast imaging for high resolution and minimal invasive live cell analysis without the need of labeling or complex sample preparation. However, due to the rather homogenous intracellular refractive index, the phase contrast of subcellular structures is limited and often low. We analyzed the impact of specific intracellular refractive index manipulation by microinjection of refractive index changing agents on the DHM phase contrast. Glycerol was chosen as osmolyte, which combines high solubility in aqueous solutions and cellular compatibility. We present data showing that the intracellular injection of glycerol causes a contrast enhancement that can be explained by a decrease of the cytosolic refractive index due to a water influx. The underlying principle was proven by experiments inducing cell shrinkage and protein concentration. The integrity of cell membranes is considered as a prerequisite and allows a reversible cell swelling and shrinking within a certain limit. The presented approach to control the intracellular phase contrast demonstrated for DHM opens also prospects for application with other quantitative phase contrast imaging technologies.

Cell death measured with optical coherence tomography

D. M. M. de Bruin, M. Broekgaarden, D. J. Faber, T. G. van Leeuwen, Univ. van Amsterdam (Netherlands)

Cell death is usually divided into two subclasses known as Apoptosis and Necrosis. Apoptosis, or programmed cell death, is a controlled mechanism for a cell to commit suicide. Necrosis is characterized as accidental cell death, induced by extreme exposure to toxic compounds, heat, or mechanic cell damage.

In this study, fluorescence-activated cell sorting (FACS) and functional or quantitative optical coherence tomography (OCT) by means of µt is used to measure apoptosis in cultured human retina pigment epithelial (RPE) cells [ARPE 19].

Control experiments were performed on healthy cells only exposed to medium. Both FACS and OCT show that no increase in cell death was measured. OCT measurements of an apoptotic cell pellet induced by a constant exposure of 10% EtOH caused a distinct change in µt; an initial increase (µt = 5.5 ± 0.5 at highest point at 4hours), followed by a gradual decrease. All measurements were in agreement with FACS data.

The results of this study have demonstrated that exposure to cell death inducers affects the light scattering properties of cells. Necrosis appears to cause an increase in light penetration and therefore a lower µt in comparison to healthy cells. This phenomenon might be due to a complete loss of cellular structure. Apoptosis appears to cause a distinct rise in µt compared with healthy cells. Oxidative stress-induced mitochondrial swelling could be responsible for the initial increase, while cell blebbing and secondary necrosis may be responsible for the decrease.

Dynamic Raman imaging of cytochromes in apoptotic cells

M. Okada, Osaka Univ. (Japan); K. Fujita, Osaka Univ. (Japan) and Japan Science and Technology Agency (Japan); N. I. Smith, Osaka Univ. (Japan); S. Kawata, Osaka Univ. (Japan) and RIKEN (Japan)

Raman microscopy has been used for label-free molecular imaging of biological samples because Raman scattering detect the molecular vibration. In this work, we applied this technique to observe dynamics of intracellular molecules during apoptosis. We used a slit-scanning Raman microscope, where a line-shaped laser focus illuminates a sample to detect Raman scattering from multiple points in the sample simultaneously, resulting in an image acquisition rate around 100 times higher than that of conventional confocal Raman microscopy. Apoptosis was induced to HeLa cells by being incubated with Actinomycin D for 2 hours. We found that Raman peak at 753 cm-1, which can be assigned to cytochromes, is distributed in the cytosol with Actinomycin D. On the other hand, in the cells incubated without Actinomycin D, 753 cm-1 peaks were observed at mitochondria. We also observed cytochromes diffused from mitochondria into cytosol gradually between 15 to 25 minutes after the addition of Actinomycin D. At the same time, Raman peak intensities at 753, 1127, 1314 and 1585 cm-1 (These are also assigned to cytochromes) were decreased. These results indicate that Raman images at 753 cm-1 gives the distribution of cytochrome c because release of cytochrome c from mitochondria to cytosol is one of the characteristics in apoptosis. Furthermore, we confirmed a Raman image reconstructing by Raman peak at 753 cm-1 shows a similar image contrast to an immunostaining fluorescence image of cytochrome c.

Long-term, time-lapse, multimodal microscopy for tracking cell dynamics in live tissue

B. W. Graf, E. J. Chaney, M. C. Valero Quiros, M. Marjanovic, M. D. Boppart, S. A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

High speed intravital microscopy has emerged as an essential tool for studying cellular dynamics in live tissue. A limitation of this technique, however, is that the timescale that a sample can be continuously imaged is limited by practical considerations to several hours. Long term observation of live tissue is of great interest for a variety of research areas. We present methods for observing long term cellular dynamics in live tissue based on three-dimensional registration of time-lapse intravital microscopy images.

For these experiments we utilized a custom multimodal microscope that allows simultaneous and co-registered acquisition of optical coherence (OCM) and multiphoton (MPM) microscopy images. OCM allows the structure of a sample to be visualized based on backscattered light while MPM excited fluorescence allows individual cells and cell function to be visualized. The OCM images of tissue structure are used to register data sets taken at different time points. The transformations of the OCM images are applied to MPM images to determine the migration of cell populations. This method of image registration is applied to in vivo tracking of bone-marrow derived GFP-labeled stem cells in mouse skin following bone marrow transplants from GFP donors into species-matched wildtype hosts. The use of three-dimensional image registration of time-lapse microscopy images enables tracking these cells after local cutaneous injury, and for investigating the role of skin stem cells in wound healing.
Multiscale optical measurements of bacterial growth

M. A. H. Mir, Z. Wang, M. Bednarz, Univ. of Illinois at Urbana-Champaign (United States); I. Golding, Baylor College of Medicine (United States); G. Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Elucidating the phenomenon of cell growth is a fundamental problem in cell biology that remains unsolved due to the extreme accuracy required in measuring the mass of individual cells [1]. Current methods of measuring growth either use a surrogate measurement for mass, such as volume, in the case of flow cytometry, or require complex experimental setups, as in the case of micro-resonators [2-3]. Flow cytometric measurements only provide population level statistics, whereas current single cell methods don’t allow for long term measurement and only provide data on individual cells. Using Spatial Light Interference Microscopy (SLIM) [4] to measure growing colonies of Escherichia Coli (e.coli) we demonstrate that is possible to accurately measure growth in mass and size simultaneously, at both the single cell and population level. By measuring the optical path length through single cells interferometrically, we can assess the dry mass (protein) content of single cells with femtogram accuracy on timescales from milliseconds to weeks. Furthermore, since this is an imaging based technique, we can study growth both spatially and temporally. This provides a complete picture of how individual cells grow in a colony, based on their mass, age, morphology and physical location within a colony. This technology thus has the potential to finally bridge the gap between what appears to be well ordered and highly controlled growth at the population level, and the inherent variability amongst single cells.


Hyperspectral imaging of the olfactory bulb activation: influence of realistic differential path-length correction factors on the derivation of oxygenation and total hemoglobin concentration maps

R. Renaud, C. Romain, Univ. Paris-Sud 11 (France); M. Bendahmane, Ctr. National de la Recherche Scientifique (France) and Univ. Paris 11 et 7 (France) and INRA (France); C. Martin, Ctr. National de la Recherche Scientifique (France) and Univ. Paris 11 et 7 (France); H. Gurden, Ctr. National de la Recherche Scientifique (France) and Univ. Paris Sud 11 (France); F. Pain, Univ. Paris-Sud 11 (France) and Ctr. National de la Recherche Scientifique (France)

Spectroscopic reflectance imaging has been used extensively in rodent cortices to obtain maps of oxygenation and total blood volume changes during brain activation. The aims of the present study are first to validate a new ultra fast illumination source for these studies and second to extend the technique to the study of the olfactory bulb (OB) during physiological activation. We have built a system based on a digital micromirrors device to sweep sequentially eight wavelengths with a temporal resolution down to 5 ms. The performance of the illumination source in terms of wavelength selectivity, available luminous power, stability and speed of switching were assessed as well as the overall set up performance in the context of the speed/sensitivity trade-off. The analysis of functional multispectral reflectance images is based on the modified Beer Lambert law which requires normalizing the path lengths for photons at different wavelengths due to the wavelength dependence of the optical properties of tissues. The differential path length factor (DFP) is difficult to measure experimentally and is generally achieved using over-simplified Monte Carlo simulations or phantoms that mimic roughly the properties of brain tissues. We will discuss for two brain structures the influence of a more realistic evaluation of the DFP on the derivation of maps of total hemoglobin concentration and oxygenation from multispectral data. In vivo experimental data, which are the first recorded in the olfactory bulb on the time course of the haemodynamic changes during sensory activation will be presented.

Wide-field in-vivo spectral and fluorescence imaging microscopy of microvessel blood supply and oxygenation

J. Lee, M. Wankhede, B. S. Sorg, Univ. of Florida (United States)

Abnormal microvascular function and angiogenesis are key components of various diseases that can contribute to the perpetuation of the disease. Several skin diseases and ophthalmic pathologies are characterized by hypervascularity, and in cancer the microvasculature of tumors is structurally and functionally abnormal. Thus, the microvasculature can be an important target for treatment of diseases characterized by abnormal microvasculature. Motivated largely by cancer research, significant effort has been devoted to research on drugs that target the microvasculature. Several vascular targeting drugs for cancer therapy are in clinical trails and approved for clinical use, and several off-label uses of these drugs have been reported for non-cancer diseases. The ability to image and measure parameters related to microvessel function preclinically in laboratory animals can be useful for development and comparison of vascular targeting drugs. For example, blood supply time measurements give information related to microvessel morphology and can be measured with first-pass fluorescence imaging. Hemoglobin saturation measurements give an indication of microvessel oxygen transport and can be measured with spectral imaging. While each measurement individually gives some information regarding microvessel function, the measurements together may yield even more information since theoretically microvessel morphology can influence microvessel oxygenation, especially in metabolically active tissue like tumors. However, these measurements have not yet been combined. In this study, we report the combination of blood supply time imaging and hemoglobin saturation imaging of microvessel networks in tumors using widefield fluorescence and spectral imaging, respectively. The correlation between the measurements in a mouse mammary tumor is analyzed.

Water deficit and salt stress diagnosis through LED induced chlorophyll fluorescence analysis in jatropha curcas L. oil plants for biodiesel

E. A. Arcanjo da Silva, Jr., P. Cunha, R. Oliveira-Filho, A. S. Gouveia-Neto, E. Costa, T. J. Camara, L. G. Willadino, Univ. Federal Rural de Pernambuco (Brazil)

Chlorophyll Fluorescence (ChIF) represents an intrinsic signal emitted by plants that can be employed to monitor their physiological state including changes of the photosynthetic apparatus, developmental processes of plant growth and development.
leaves, state of health, stress events, stress tolerance, and also to detect diseases or nutrient deficiency of plants. Jatropha curcas (Linnaeus) is a multipurpose plant with many attributes and notable potential. Of particular scientific and/or technological interest is that, the fruit of jatropha contains viscous oil that can be used for soap making, in the cosmetic industry and mainly can be employed as a petro-diesel/ kerosene substitute or extender.

LED induced chlorophyll fluorescence analysis is employed to investigate the effect of water and salt stress upon the growth process of physicnut (jatropha curcas) grain oil plants for biodiesel. Red(Fr) and far-red (FFr) ChlF emission signals around 685 nm and 735 nm, respectively, were observed and examined as a function of the stress intensity (salt concentration and water deficit) during a 30 days period of time. The Chl fluorescence ratio Fr/FFr which is a valuable nondestructive and noninvasive indicator of the chlorophyll content of leaves was exploited to monitor the level of stress experienced by the plants. The ChlF technique data indicated that the salinity plays a main role in the chlorophyll concentration of leaves tissues for NaCl concentrations in the 25 to 200 mM range, and results agreed quite well with those obtained using conventional destructive spectrophotometric methods. The Chl fluorescence analysis permitted detection of damage caused by salinity and either lack or water deficit in the early stages of the plants growing process, and can potentially be used as an early-warning indicator of stress.

7902-09, Session 3

High-throughput in-vivo vertebrate imaging and screening

M. F. Yanik, C. Pardo, T. Chang, B. Koo, C. Gilleland, S. Wasserman, Massachusetts Institute of Technology (United States)

We demonstrate the first high-throughput platform for cellular-resolution in vivo pharmaceutical and genetic screens on vertebrates (cover, Nature Methods, August 2010). Small size, optical transparency of highly complex organs and ease of culture make zebrafish (Danio rerio) larva an ideal organism for large-scale in vivo genetic and chemical studies of many processes that cannot be replicated in vitro. Zebrafish models of several human diseases have been developed. Lead compounds discovered by screening chemical compound libraries for efficacy in zebrafish disease models have been useful for pharmaceutical discovery owing to the high level of conservation of drug activity between mammals and zebrafish. However, existing zebrafish screens are done either manually or with coarse imaging with highly limited capabilities and throughput. Our system dramatically accelerates both throughput and complexity of pharmaceutical screens on whole vertebrates. The system automatically loads zebrafish from reservoirs or multwell plates, and positions and rotates them in 3-dimensions for high-speed multifocal confocal imaging and laser manipulation of both superficial and deep organs within 19 seconds without damage. We show screening of retinal axon guidance mutants. We also show neuronal regeneration assays in combination with femtosecond laser microsurgery. Our technology can permit large-scale in vivo drug discovery on complex processes such as organ development, neural degeneration and regeneration, stem cell proliferation, cardiovascular, immune, endocrine and nervous system functions, pathogenesis, cancer and tissue specificity, and toxicity of drugs.

7902-10, Session 3

Quantifying thermal modifications on laser welded skin tissue

H. O. Tabakoglu, Fatih Univ. (Turkey); M. Gülsoy, Bogazici Univ. (Turkey)

Laser tissue welding is a potential medical treatment method especially on closing cuts implemented during any kind of surgery. Photothermal effects of laser on tissue should be quantified in order to determine optimal dosimetry parameters such as power, time, pulsed or continuous regime for any application. Recovery period depends on the thermal harm given to the skin tissue. Thermal effect can be determined under light microscope. Polarized light and phase contrast method give information about collagen structural changes on hematoxilen and eosine stained tissue samples. In our study, 3 different near infrared laser wavelengths (809 nm, 980 nm and 1070 nm) were used for skin welding. Experiments were performed in vivo on Wistar rats dorsal skin, 1 cm long cuts were treated spot by spot laser application. In all laser applications, 0.5 W of power was delivered to the tissue in 5 s continuously, resulting in 79.61 J/cm² energy density (15.92 W/cm² power density) for each spot. The recovery process was followed up for 21 days. The 1st, 4th, 7th, 14th, and 21st days of this period were determined as control days, and skin samples needed for histology were removed on these particular days. The samples were embedded in paraffin blocks and sectioned to 5-8 µm-thick samples. The slides were stained with H&E for general histology. The stained samples were examined under a light microscope (Eclipse 80i, Nikon Co.). Images were taken with a CCD camera (DS-Fi1, Nikon Co.) and examined with imaging software (NIS Elements-D, Nikon Co.).
For evaluating effects of optical absorption by blood, reflection images were taken at 550 nm (R550) and 610 nm (R610).

Results: The average LN ratio of 0.356x/405x in adenomas was significantly different from 1.0, indicating broad distinction between adenomatous and normal mucosa. Small adenomas as well as large adenomas could be effectively detected by this method. The value of F365x/F405x was not correlated with the index of haemoglobin, log(R610/R550), at normal mucosal sites, while F365x and F405x were negatively correlated.

Conclusion: These results showed that the autofluorescence ratio imaging is a promising technique for detecting adenomas in colonic mucosa.

7902-13, Session 3

**Multimode optical imaging for translational chemotherapy: tumor detection and delineation by targeted gallium corrole**

J. Hwang, L. K. Medina-Kauwe, Cedars-Sinai Medical Ctr. (United States); Z. Gross, Technion-Israel Institute of Technology (Israel); H. B. Gray, California Institute of Technology (United States); D. L. Farkas, Cedars-Sinai Medical Ctr. (United States)

We here report the feasibility of tumor detection and delineation using multimode optical imaging of targeted gallium corrole (HerGa). HerGa is highly effective for HER2+ tumor targeted elimination in vivo as well as emits intense fluorescence. In addition, it has the additional potential as a photosensitizer for photodynamic therapy besides its inherent cytotoxicity. Thus, these unique characteristics of HerGa prompt us to investigate the potential of HerGa for tumor detection and delineation. In order to investigate it, we here performed multimode optical imaging ex vivo and in vivo, including fluorescence intensity, spectral, lifetime, and two-photon excited fluorescence imaging, using our advanced custom-built imaging system. While the fluorescence intensity imaging provided information about tumor targeting capacity and tumor retention of HerGa, ratiometric spectral imaging offered more quantitative and specific information about HerGa location and accumulation. Most importantly, the fluorescence lifetime imaging allowed us to discriminate between tumor and non-tumor regions by fluorescence lifetime difference of HerGa. Finally, two-photon excited fluorescence images provided high resolved and detailed information around the tumor regions where HerGa accumulates. Taken together, the results shown in this report suggest the feasibility of tumor detection and delineation by multimode optical imaging of HerGa. In particular, the multimode optical imaging can offer multiple/complementary information simultaneously in the tumor detection and delineation by HerGa, thus enhancing contrast.

7902-14, Session 4

**Simultaneous multimodality optical and MR imaging of tumor micro-environment within implanted window chambers**

M. F. Shayegan Salek, College of Optical Sciences, The Univ. of Arizona (United States); D. Jennings, Harvard-Massachusetts Institute of Technology (United States); T. Wu, A. F. Gmitro, The Univ. of Arizona (United States)

Optical imaging and MRI have both been used extensively to study tumor microenvironment. However the two imaging modalities are complementary and could be used to cross-validate one another. Window chambers are support structures which are widely used in studying tumor microenvironment with optical microscopes. MR imaging of window chambers has also been reported. We have developed a modular platform which is capable of doing optical microscopy inside an MRI instrument. To do this, an optical relay system transfers the image to outside of the MR bore to a commercial grade camera. This enables simultaneous optical and MR imaging of the same tissue and thus creates the ideal situation for comparative or complementary studies using both modalities. Initial experiments have been done using GFP labeled prostate cancer cells implanted in mouse dorsal skin fold window chamber. Vascular hemodynamics and vascular permeability were studied using our imaging system and also separately under confocal microscopy for further comparison. Towards this goal, we developed a dual MR-Optical contrast agent by labeling biotinylated BSA with both Gd-DTPA and Alexa Fluor. Overall system design and results of these preliminary vascular studies will be presented.

7902-15, Session 4

**Small animal Cerenkov luminescence imaging**

R. K. Gill, G. S. Mitchell, C. Li, S. R. Cherry, Univ. of California, Davis (United States)

We propose and demonstrate an optical imaging method, Cerenkov Luminescence Imaging (CLI), based on the detection of Cerenkov radiation to noninvasively image beta-emitting radionuclides inside small animals. At present, there is no sensitive in vivo imaging method for monitoring the biodistribution of β-emitting radionuclides used for radioimmunotherapy. CLI will allow in vivo imaging of the biodistribution of these radionuclides, such as Y-90, with high sensitivity. We have successfully detected Cerenkov light from F-18 and Y-90 in small animals using reasonable doses of ~200 µCi. For example, a peak signal of 2.3x10^5 photons/s/cm^2/sr were detected from a mouse study where a capillary tube containing 274 µCi of Y-90 was inserted down the esophagus. Based on GEANT4 Monte Carlo calculations for Y-90 in tissue, we expect to observe ~70 visible light photons per decay. Currently, we are characterizing the intensity and optical emission spectra of Cerenkov radiation at different depths using agar and hemoglobin phantoms. Using a phantom of 5 mm thickness, we are able to detect 3.1x10^4 photons/s/cm^2/sr from a capillary tube containing 80 µCi of F-18. Since mice are typically no thicker than 1.5 cm, we expect that we can image the biodistribution of beta minus emitters in a whole mouse by viewing both the dorsal and ventral sides. By measuring the emitted light from several views of the mouse, it is possible to reconstruct 3D images of the Cerenkov light distribution using techniques analogous to those used for bioluminescence tomography.

7902-17, Session 4

**The new hyperspectral microscopic system for cancer diagnosis**

Y. Hsieh, Y. Chen, T. Huang, O. Mang, National Central Univ. (Taiwan); J. Chiou, Y. Lin, M. Tsai, D. Bau, C. Chiu, G. Teseng, N. Chang, China Medical Univ. (Taiwan); W. Kao, S. Wu, National Taiwan Normal Univ. (Taiwan)

Presently, the cancer was examined by diagnosing the pathological changes of tumor. If the examination of cancer can diagnose the tumor before the cell occur the pathological changes, the cure rate of cancer will increase. This research develops a human-machine interface for hyper-spectral microscope. The hyper-spectral microscope can scan the specific area of cell and records the data of spectrum and intensity. These data is helpful to diagnose tumor. This research aims to develop a new system and a human-machine interface to control the hyper-spectral microscope. This interface can control the moving speed of motor, the exposure-time of hyper-spectrum, real-time focus, image of fluorescence, and the data of spectral intensity and position.
Multispectral line confocal imaging microscope for biomedical fluorescence applications

M. M. Meyers, GE Global Research (United States)

A Line Confocal Microscope has been developed, and tested, which is capable of simultaneous, high resolution, fluorescence imaging of biological samples stained with 3 - 5 fluorophores. The system uses 3 - 5 solid-state laser module coupled into one single mode fiber as illumination input to a line scanning confocal microscope. The excitation illumination beam is diffraction limited in the scanning direction and covers the full width of the sample for the cross scan direction. The system works with microscope objectives with NA's ranging from 0.25 to 0.75 in air. The line confocal configuration provides the majority of the benefits of confocal microscopes, such as deep sectioning and improved contrast, while allowing for a much greater fraction of the excitation illumination to reach the sample area, resulting in reduced exposure times.

One configuration of the instrument is designed to work as a parallel fluorescent microscope with 3 to 5 discrete cameras, while the alternate configuration allows the instrument to act as a hyperspectral microscope that samples the spectral emission output over the visible and NIR wavelengths. The hyperspectral image data can be used to extract fluorescent images from each fluorophore, while also allowing for the determination and subsequent subtraction of background autofluorescence in a single operation.

The instrument allows for increased fluorescent imaging throughput, due to its higher excitation transmission, and parallel operation. While in the hyperspectral mode, it allows for extraction of more detailed information on the state of the biological tissue through analysis of the emitted spectral intensity distribution.

Polarization-sensitive dermoscopy for image processing-assisted evaluation of atypical nevi: towards step-wise detection of melanoma

L. Yu, Windward School (United States); A. Joseph, Univ. of Southern California (United States); E. H. Lindsley, Spectral Molecular Imaging, Inc. (United States); D. L. Farkas, Spectral Molecular Imaging, Inc. (United States) and Univ. of Southern California (United States)

Non-invasive detection of melanoma, especially early in the evolution of this deadly disease, is extremely important for outcomes: most lesions caught early and removed surgically yield a likelihood of cancer eradication of nearly 100%. In contrast to this, late detection leads to very high (>90%) mortality rates. Clearly, reliable early detection and diagnosis are critically important, and more likely to be attainable by advanced optical imaging, but currently (1) no FDA-approved commercial device achieves this task; (2) the bulk of the clinical assessment is done by physicians (typically dermatologists), assisted by pathologists, in a very simple, observation-based way. Using the ABCD A for asymmetry, B for borders, C for color and D for dimension, (2) criterion for classifying the pigmented nevi, decisions are reached on relative risk and thus need for removal. Sometimes (too infrequently), nevus images are recorded digitally using a dermoscope [1], an optical skin imaging device with a color camera. Another major problem is that the patients at risk, for various reasons, do not see a doctor in time.

We have taken a two-pronged approach to improving this current standard: (a) we are developing a new, hyperspectral imaging-based device aimed at non-invasively detecting melanoma early (to be presented separately) and (b) we are using a commercially available dermoscope, polarized imaging and ABCD-inspired image processing for establishing an imaging capability that could assess the risk of a particular lesion (pigmented nevus) harboring melanoma. The latter is the focus of the work presented here, with its main goal being the investigation of a relatively inexpensive device for patient self-evaluation that could enhance the logistic connection between patients in need and their physicians: the idea consists in obtaining images of the nevi and sending them digitally- via the internet - to a dermatologist of choice, so that the latter, after image processing and evaluation, can determine the need for seeing the patient for assessment and possible intervention. If the risk determined warrants it, the physician, hopefully in possession of significant experience and better technologies, will see the patient for a more thorough consultation.

In this study, we used the ProScope HR (Bodelin Technologies, Lake Oswego OR), a commercially available hand-held microscopic imaging device with polarization control that magnifies skin lesions typically about 50x (but higher magnifications were also tested). The detector is a relatively standard 3-color camera with 1.3 megapixel resolution, and the connection to a (Apple Macintosh) laptop computer is via USB. We imaged a total of 18 volunteers, 6 each from Caucasian, Asian and African-American ethnic background, each with 1-3 nevi of interest. Control skin images were also obtained from all subjects. We stored the images digitally, and developed image analysis methods customized to applying the ABCD criteria and generating a single risk number (Total Dermoscopy Score, TDS), ranging between 1 and 100, that reflects the assessment of the need and urgency to see a specialist for additional evaluation. All our studies will be, of course, shared with specialists (including pathologists and dermatologists) for their feedback. Results will also be compared with our new data obtained via hyperspectral imaging and customized segmentation.

The two-step approach described here for assessing nevi will hopefully provide a useful alternative to current procedures [3], and an improved ability to bring the patients to the clinic, to diagnose melanoma early.

Live atomic force microscopy imaging of laser microbeam-assisted cellular microsurgery

N. D. Ingle, S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Since the invention of lasers, laser microbeam has been employed to cause highly localized damage to cellular and sub-cellular organelles. With the advent of ultrafast lasers, the spatial extent of damage has been reduced to sub-wavelength dimensions, thus enabling precise nano-surgery with the least amount of collateral damage. This has specifically enhanced efficiency of optoporation-injection of exogenous impermeable substances into the cell by formation of a transient hole. However, the kinetics of hole formation and sealing of membrane has not been visualized at nanoscale resolution. Here, we report the realization of live atomic force microscopy (AFM) imaging of cellular microsurgery caused by ultrafast tunable Ti: Sapphire laser microbeam. AFM imaging was carried out using a Nanonics Multiview system in parallel to exposure of the laser beam, controlled by Uniblitz shutter. Red blood cells (RBCs) were chosen for micro-surgery due to their smooth surface topography. The transparent nature of the Nanonics fiber-optic AFM cantilever allowed simultaneous bright field/phase contrast imaging of the RBC. Further, simultaneous fluorescence imaging could be realized during laser exposure and AFM imaging. AFM imaging revealed subtle trenches...
created by defocusing or aberration, which could not be obtained by conventional microscopy. Further, no resealing was observed in dry RBC samples. We will present the dynamics of hole formation and resealing on live RBC membrane.

7902-19, Session 5

Monitoring single-membrane protein dynamics in a liposome manipulated in solution by the ABELtrap

T. Rendler, M. Renz, S. Ernst, M. Börsch, Univ. Stuttgart (Germany)

FoF1-ATP synthase is the essential membrane enzyme maintaining the cellular level of adenosine triphosphate and comprises two rotary motors. We measure subunit rotation by intramolecular fluorescence resonance energy transfer (FRET) between two fluorophores at the rotor and at the stator of the enzyme. Confocal FRET measurements of freely diffusing single enzymes in lipid vesicles are limited to hundreds of milliseconds by the transit times through the laser focus. A E Cohen and W E Moerner (Stanford) have recently developed an Anti-Brownian electrokinetic trap (ABELtrap) which is capable to immobilize single molecules, proteins, viruses or vesicles in solution. Trapping of fluorescent particles is achieved by applying a real time, position-dependent feedback to four electrodes in a microfluidic device. The standard deviation from a given target position in the ABELtrap is smaller than 200 nm. We report a combination of the ABELtrap with confocal FRET measurements and have monitored single membrane enzyme dynamics by FRET for more than 10 seconds in solution.

7902-20, Session 5

Optical trapping forces on biological cells on a waveguide surface

P. Levhaugen, B. S. Ahluwalia, Univ. of Tromso (Norway); T. R. Huser, UC Davis Medical Ctr. (United States); P. McCourt, O. G. Hellesø, Univ. of Tromso (Norway)

A three dimensional finite element method is used to model the forces acting on biological cells trapped on an optical waveguide surface. Gradient and propagation forces experienced by the cells are studied for different waveguide configurations. We describe how inner cell structures react to the optical waveguide forces. Red blood cells take on different shapes depending on the osmotic pressure. In a slightly hypotonic medium, red blood cells become spherical. We compare the optical forces working on spherical and biconcave disk shaped cells. Experiments on red blood cells trapped on waveguides are compared with the simulation results.

7902-21, Session 5

2D freeform plasmonic trapping via spatial light modulator

S. Chen, H. Su, National Cheng Kung Univ. (Taiwan)

Optical tweezers is an important technique which can be utilized to study the dynamics of single macromolecule, probe subcellular compartments of living cells, and construct structures from nanoscale objects. In this study, an objective-based two-dimensional (2D) surface plasmon (SP)-enhanced optical trapping system with a spatial light modulator (SLM) has been developed to trap dielectric particles in freeform. Through a gold film with a thickness of 45 nm in the near infrared region, the 40-fold electric field enhancement is reached and hence strong 2D trapping force distribution with the SP excitation has been demonstrated. Furthermore, the trapping patterns are based on SLM intensity modulation to control the trapping force distribution, and then a freeform trapping is built.

7902-22, Session 5

High-speed fret screening for optical proteomics in microfluidic format

V. Viskitkul, King’s College London (United Kingdom)

Cancer studies require a thorough understanding of how human gene expressions and DNA modifications are translated in the proteome level. In order to unravel the large and complex interaction of the proteome, we have developed a compact lifetime-based flow cytometer, using a commercialized microfluidic chip, to screen large non-adherent cell populations. Fluorescent signals from samples at the focal volume are detected using time correlated single photon counting (TCSPC) in the burst integrated fluorescence lifetime (BIFL) mode for lifetime calculation. The practicality of the system was validated with human cancer cell lines (293T and A431) that were transiently transacted with FRET standard, consisting of GFP-19 Amino-acid linker-RFP. An analysis Software was written in Matlab to process the lifetime of detected samples using the newly developed Bayesian fitting algorithm and chronologically save the information in a multi-dimensional image file. A sorting possibility of the system is also demonstrated by using the lifetime read-outs of the FRET and non-FRET cell population in the image file to modulating an optical trap around the entrance of either outlets of a multi-output fluidic chip.

7902-23, Session 5

Parallel analysis of biological cells using multifocal laser tweezers Raman spectroscopy

R. Liu, D. S. Taylor, D. L. Matthews, J. W. Chan, Ctr. for Biophotonics Science and Technology (United States)

Laser tweezers Raman spectroscopy (LTRS) has proven to be a powerful label-free single cell analytical technique for cancer cell detection and cellular dynamics investigation. However, one major drawback of the technique is its low analytical throughput because long interrogation times are needed to detect the weak Raman signals and only one cell can be probed at a time. This makes it difficult to use LTRS in time-dependent studies characterizing the real-time response of many cells to external stimuli. To address these limitations, we report on the development and characterization of a multifocal laser tweezers Raman spectroscopy (M-LTRS) technique for parallel Raman spectral acquisition of multiple individual biological cells or multiple biological cells as a whole. For the first detection approach, multiple laser foci are generated in a linear pattern using a time-sharing trapping scheme to optically trap multiple single suspension cells. Raman signals from the trapped objects are simultaneously projected through the slit of a spectrometer and spatially resolved on a CCD detector with minimal signal crosstalk between neighboring cells. For the second detection approach, an array of laser foci are generated in the same way to immobilize N×N cells and the backscattered Raman signals from multiply trapped cells are desanned and delivered to the Raman spectrometer through a multimode optical fiber so that the Raman signatures from a large number of cells can be recorded. By improving the rate of single cell analysis, M-LTRS is expected to be a valuable method for studying single cell dynamics of cell populations.
Evaluation of the collateral damage during a femtosecond-laser axotomy by using a multimodal microscopy workstation

O. E. Olarte, S. I. C. O. Santos, M. Mathew, S. Psilodimitrakopoulos, P. Loza-Alvarez, ICFO - Instituto de Ciencias Fotónicas (Spain)

Using a femtosecond laser, it is possible to dissect individual nerve axons within living Caenorhabditis elegans (C. elegans), without compromising the life of the organism. Nonlinear absorption at the focus of a microscope objective is the mechanism responsible for the high precision neural nanosurgery. This also prevents the tissue surrounding the severed axon to be damaged. Having such precision tool for performing the axotomy has the potential for studying neuron functionality and nerve regeneration in such basic model organisms. Furthermore, the assessment of the damage around the wound after the laser operation is important because it allows understanding any possible side effect during axon regeneration. To do that, both the surgery tool and the imaging system should be running simultaneously in order to observe all the triggered processes. Nevertheless, the imaging methods that have been used to assist the cutting process, mostly based on linear fluorescence, do not allow to reveal accurately the collateral damage induced at the neighborhood of the dissected axon. In this study we provide a multimodal approach for simultaneous axotomy and high resolution imaging. Here the collateral damage is assessed by direct visualization of anatomical references of the nearby tissues and regions. For this, we use a wide-ranging set of linear and nonlinear microscopy techniques capable of high resolution imaging, some of them running simultaneously. This offers the unique possibility to observe amongst other things, the dynamics of the related effects caused in the damaged region during a nano-surgery intervention.

Microtubule traffic in filamentous fungi confined in microfluidics devices

M. Held, D. V. Nicolau, Univ. of Liverpool (United Kingdom)

No abstract available

High-throughput sheathless flow cytometry using inertila microfluidics

A. A. S. Bhagat, S. S. Kuntaegowdanahalli, I. Papautsky, Univ. of Cincinnati (United States)

Flow cytometer is a powerful single cell analysis tool that allows multi-parametric study of suspended cells. Most commercial flow cytometers available today are bulky, expensive instruments requiring high maintenance costs and specially trained personnel for operation. Hence, there is a need to develop a low cost, portable alternative that will aid in making this powerful research tool more accessible. In this work we describe a sheath-less, on-chip flow cytometry system based on the principle of Dean curl coupled inertial microfluidics. The design takes advantage of the Dean drag and inertial lift forces acting on particles flowing through a spiral microchannel to focus them in 3-D at a single position across the microchannel cross-section. Unlike the previously reported micro-flow cytometers, the developed system relies entirely on the microchannel geometry for particle focusing, eliminating the need for complex microchannel designs and additional microfluidic plumbing associated with sheath-based techniques. In this work, a 10-loop spiral microchannel 100 µm wide and 50 µm high was used to focus 6 µm particles in 3-D. The focused particle stream was detected with a laser induced fluorescence (LIF) setup. The microfluidic system was shown to have a high throughput of 2,100 particles/sec. Finally, the viability of the developed technique for cell counting was demonstrated using SH-SY5Y neuroblastoma cells. The passive focusing principle and the planar nature of the described design will permit easy integration with existing lab-on-a-chip (LOC) systems.

Digital holographic microscopy combined with optical tweezers

N. Cardenas, The Univ. of Texas at Arlington (United States); L. Yu, Nanoscope Technologies LLC (United States); S. K. Mohanty, The Univ. of Texas at Arlington (United States)

While optical tweezers have been widely used for the manipulation and organization of microscopic objects in three dimensions, observing the manipulated objects along axial direction has been quite challenging. In order to visualize organization of objects along axial direction, we report development of a Digital holographic microscopy combined with optical tweezers (DHOT). Digital holography is achieved by use of a modified Mach-Zehnder interferometer with digital recording of interference pattern of the reference and sample laser beams by use of a single CCD camera. Since phase changes observed in DHOT is very sensitive to optical thickness of objects, estimation of number of particles trapped in the axial direction could be obtained with high precision. In this method, quantitative phase information is retrieved dynamically with high temporal resolution, only limited by frame rate of the CCD. Digital focusing, phase-unwrapping as well as online analysis and display of the quantitative phase images was performed on a software developed on LabView platform. Since in diseases such as malaria and diabetics, change in refractive index of blood cells occurs, this system would be employed to map refractive index of biological samples immobilized by optical tweezers.

Dynamics of optically trapped red blood cells by phase contrast microscopy

M. C. Potooa, E. E. Hoover, G. Riccota, K. Roth, J. A. Squier, Colorado School of Mines (United States); R. Jimenez, JILA (United States); D. W. Marr, Colorado School of Mines (United States)

We report red blood cell (RBC) stretching using a Zeiss Axioplan microscope, modified for phase contrast and optical trapping using a 915 nm diode laser bar, as a tool to characterize RBC dynamics along a linear optical trap. Phase contrast offers a convenient method of converting small variations of refractive index into corresponding amplitude changes, differentially enhancing the contrast near cell edges. We have investigated the behavior of RBCs within both static and dynamic microfluidic environments with a linear optical stretcher. Studies with microfluidic systems allow characterization of cell interactions with the line optical force field without the complicating forces associated with hydrodynamics. In flowing, dynamic systems, cells stretch along the optical trap down microfluidic channels and are eventually released to recover their original shape. We record the dynamic cell response with a CCD camera at 250 fps and extract cell contours with sub-pixel accuracy using derivative operators. To quantify cell deformability, we measure the major and minor axes of individual cells both within and outside of the trap which also allows measurement of cell relaxation. In these studies, we observe that cell rotation, stretching, and bending along the linear optical trap, are tightly coupled to the modulation of optical power and cell speed inside our microfluidic systems.
Laser protein patterning

J. M. Belisle, L. A. Levin, S. Costantino, Univ. de Montréal (Canada)

Retinal ganglion cells (RGC) are the neurons responsible for transmitting visual information from the eye to the brain. During development, RGCs need to extend their axons along the optic pathway from the retina to specific targets. The growth cone, located at the tip of the extending axon, is sensitive to changes in concentration of molecules called guidance cues. Such guidance cues direct axonal growth and can either be attractive or repulsive. The spatial and temporal distribution of these molecules is crucial for appropriately connecting not only the visual pathway, but the entire nervous system.

A specific example is the presence at the optic nerve head of laminin, which is required for the axons to correctly exit the retina. To study axonal guidance in vitro, we developed an optical method to produce substrate-bound protein patterns named LAPAP (Laser-assisted protein adsorption by photobleaching). This method relies on the photobleaching of molecules conjugated with fluorescent dyes by using a laser to adsorb them on a glass substrate and therefore allows producing arbitrary protein patterns with micrometer resolution. We used RGC-5 cells, a neuronal cell line, which can be differentiated with staurosporine to trigger axonal growth. We studied in vitro the role of laminin guiding such cells using concentration gradients produced with LAPAP in order to better understand the development of the visual system.

Depth-targeted transvascular drug delivery by using annular-shaped photomechanical waves

T. Akiyama, Keio Univ. (Japan); S. Sato, H. Ashida, National Defense Medical College (Japan); M. Terakawa, Keio Univ. (Japan)

Targeted transvascular drug delivery is one of the most effective approaches to treat many diseases and injuries. However, safe, efficient and versatile methods for delivering drug molecules to targeted tissue through blood vessels have not been established. Recently, we found that photomechanical waves (PMWs) can transiently increase the permeability of blood vessels in skin, muscle and brain of rats. In this study, we examined the use of annular-shaped PMWs to increase the pressure at target depths due to the superposition of pressure waves. This can increase the permeability of blood vessels located in the specific depth regions, enabling depth-targeted transvascular drug delivery. Annular PMWs were produced by irradiating a laser target (a rubber disk covered with a transparent plastic sheet) with annular pulsed laser beams (Q-switched Nd:YAG laser; 532 nm; 6 ns) which were obtained with an axicon lens. We first examined propagation and pressure characteristics of annular PMWs in tissue phantoms and confirmed an increased pressure at a target depth, which can be controlled by changing laser parameters. We injected Evans blue (EB) into a rat tail vein and a laser target was placed on the fascia of the anterior tibialis muscle. The target was irradiated with three annular laser pulses (inner diameter, 3 mm; outer diameter, 5 mm) at a laser fluence of 2.5 J/cm². After perfusion fixation, we observed fluorescence originating from EB at a target depth of around 5 mm in the tissue, demonstrating the capability of annular PMWs for depth-targeted transvascular drug delivery.

Simultaneous measurements of fluorescence lifetimes, rotational correlation times and FRAP recovery times

J. A. Levitt, P. Chung, D. R. Alibhai, K. Suhling, King’s College London (United Kingdom)

We demonstrate an experimental arrangement for simultaneous measurements of fluorescence lifetimes, rotational correlation times and fluorescence recovery times after photobleaching. The method uses time-correlated single-photon counting (TCSPC)-based fluorescence lifetime imaging (FLIM) and an inverted confocal laser-scanning microscope. We have recorded a series of polarization-resolved fluorescence images during a time-lapse fluorescence recovery after photobleaching (FRAP) experiment. The image series has been used to measure rotational and translational diffusion coefficients of fluorescent dyes in living cells simultaneously. The resulting polarization-resolved images have been used to create a map of rotational correlation times, and rotational diffusion coefficients. The translational diffusion coefficient in a region of interest was determined using the FRAP recovery curve calculated from the fluorescence intensity image series. Thus, a data set which contains time-resolved and polarization-resolved fluorescence data in every pixel of an image for a FRAP recovery series was collected with an acquisition time similar to that of a standard time-domain FLIM experiment. By making these measurements simultaneously we significantly reduce the acquisition time and minimise the effects of photobleaching of the sample. Moreover, we have successfully eliminated the need for several sequential measurements to determine the diffusion characteristics and local variations in fluorescence lifetimes in live cells.

Time-gated spontaneous and resonance Raman spectroscopy for biomedical applications

Z. J. Smith, F. Knorr, C. V. Pagba, S. Wachsmann-Hogiu, UC Davis Medical Ctr. (United States)

Spontaneous and Resonance Raman scattering are techniques that, due to their high chemical specificity, have the potential to diagnose diseases, analyze and characterize biological samples, study interactions between macromolecules, and to monitor biological functions. However, since Raman scattering is a very weak process and the measured spectra overlap a stronger fluorescence background, separation of the two signals is desirable. Fluorescence lifetimes in biological samples are typically of the order of 1ns. Virtual state lifetimes by contrast are on the order of femtoseconds. Therefore, an efficient way to reject the fluorescence signal in Raman measurements is by using an ultrafast shutter. Previous attempts used lasers at low rep rate and high pulse energies that are damaging for biological materials. Here we present an ultrafast gate that utilizes nanjoule level pulse energies and 80 MHz rep rates, well below the nonthermal ablation threshold, and average powers low enough to avoid thermal damage. Using a novel nonlinear medium and a co-axial Kerr shutter design, we will show fluorescence free spontaneous and Resonance Raman spectra obtained from highly fluorescent biological and nonbiological samples.
Bioluminescence Imaging (BLI) is an increasingly useful and applicable technique that allows for the non-invasive observation of biological events in intact living organisms, ranging from single cells to small rodents. Though the photon production occurs within the host, significant exposure times can be necessary due to the low photon flux compared to fluorescence imaging. In animal models, the constant presence of hemoglobin, whose optical absorption spectrum strongly overlaps most bioluminescent emission spectra, leads to a further decrease in detectable photons. We have developed and validated a technique that is able to red-shift the bioluminescent photons to the more desirable optical region of >650nm, a region of minimal absorbance by hemoglobin. This red-shift occurs by using bioluminescence as an internal light source capable of exciting a fluorophore, such as a fluorescent protein or a quantum dot, that emits in the red. Interestingly, in the absence of an absorber, this excitation can occur over substantial distances (microns to centimeters), far exceeding distances associated to, and thereby precluding, resonance energy transfer phenomena. We show this novel technique yields a substantial increase in the number of red photons for in vitro and in vivo conditions, both in isolated single cells and intact living mice.

**7902-34, Session 7**

**Characterization and application of a redox-sensitive GFP-mutant roGFP**

K. Eigass, S. Wierer, S. Peter, S. Bieker, U. Zentgraf, F. Schleifenbaum, Eberhard Karls Univ. Tübingen (Germany)

For the quantitative analysis of molecular processes in living (plant) cells, such as the perception and processing of environmental and endogenous signals, new combinatorial approaches in optical and spectroscopic technologies are required. The use of green fluorescent protein (GFP) and its variants, to create fluorescent fusion proteins has revolutionized the in vivo analysis of cell biological processes. Fluorescence intensity measurements provide live-cell images with spatio-temporal resolution but usually no information about dynamics in physico-chemical parameters, which might occur during cell physiological processes, in the immediate environment of the fluorophore-tagged protein. Recent progress has been made in generating a redox-sensitive mutant of GFP (roGFP), which shows changes in its optical properties in response to changes in the redox state.

We characterized the optical properties of roGFP in vitro by determining shifts in the absorption and emission spectra and changes in the fluorescence decay time of purified roGFP. For this, we applied spectrally and temporally resolved fluorescence spectroscopy on purified roGFP exposed to different environmental redox potentials. Based on these in vitro findings, we demonstrate and compare several applications of roGFP for the in vivo analysis of cell biological processes. Knowledge of the optical properties of roGFP enables the quantitative analysis of the intracellular redox potential during complex processes taking place in the course of a plant’s life e.g. responses to drought, salt stress, high or low temperature stress or the aging process of living plants.

**Quantitation of cellular autophagy using 4D image-based cytometry**

F. Chuang, NSF Ctr. for Biophotonics Science and Technology (United States)

The advent of biotechnology including polymerase chain reaction (PCR), gel electrophoresis, immunoassays, DNA/protein microarrays, and bioinformatics - have enabled medical scientists to make tremendous progress in understanding the molecular basis of human health and disease. However, the relative lack of tools and methods to visualize and manipulate living biological systems at the subcellular and molecular level has become a major obstacle to further progress in practically all fields of biomedicine. We present one example of ongoing research at the NSF Center for Biophotonics to better understand molecular mechanisms in cancer biology.
Autophagy is a universal, intracellular recycling program that is triggered when cells undergo environmental stress, such as nutritional starvation. Based on previous research, we hypothesized that autophagy plays an important role in modulating breast and prostate tumor cell killing by arginine deminilase (ADI) - we used deconvolution fluorescence microscopy to obtain 3D image sequences, from which we could extract statistical information about the number, distribution, and degree of colocalization or fusion between autophagosomes and lysosomes. This information not only helps us to understand the basic relationship between autophagy and apoptosis, but may allow us to identify new cancer pharmaceuticals whose efficacy is enhanced through the precise modulation of autophagy.

7902-39, Session 8

Study of cell classification with a diffraction imaging flow cytometer method
K. Dong, TEO Systems, Inc. (United States); K. Jacobs, East Carolina Univ. (United States); Y. Sa, Y. Feng, Tianjin Univ. (China); J. Q. Lu, X. Hu, East Carolina Univ. (United States)

Morphological features provide powerful markers for cell classification. Investigation of cell morphology can yield insights on many biochemical processes underlying various cellular activities in life science and clinical studies. Nearly all existing flow cytometers, however, are based on detection of angularly integrated signals of scattered light and fluorescence for cell classification. This approach yields very limited morphology information and relies mainly on fluorescence signals to label and classify cells. The method of diffraction imaging flow cytometry provides a label-free approach to extract 3D morphological features rapidly on a single-cell basis. With a diffraction imaging flow cytometer, we have acquired and analyzed the diffraction imaging data from 5 types of cultured cells. A gray level co-occurrence matrix (GLCM) algorithm was applied to extract the interference fringe related textures in the diffraction image data. In this algorithm the image textures are captured by constructing a co-occurrence matrix of the conditional joint probabilities of all pair-wise combinations of grey levels in the image at pre-determined inter-pixel distance and orientation. Six statistical features have been selected to demonstrate the potential of this new method for rapid cell assay. The quantitative values of the GLCM features were imported into a support vector machine algorithm for automated cell classification to classify 30 cells for each of the 5 cell types. We further studied the 3D morphology of these cells using a confocal microscopy method to establish the strong correlation between the morphological features and diffraction image textures.

7902-40, Session 8

Extraction of multiple fluorescence lifetimes from cytometric data
P. Jenkins, New Mexico State Univ. (United States); J. P. Freyer, National Flow Cytometry Resource (United States); M. S. Naivar, Darkling Simulations, LLC (United States); J. P. Houston, New Mexico State Univ. (United States)

Recent developments in phase-sensitive flow cytometry have led to a digital extraction of the fluorescence lifetime of a cell/particle for both analysis and sorting applications. The lifetime is acquired using a digital Fourier analysis technique performed subsequent to fluorescence measurements. This method uses an intensity-modulated laser source to excite the samples and a high speed data acquisition system to capture the modulated Gaussian signals and digitally analyze in Fourier space. Instead of assuming single exponential fluorescence decay kinetics when analyzing data, indicating average lifetime of the fluorescence, multi-exponential decay kinetics, indicating individual fluorophore lifetimes, are investigated. By modulating at multiple frequencies simultaneously, we hypothesized that multiple lifetimes can be extracted in real time with simple changes to the modulation source. Our approach involves shifting from a single modulation frequency to multiple by exploiting square-wave modulation, and using Fourier analysis of multi-exponential fluorescence decays to extract their phase shift at multiple harmonics. We have developed computer programs which simulate a single fluorescence event and allow for an idealized simulation of data collection. Using these programs we can extract individual phase lifetimes from multiple fluorophores producing a single fluorescence signal by exploiting the multiple harmonics present in the excitation.

7902-41, Session 8

CytometryML, DICOM, and FCS-ACS
R. C. Leif, S. H. Leif, Newport Instruments (United States)

Introduction-Background: The integration of cytometry data with clinical, pharmacological, and research data requires that these software systems interoperate. Diverse groups with minimal interaction are creating components for their specific applications. This is expensive in terms of money and human effort. The Digital Imaging and Communications in Medicine (DICOM) standard includes pathology supplements; but, does not include list-mode data and has a unique format. CytometryML is an XML schema based translation, extension, amalgamation and augmentation of DICOM and International Society for Advancement of Cytometry (ISAC) standards.

Methods: DICOM and the ISAC Flow Cytometry standards are translated into XML schemas. CytometryML presently consists of 4 major schemas: Series, Instance, Instrument, and Specimen; it also includes Image and List-Mode schemas. Series metadata, which is specific for an entire collection of images and/or list-mode files produced by a single instrument and derived from a single specimen, as well as the storage locations of the individual instance files, are stored together with associated metadata files in a container (ZIP) file. Each instance file includes the metadata and binary image and/or list-mode files that are specific for a single or closely related group of instrument runs from a single specimen.

Results and Conclusions: CytometryML when combined with the ISAC ACS schemas should permit the integration of Cytometry data with the necessary XML based medical informatics to create a reliable, efficient, interoperable, public, international cytometry-pathology software infrastructure.

7902-42, Session 9

Ultra-wide-field lensfree fluorescent imaging of caenorhabditis elegans on a chip
A. F. Coskun, I. Sencan, T. Su, A. Ozcan, Univ. of California, Los Angeles (United States)

Caenorhabditis elegans (C.elegans) is a multi-cellular organism that is widely explored to unravel the causes of various diseases including cancer. Together with its well characterized nervous system and genome, it has become a powerful model-organism that can be rapidly and cost-effectively cultured in lab-environment. Its transparency also permits the use of light-microscopy to study the physiology of the worm in response to different stimuli, such as drugs. Motivated by these, there has been extensive research on micro-fluidics enabled high-throughput screening tools for study of C. elegans.

However, conventional light-microscopy does not provide a good match for the throughput and field-of-view of existing micro-fluidic platforms. To mitigate this limitation, here we introduce a high-throughput lensfree fluorescent imaging modality that can monitor transgenic or fluorescently
labeled C. elegans samples over an ultra-wide field-of-view of >8cm² with a resolution ~10um. Furthermore, this on-chip fluorescent imaging platform does not use bulky components such as lenses, thin-film filters or mechanical scanners; and therefore provides a better match to lab-on-a-chip tools available for C. elegans research.

In our approach, the fluorescent emission from the nematode is collected by a fiber-optic faceplate and is delivered to a wide-field CCD sensor-array without the use of any lenses. To make up for the lack of lenses, the detected fluorescent signal is then digitally reconstructed using compressive decoding to yield ~10um resolution over the entire imaging field-of-view (~8cm²). Such a high-throughput lensfree on-chip fluorescent imaging platform might be especially valuable for drug discovery, genome analysis and cancer research.

**7902-43, Session 9**

**Adaptive optics in sectioning microscopes: the practical implementation**

J. Andilla, Imagine Optic SA (France); J. Ballesta, Imagine Optic Inc. (United States); X. Levecc, Imagine Optic SA (France)

Deep imaging in biological tissues suffers from refractive aberrations. Those aberrations reduce the image quality of the biological samples and also reduce the signal to noise ratio of the sectioning microscopes. During the last years, adaptive optics has been demonstrated as a powerful tool to correct those problems by using deformable mirrors. The methods proposed in those demonstrations can be usually classified in three groups: Sensor-based, Sensor-less, and Model-based. The first one uses a wavefront sensor to determine the aberrations; the measure given by the sensor is used to directly compute a correction shape for the deformable mirror. In the second group, the optimizations are done by testing the quality of the resulting image of a known shape of the mirror and iteratively optimize it. For the last one the improvement is given by assuming a known aberration and to be able to pre-compensate it. Here, we present a complete study of each strategy of correction with its advantages and drawbacks and finally we will propose a complete protocol to improve the image quality when doing deep imaging microscopy. On the other hand, each technique of sectioning microscopy has its own characteristics. In can be shown that the optical aberrations introduced by the system have different effects and, then, corrections should be applied differently in each case. We also demonstrate how the adaptive optics system should be implemented in each technique. Finally we summarize the most relevant characteristics which define, in the adaptive optics point of view, each system.

**7902-44, Session 9**

**Analysis of time-gated FLIM data by means of the phasor approach**

F. Fereidouni, D. van den Heuvel, J. Voortman, E. Hofman, H. C. Gerritsen, Utrecht Univ. (Netherlands)

Fluorescence lifetime imaging is a versatile tool which can be utilized to distinguish and identify the molecular environment. Both time correlated single photon counting (TCSPC) and time gating methods are used for lifetime imaging but to obtain high accuracies high signal-to-noise ratios are required.

The phasor approach is a graphical global analysis method that increases the S/N ratio of the analysis. This method simplifies the analysis of FLIM data and avoids difficulties of nonlinear regression fitting. It has been successfully employed for analyzing both frequency domain and time domain lifetime images. Time gating detection methods run at very high count rates (~10 MHz) but use of the phasor approach to analyze the data is complicated by truncation and under sampling of the decay curve due to the limited number of gates. In this paper we present a modification to the phasor analyses method that takes into account the cut-off and sampling problem. This approach is tested on both simulated lifetime images and on real data. We demonstrate that this method can be applied to retrieve two lifetimes from time gating data that can not be resolved using standard (non global) fitting techniques.

**7902-45, Session 9**

**Quantum cascade laser-based replacement for FTIR microscopy**

M. Weida, B. Yee, Daylight Solutions, Inc. (United States)

Infrared (IR) microscopy has shown itself to be an important diagnostic tool for tissue analysis.[1, 2] To date, the main tool for performing IR microscopy has been the Fourier transform infrared (FTIR) microscope. FTIR microscopes utilize incandescent bulbs for light sources, and require cryogenically cooled detectors for the weak, optically poor probe signals. Image acquisition times can be tens of minutes even for sophisticated instruments, and the size and cost of FTIR microscopes precludes their broader clinical use. The development of broadly tunable, external cavity quantum cascade lasers (ECqCL™) has created a light source in the mid-IR that is ideal for IR microscopy. Spectrally brilliant probe beams that are diffraction limited, with intensities many orders of magnitude higher than incandescent sources, can be generated from compact, room temperature ECqCL™ devices. Moreover, the increase in intensity allows the use of room temperature microbolometer focal plane arrays (FPAs) for detection. The combination of ECqCLs™ and microbolometer FPAs opens the possibility of producing low cost, compact, room temperature IR microscopes with acquisition speeds thirty times that of state-of-the-art FTIR microscopes. The present study explores the challenges of creating this new generation of IR microscopes, and demonstrates the capabilities of the technology.

Bibliography


**7902-35, Session 10**

**Diesel exhaust particle toxicity to human lung adenocarcinoma epithelial cell line: combined instrumental approaches to study morphological, biochemical, and biomechanical alterations at the cellular level**

A. Zhou, Y. Wu, G. D. McEwen, Utah State Univ. (United States)

A multi instrumental approach using electric cell-substrate sensing (ECIS), Raman microspectroscopy (RM), and atomic force microscopy (AFM), to further understand links between morphological, biochemical, and cytoarchitectural/biomechanical changes of human lung carcinoma epithelia (A549) induced by diesel exhaust particles (DEP). ECIS indicated a range of DEP cause apoptotic responses in attached cells and decreased attachment of suspended cultures. Raman results show changes in cellular bio constituent ratios between treated and untreated A549. AFM illustrates variations in the cellular membrane surface hydrophobicity and decreased membrane surface adhesion forces with treatment times. These findings suggest morphological links to biochemical, and biomechanical changes caused by DEP.

**7902-46, Session 10**

**An on chip Fresnel zone plates enabled optofluidic microscope for sectional cell imaging**

L. M. Lee, S. Pang, J. Wu, S. A. Lee, G. Zheng, C. Yang,
Surface plasmon enhanced high-resolution total internal reflection fluorescence microscopy

K. Kim, Y. Oh, W. Lee, D. Kim, Yonsei Univ. (Korea, Republic of)

Conventional imaging techniques, such as epi-fluorescence and confocal microscopy, have been widely used to observe various biological phenomena. In recent years, there have been new approaches for super resolution imaging including STED microscopy, field optical superlens imaging, and photo-activated light microscopy.

In this paper, we have studied imaging resolution of plasmon enhanced total internal reflection fluorescence microscopy (TIRFM) by exciting localized hot spots of surface plasmon associated evanescent fields. The hot spots are activated by nanostructures: for example, nanoislands and periodic nanopatterns. Random islands were synthesized by thermal annealing method. A metal film that is initially evaporated on a glass substrate is annealed under high temperature, whereby the metal film substrate is transformed to island structures. The size distribution of nanoislands can be adjusted by initial thickness of metal film and the annealing temperature and time in the fabrication process. The distribution of hot spots for efficient excitation of fluorescent molecules can be controlled by changing the size distribution of nanoislands. Periodic nanostructures were fabricated by e-beam lithography.

The concept was experimentally confirmed by imaging fluorescent beads and visualizing endocytotic internalization of GFP-tagged adenoviruses in cells. The results confirm the enhancement of resolution, which was more prominent at higher concentration of fluorescent molecules. In this enhanced TIRFM, the imaging resolution is mainly affected by the size of hot spots, irrespective of the randomness of the nanostructure-based imaging platform, while conventional TIRFM remains to be diffraction-limited. Further investigation on the dependence of imaging resolution on specific nanostructures is currently under way.

Instantaneous spatial light interference microscopy (iSLIM)

H. Ding, G. Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Quantitative Phase Imaging (QPI) has become a rapidly emerging area of study. The range of QPI applications in biology includes red blood cell imaging, cell growth, average refractive index, and optical properties of tissues. In addition to the resolution and contrast that characterize typical, intensity-based microscopes, phase sensitivity is essential for the performance of a quantitative phase microscope. Diffraction Phase Microscopy (DPM) is a common-path interferometric method that was developed to provide highly stable phase measurements without degrading the diffraction limited resolution. Here we developed Instantaneous Spatial Light Interference Microscopy (iSLIM) as novel white light-based quantitative phase imaging method, which provides single-shot, speckle-free imaging. iSLIM combines the benefits of common-path phase stability associated with diffraction phase microscopy and that of white light illumination associated with phase contrast microscopy.

iSLIM is implemented as an add-on module to a commercial phase contrast microscope, which is consequently transformed into a quantitative phase microscope. iSLIM employs the spatially coherent white light illumination from a commercial PCM (Axio Observer Z1, Zeiss) in a common path geometry specific to DPM and, as a result, provides not only high phase sensitivity and diffraction limited transverse resolution, but also high contrast to noise. iSLIM’s features advance the field of quantitative phase imaging by providing speckle-free images, which allows for spatially sensitive optical path-length measurement, by enabling temporally sensitive optical path-length measurement, and by providing phase dispersion imaging due to the broad band illumination. We foresee that iSLIM is likely to make a broad impact by its low-maintenance and straightforward implementation with existing phase contrast microscopes.
observed an increase in the in situ fluorescence as well as structural alterations within these materials during the course of glycation. The two-photon fluorescence emission maximum was observed at about 460 nm with a shoulder at 520 nm. The emission maximum in the one-photon excitation experiment (λex=360 nm) was at 445 nm. This maximum had shifted to about 460 nm during the course of glycation with glyceraldehyde. For the ribose and glucose, in addition to the 460 nm peak, the 445 nm component persisted. As determined from measuring the fluorescence intensity at 460 nm maximum, glycation with glyceraldehyde was faster compared to ribose and glucose. Moreover, glyceraldehyde-glycated collagen hydrogels generated stronger fluorescence signals compared to ribose and glucose-glycated samples. Upon excitation of glycated collagen hydrogels with 330 nm light, we measured the fluorescence intensity at 460 nm maximum, glycation with glyceraldehyde was faster compared to ribose and glucose. Moreover, glyceraldehyde-glycated collagen hydrogels generated stronger fluorescence signals compared to ribose and glucose-glycated samples. Upon excitation of glycated collagen hydrogels with 330 nm light, different emission peaks were detected.

7902-51, Session 11

Dynamical study of cell motility by simultaneous light microscopy and surface plasmon resonance imagery

J. Moreau, Lab. Charles Fabry (France); J. Allain, R. Gulvady, Ecole Polytechnique (France); M. T. Canva, Lab. Charles Fabry (France)

Living cells interact with their surrounding environment in a complex but carefully controlled way. In particular, they are able to develop strong adhesion with either a substratum or other cells in a tissue, but also to detach in certain conditions. This is one of the key components for fascinating behaviours, such as cell motility or embryo morphogenesis. Investigating the interaction between a cell and its substratum is then an important question in biology. In particular, it is important to be able to study the strength of this contact in a dynamic approach. In this paper we described a novel optical instrumentation which combines classical reflection microscopy with surface plasmon resonance imagery (SPRI). SPRI is based on the detection of evanescent surface waves created on the surface of a thin gold layer. The penetration depth of these surface plasmon waves in the aqueous medium above the gold being limited to 100 nm, near field image of events happening on the surface can be obtained in real time, without any label. Simultaneous acquisition of a far field microscopic image of the surface allows comparison of these two imaging mode. Cell motility was studied on the model case of Dictyostelium cells. We show that quantitative information on the cell-surface interaction during the cell movement can be extracted from SPRI images. Influence of different environments on the plasmonic signal of cells was also studied.

7902-52, Session 11

Validation of autoLF: a platform for quantifying near-infrared fluorescent images of lymphatic propulsion in humans

J. C. Rasmussen, M. Bautista, G. Dickinson, B. Niccum, I. Tan, K. E. Adams, M. B. Aldrich, M. V. Marshall, The Univ. of Texas Health Science Ctr. at Houston (United States); C. E. Fife, E. A. Maus, L. A. Smith, The Univ. of Texas Health Science Ctr. at Houston (United States) and Memorial Hermann Hospital (United States); J. Zhang, X. Xiang, K. Zhou, Siemens Corporate Research (United States); E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

Recently, we demonstrated near-infrared (NIR) fluorescence imaging for quantifying real-time lymphatic propulsion in humans following intradermal injections of microdose amounts of indocyanine green. However computational methods for image analysis are underdeveloped, hindering the translation and clinical adaptation of NIR fluorescent lymphatic imaging. In our initial work we used ImageJ and custom MATLAB programs to manually identify lymphatic vessels and individual propulsion events using the temporal transit of the fluorescent dye. In addition, we extracted the apparent velocities of cell propulsion and time periods between propulsion events. Extensive time and effort were required to analyze the 8-6 gigabytes of NIR fluorescent images obtained for each subject. To alleviate this bottleneck, we commenced development of AutoLF, an integrated software platform which will permit automated, near real-time analysis of lymphatic function using NIR fluorescent imaging. However, prior to automation, the base algorithms calculating the apparent velocity and period must be validated to verify that they produce results consistent with the proof-of-concept programs. To do this, both methods were used to analyze NIR fluorescent images for ten subjects and the number of propulsive events identified, the average apparent velocities, and the average periods for each subject were compared. Paired Student's t-tests indicate that the differences between their average results are not significant. With the base algorithms validated, further development and automation of AutoLF can be realized, significantly reducing the amount of user interaction required, and potentially enabling the near real-time, clinical evaluation of NIR fluorescent lymphatic imaging.

7902-53, Session 11

Quantitative analysis of lipid droplets accumulation in living macrophages by coherent anti-Stokes Raman scattering microscopy

W. Chen, C. Chien, National Yang-Ming Univ. (Taiwan) and Academia Sinica (Taiwan); T. Chang, Academia Sinica (Taiwan) and National Yang-Ming Univ. (Taiwan)

The over-uptake of low density lipoproteins (LDLs) by macrophages will lead to lipid droplets accumulation, followed by foam cell formation, and finally result in atherosclerosis. Fluorophore-labeled acetylated LDL has been used to investigate the uptake of lipoprotein by macrophages. However, lipid droplets accumulation in macrophages can not be directly observed in real time. Here we introduce coherent anti-Stokes Raman scattering (CARS) microscopy to achieve the label-free real-time monitoring of lipid droplets accumulation during the formation of foam cells. To quantitatively estimate the amount of lipid accumulated in macrophages, we have used the image analysis method based on maximum entropy thresholding. Our results show that this analytic method is consistent with traditional lipid staining method and biochemical assay. We further characterize and evaluate the effects of some anti-oxidants and related drugs on lipid droplets accumulation. It appears that CARS microscopy is a powerful tool for the screen of potential therapeutic agents for atherosclerosis.

7902-60, Poster Session

The heavy metals influence on spectroscopic characteristics of sulfur cycle bacteria Desulfuromonas acetoxidans

O. M. Vasyliv, O. I. Bily, S. O. Hnatush, Ivan Franko National Univ. of L'viv (Ukraine)

Spectroscopic characteristics of bacterial cells, as usual, depend on their sizes, refractive indexes of their components, such as membrane, nucleoid and cytoplasm, and surrounding environment. Interaction between bacterial cells and heavy metals ions should causes to their optical characteristics' changes. In this work the influence of cadmium, copper, zinc and lead salts on the light-diffusion properties and growth of sulfurreducing bacteria Desulfuromonas acetoxidans has been investigated. Concentration changes and relative content of cells D. acetoxidans in the set intervals of sizes under the influence of heavy metals have been observed. Correlation between changes of light-diffusing properties and growth of bacterial cells Desulfuromonas.
acetoxydans under the influence of heavy metals has been shown. These bacteria are considered to be used as microbial-anode fuel cells with high electron recovery (>80%) from oxidized acetate to electric current. The investigations have been carried out by measuring sizes’ distribution of bacterial cells and spectral dependence of solutions’ turbidity under the influence of heavy metals. In this work for the estimation of light-diffusing properties of bacterial cells a method which is based on registration of the intensity dissipated light changes by the cells is offered. This method includes the statistical set of amplitude changes and duration of impulses for the particles of the set size, construction of the cross-correlation function on the basis of the data of measuring, which expresses statistical characteristics of light dispersion intensity by the investigated particles, and gaining of particles distributing after sizes, by solution of integral equalization of Fredholm the first kind.

7902-61, Poster Session

Heating device for 96-well microtiter culture plates

T. Bruns, C. Berchtold, H. Schneckenburger, Hochschule Aalen (Germany)

Since their launching in the 1960s, 96-well microtiter plates gained global popularity in medicine, chemistry and biotechnology. Main fields of application are found in the area of diagnostics, serology, cell culture- and immunological research. Generally, specimens within the wells, e.g. proteins or vital cells, have ambient temperature, which is often subject to considerable fluctuations and inhibits comparative measurements. Therefore, accurately defined, homogenous and fast heating of the specimen without interfering and falsifying impact of ambient air and apparatus temperature is essential for quantitative measurements of temperature dependent parameters.

The present heating device is attached on top of the microtiter plate, permitting individual and fast heating of the specimen by resistor elements without direct contact. All wells of the microtiter plate remain accessible from above and beyond for sensory (e.g. optical) measurements independently from the bottom geometry of the wells.

The height of a standard microtiter plate is increased by only 4 to 6 mm due to the heating device. By various circuit configurations it is possible to realise either homogenous heating all over the plate or heating of individual arrays or clusters of wells, e.g. for maintaining defined temperature gradients.

The heating device is validated by microscopic experiments as well as by a total internal reflectance fluorescence reader system for selective investigations of cell membranes. For this purpose, particularly with regard to potential pharmaceutical applications, the kinetics of cancer cells incubated with temperature-dependent fluorescent dyes, e.g. 22-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-23,24-bisnor-5-cholen-3beta-ol (22-NBD-chol) is determined.

7902-62, Poster Session

The study of the correlation properties on RBC flickering using double path interferometric quantitative phase microscopy

S. Lee, J. Lee, C. Park, D. Kim, Gwangju Institute of Science and Technology (Korea, Republic of)

The time series analysis of RBC membrane undulations provides the important information on their complex dynamic motilities in RBCs. We have demonstrated the application of detrended fluctuation analysis (DFA) for quantitative phase microscopy (QPM) by studying the correlation property of RBC flickering. DFA has showed useful in analyzing long-range correlations in time series. Our QPM imaging system has provided the nanometer path length sensitivity on a millisecond scale. We have measured the time series thickness variations of a normal RBC over the whole cell surface. The heterogeneous spatial distribution of cell membrane fluctuations was shown to reveal the temporal and spatial properties of RBC flickering motions for a normal RBC. We have applied DFA to study the correlation properties of time series thickness data obtained from QPM. We have also shown the exponents varied approximately between 0.7 and 1 for a normal RBC and the average exponent for background noise outside a cell was close to the exponent of white noise. In this way, we have shown the usefulness of the QPM system for simultaneously analyzing the correlation properties and the cell stiffness of time series RBC thickness fluctuations over the cell surface using DFA. In this regard, QPM technique will be a powerful and practical tool for studying the complex dynamics of RBC membrane fluctuations under various physiological conditions.

7902-63, Poster Session

Real time diagnosis of bladder cancer with probe-based confocal laser endomicroscopy


Probe-based confocal laser endomicroscopy (pCLE) is an emerging technology for in vivo optical imaging of the urinary tract. Particularly for Bladder cancer, real time optical biopsy of suspected lesions will likely lead to improved management of bladder cancer. With pCLE, micron scale resolution is achieved with sterilizable imaging probes (1.4 or 2.6 mm diameter), which are compatible with standard cystoscopes and resectoscopes. Based on our initial experience to date (n = 60 patients), we have demonstrated the safety profile of intravesical fluorescein administration and established objective diagnostic criteria to differentiate between normal, benign, and neoplastic urethrom. Confocal images of normal bladder showed organized layers of umbrella cells, intermediate cells, and lamina propria. Low grade bladder cancer is characterized by densely packed monomorphic cells with central fibrovascular cores, whereas high grade cancer consists of highly disorganized microarchitecture and pleomorphic cells with indistinct cell borders. Currently, we are conducting a diagnostic accuracy study of pCLE for bladder cancer diagnosis. Patients scheduled to undergo transurethral resection of bladder tumor are recruited. Patients undergo first white light cystoscopy (WLC), followed by pCLE, and finally histologic confirmation of the resected tissues. The diagnostic accuracy is determined both in real time by the operative surgeon and offline after additional image processing. Using histology as the standard, the sensitivity, specificity, positive and negative predictive value of WLC, pCLE, and WLC + pCLE are calculated. With additional validation, pCLE may prove to be a valuable adjunct to WLC for real time diagnosis of bladder cancer.

7902-64, Poster Session

High-speed fluorescence lifetime measurement by dual-channel waveform measurement

Y. J. Won, D. Kim, Gwangju Institute of Science and Technology (Korea, Republic of)

Analog mean-delay (AMD) method is a powerful alternative method in measuring the lifetime of molecules using confocal fluorescence lifetime imaging (FLIM). Even though the photon economy and the lifetime precision of the AMD method are proven to be as good as the state-of-the-art time-correlated single photon counting (TC-SPC) method, there have been some speculations and concerns about the accuracy of this method off from the absolute lifetime value of a fluorescence probe. In the AMD method, the temporal waveform of an emitted fluorescent signal is directly recorded with a slow digitizer whose bandwidth is much lower than the temporal resolution of lifetime to be measured. We have found that the drifts and the fluctuations of the absolute zero position in a measured temporal waveform are the major problems in the AMD method. We have also proposed dual channel
waveform measurement scheme that may suppress these errors. It is shown that there may exist more than 2 ns drift in a measured temporal waveform during the period of the first 12 minutes after electronics components are turned on. The standard deviation of a measured lifetime after this warm-up period can be as large as 51 ps without proposed scheme. We have shown that this error can be reduced to 9 ps with our scheme.

7902-65, Poster Session

Transillumination of subcutaneous adipose tissues using near-infrared hyperspectral imaging in the 1100-1800-nm wavelength range

K. Ishii, A. Kitayabu, Y. Kobayashi, N. Honda, Osaka Univ. (Japan); K. Awazu, Osaka Univ. (Japan) and Univ. of Fukui (Japan) and Kyoto Univ. (Japan)

Hyperspectral imaging (HSI) is a chemical imaging modality with spectroscopic information. HSI concept combines spectroscopy and imaging and allows the recording of the entire reflectance spectrum for every pixel in an entire image. This technique was often used in agricultural or pharmaceutical industries. But there have been a few reports for clinical medical applications. In near-infrared (NIR) wavelength region, the significant absorption peaks are often observed by the overtone of mid-infrared molecular vibration. In addition, NIR light has a high penetration because of low scattering and less absorption by water or protein.

In this study, we constructed the NIR-HSI system and the high-contrast subcutaneous adipose tissue imaging was conducted in-vitro. The super continuum (SC) light source (wavelength range: 1100 nm to 2300 nm) was used for a NIR broadband light. NIR SC light was delivered to a grating spectrometer for selecting an irradiation wavelength. The wavelength-limited light was irradiated to the sample. Diffuse reflection light on the sample was detected by the InGaAs charge coupled device. In the absorption spectra which are obtained by our NIR-HSI system, the characteristic absorption bands were observed around 1200 nm and 1700 nm. In the processed images using these wavelength bands, subcutaneous adipose tissue was observed through a skin. In a hyperspectral image by another processing using all wavelengths, a high-contrast image of subcutaneous adipose tissue is also obtained. NIR-HSI system is a powerful diagnostic technique for adipose tissues distribution and their morphological change on/inside a tissue.

7902-66, Poster Session

Developments of Pulse Laser Assist Optical Tweezers (PLAT) for in vivo manipulation

S. Maeda, T. Sugiiura, K. Minato, Nara Institute of Science and Technology (Japan)

Optical tweezers is a technique to trap and to manipulate micron sized objects under a microscope by radiation pressure force exerted by a laser beam. Optical tweezers has been utilized for single-molecular measurements of force exerted by molecular interactions [1] and for cell palpation [2].

To extend applications of optical tweezers we have developed a novel optical tweezers system combined with a pulse laser. We utilize a pulsed laser (Q-switched Nd: YAG, wavelength of 1064 nm) to assist manipulations by conventional optical tweezers achieved by a continuous wave (CW) laser. The pulsed laser beam is introduced into the same optics for conventional optical tweezers. In principle, instantaneous radiation force is proportional to instantaneous power of laser beam. As a result, pulsed laser beam generates strong instantaneous force on an object to be manipulated. If the radiation force becomes strong enough to get over an obstacle structure and/or to be released from adhesion, the object will be free from these difficulties. We have named this technique as Pulse Laser beam Assisted optical Tweezers (PLAT). We have successfully demonstrated to manipulate objects surface on a living cell for “in vivo manipulation.”

References;

7902-67, Poster Session

Polarized Raman studies for early detection of cancer in human cervical tissue

J. M. Jagtap, T. Singh, M. Mozumder, P. Shukla, A. Pradhan, Indian Institute of Technology Kanpur (India)

Biochemical changes occur in tissue during progression of cancer which provides important clues for early detection. Raman spectroscopy is a highly specific technique, providing useful information about molecular compositions. Polarized Raman spectroscopy has been performed on human cervical tissue to detect the precancers. Un-polarized, co and cross polarized Raman spectra were recorded in a range of 1000 - 2000 cm-1 for 28 samples taken from 14 patients. Raman spectra of cervical tissue contains peaks in the vicinity of 1071, 1130, 1280, 1365, 1454, 1505, 1660, 1780 and 1965 cm-1. In particular, it is observed that the peaks at 1130, 1280 and 1365 cm-1 are signatures of the superficial epithelial layer and not from the stromal collagen content. This decoupling of epithelial and stromal signatures is done through the polarized spectra. The peaks observed at 1365 and 1660 cm-1 in co-polarized spectra were analyzed by comparing the intensity ratio of those peaks for cancer to normal tissues and a significant difference is observed. The potential of polarized Raman Spectroscopy in discriminating normal and precancerous human cervical tissue is enhanced via principal component analysis (PCA). Interestingly, it is found that the covariance map gives information about shift of peak position in spectra of abnormal tissue compared to normal tissue.

7902-68, Poster Session

Differentiating human cervical cancer and normal tissue through wavelet domain characterization of intrinsic fluorescence

R. Gudibande, IISER Kolkata (India); M. Mozumder, R. Singh, Indian Institute of Technology Kanpur (India); P. K. Panigrahi, IISER Kolkata (India); A. Pradhan, Indian Institute of Technology Kanpur (India)

Wavelet Transform based multi-resolution analysis has been used to characterize the intrinsic fluorescence of both dysplastic and normal human cervical tissues. The fluorescence spectra corresponding to 325 nm and 370 nm excitation from cervical dysplastic tissues of 48 patients from diverse age groups are studied in detail using Morlet wavelet. The wavelet modulus maxima lines for 325 nm excitation indicated a distinct shift for dysplastic tissues towards the lower wavelengths. Patients from diverse age groups are studied in detail using Morlet wavelet. The wavelet modulus maxima lines for 325 nm excitation indicated a distinct shift for dysplastic tissues towards the lower wavelengths. Patients from diverse age groups are studied in detail using Morlet wavelet. The wavelet modulus maxima lines for 325 nm excitation indicated a distinct shift for dysplastic tissues towards the lower wavelengths. Patients from diverse age groups are studied in detail using Morlet wavelet. The wavelet modulus maxima lines for 325 nm excitation indicated a distinct shift for dysplastic tissues towards the lower wavelengths. Patients from diverse age groups are studied in detail using Morlet wavelet.
Infrared spectroscopic imaging of cancerous kidney tissue was performed by means of FTIR microscopy. The spectra of thin tissue cryosections were collected with 64x64 MCT FPA detector and imaging area was increased up to 5.4x5.4 mm by mapping by means of PC controlled x,y stage. Chemical images of the samples were constructed using statistical treatment of the raw spectra. Several unsupervised and supervised statistical methods were used. The imaging results are compared with results of the standard histopathological analysis. It was concluded that application of method of cluster analysis ensures the best contrast of the images. It was found that border between cancerous and normal tissues visible in the infrared spectroscopic image corresponds with the border visible in histopathological image. Closer examination of the infrared spectroscopic image reveals that small domains of cancerous cells are found beyond the border in areas distant from the border up to 3 mm.

Automated optical cell detection, sorting, and temperature measurements

J. D. Kindt, M. Naqbi, T. Kiljan, W. Fuller, W. Wang, Colorado State Univ. (United States); D. W. Kisker, eOptra LLC (United States); K. L. Lear, Colorado State Univ. (United States)

An automated cell detection and sorting system was developed, combining both the optofluidic intracavity spectroscopy (OFIS) technique and dielectrophoresis (DEP). The OFIS method utilizes a microfluidic channel as a Fabry-Perot cavity to produce characteristic transmission spectra of individual cells. The concept behind optical detection is that a decrease in spectrum intensity beyond a threshold indicates that a cell is present. Upon detection, an RF voltage is automatically applied to electrodes, trapping the cell with DEP forces. The automated system is controlled by a computer with custom LabVIEW code, which changes the spectrometer between two different integration times. First, a shorter integration time of 100ms is utilized to detect cells moving through the channel at rates up to 200μm/s. Once the cell is trapped, the integration time is changed to 6s for further optical analysis with a higher signal-to-noise ratio. The additional optical analysis has been previously described and is able to distinguish between cancerous and non-cancerous cells. Based on the classification results, the system then sorts the cell with steering electrodes into one of two microfluidic channels. The real-time cell detection, trapping, and sorting has improved throughput over manual measurements by a factor of 15 to 20. A further advantage is that RF Joule heating can be measured from known dn/dT values of the medium. Since the OFIS spectrum is sensitive to refractive index in the microfluidic channel, a percent decrease in peak wavelength indicates a temperature increase. Accurate knowledge of temperature rise is useful for investigating cell viability issues.

Modeling and Tissue Parameter Extraction Challenges for Free Space Broadband fNIR Brain Imaging Systems

E. Sultan, Drexel Univ. (United States) and School of Biomedical Engineering and Health Systems, Drexel Univ. (United States) and National institute of Health (United States); K. Manseta, A. Khwaja, Drexel Univ. (United States); L. Najafizadeh, A. H. Gandjbakhche, National Institutes of Health (United States); K. Pourrezaei, A. S. Daryoush, Drexel Univ. (United States)

Fiber based functional near infra-red (fNIR) spectroscopy has been considered as a cost effective imaging modality. To achieve a better spatial resolution and greater accuracy in tissue optical parameters (i.e., $\mu_a$ and $\mu_s$) extraction, broadband frequency modulated systems covering multi-octave frequencies of 50-1000MHz is considered. A helmet mounted broadband free space fNIR system is considered as significant improvement over bulky commercial fiber fNIR realizations that are inherently dispersive for broadband operation. Accurate measurements of amplitude and phase of the frequency modulated NIR signals (670nm, 795nm, and 850nm) is reported here using free space optical transmitters and receivers realized in a small size and low cost modules. The tri-wavelength optical transmitter is based on vertical cavity semiconductor lasers (VCSEL), whereas the sensitive optical receiver is based on either PIN or APD photodiodes combined with transimpedance amplifiers. This paper also has considered brain phantoms to perform detection of modulated light for separations of up to 5cm. Analytical transmittance and reflectance of modulated photons in diffused media has been modeled using Diffusion Equation (DE). The robustness of the DE modeling and parameter extraction algorithm was studied by experimental verification of multi-layer diffused media phantoms. In particular, comparison between analytical and experimental models for narrow band and broadband has been performed to analyze the advantages of our broadband fNIR system. Moreover, performance limitations of the modified standard diffusion equation are identified through comparison of the extracted with the ones extracted using the gold standard of Monte Carlo simulation.

Microfluidic isolation and manipulation of microscopic objects using optical trap with geometric distortion

S. Shivalingaiah, S. K. Mohanty, The Univ. of Texas at Arlington (United States)

The innumerable possibilities offered by combining optical forces with micro-fluidic systems presents a growing interest. Several approaches have been proposed for trapping and manipulating, guiding, sorting or analyzing biological particles within micro-fluidic devices. Though optical tweezers using novel beams have been utilized in the past, integration of these beams into microfluidic devices for lab-on-a-chip applications requires efficient actuation of microscale samples. We report development of an optical isolator based on pincushion distortion introduced into astigmatic optical tweezers. While objects in the range of 1 to 5 microns (polystyrene particles and bacteria) were transported away along the curvilinear trajectories of the pincushion profile, objects in 10-20 micron range (e.g. cells) could easily be trapped in the center of the pincushion profile. This enabled efficient isolation of cell(s) from its surrounding with high spatial and temporal precision and thus opens up new possibility to control and study interaction of cells (and other microscopic objects) with surrounding objects without requiring presence or actuation of physical valves. The trapped and isolated cell(s) could be transported by maneuvering the sample stage or beam. Further, optical clearing of wide microscopic area was achieved without requiring beam scanning or sample movement. Introduction of coma into the optical isolator profile resulted into a virtual unidirectional valve that allowed efficient transportation of microscopic objects in the desired direction along the microfluidic channel. We will present theoretical simulation of force exerted by such beam profiles and experimental demonstration of its potential in microfluidic isolation and manipulation.
Development of a stigmatic imaging mass spectrometer using laser desorption/ionization

K. Awazu, Osaka Univ. (Japan) and Univ. of Fukui (Japan) and Japan Science and Technology Agency (Japan); H. Hazama, H. Nagao, H. Yoshimura, J. Aoki, Osaka Univ. (Japan) and Japan Science and Technology Agency (Japan); K. Fujii, Osaka Institute of Technology (Japan) and Japan Science and Technology Agency (Japan); T. Tashima, Japan Science and Technology Agency (Japan); K. Masuda, Suntory Institute for Bioorganic Research (Japan) and Japan Science and Technology Agency (Japan); M. Toyoda, Osaka Univ. (Japan) and Japan Science and Technology Agency (Japan); Y. Naito, Graduate School for the Creation of New Photonics Industries (Japan) and Japan Science and Technology Agency (Japan)

Imaging mass spectrometry (IMS) using laser desorption/ionization allows direct investigation of the spatial distributions of molecular contents in a tissue section. Conventional IMS is performed by scanning a focused laser on the sample. This method loses spatial information within the laser spot, and it takes a few or few tens of hours for a measurement. In order to achieve higher spatial resolution (<1 µm) and shorter measurement time (a few tens of minutes for a sample), we are developing a stigmatic imaging mass spectrometer, where an entire sample surface is irradiated with a laser and the distributions of generated ions are projected onto a position- and time-sensitive ion detector. A mesh pattern image was obtained with spatial resolution of 3-4 µm. For analysis of complex biological samples with a high mass resolution, the instrument has a multi-turn time-offlight mass spectrometer (MULTUM-IMG). An ion image of a micro-dot pattern sample was observed after ten circulations in the multi-turn circuit of MULTUM-IMG maintaining the pattern of the sample. And stigmatic ion images of the ions of crystal violet and methylene blue produced from the stained section of a brain were observed. An ion image of whole part of a hippocampus (3.25 mm x 1.5 mm) in the brain section can be obtained within 13 minutes. In the future, the ion images of molecules existing in tissues such as lipids, peptides, proteins and drugs introduced into a body will be observed.

Utility of fallopian tube imaging techniques for tubal intraepithelial carcinoma detection

S. El Hallani, S. Lam, J. McAlpine, B. Gilks, C. E. MacAulay, The BC Cancer Agency Research Ctr. (Canada); P. M. Lane, The BC Cancer Agency Research Ctr. (United States)

PURPOSE: To compare the ability of Optical Coherence Tomography (OCT) and autofluorescence visualization to detect Tubal Intraepithelial Carcinoma (TIC) in susceptible women. DESIGN: Ex-vivo imaging of fallopian tubes after salpingo-oophorectomies from women with BRCA mutations or pelvic serous carcinoma. METHODS: A fiberoptic probe was inserted into the lumen of surgically removed fallopian tube. Radial scanning of the mucosa was done to generate OCT images in real time. Following OCT imaging, the fallopian tube was cut along the lumen and the mucosa was submitted to direct visualization of autofluorescence alterations. Tubes were formalin fixed and tissue imaging data were correlated to histology. RESULTS: Fluorescence images were captured using 405nm alone and then 405/436nm excitation. Clinically occult TIC and serous carcinoma implants resulted in loss of autofluorescence. The surrounding mucosa, as an internal anatomic control, showed normal green autofluorescence. Using OCT, the TIC fields had a thicker wall compared to normal tubal mucosa. CONCLUSION: Autofluorescence visualization and OCT are promising tools for in vivo TIC detection in susceptible women.

Linear spectral unmixing through multispectral fluorescence excitation imaging

M. Khojasteh, C. E. MacAulay, The BC Cancer Agency Research Ctr. (Canada)

Fluorescence imaging and analysis of fluorescent structures is a potential candidate for tissue diagnostics in a wide variety of clinical situations. When more than one fluorophore is present in the sample or in case of presence of unwanted autofluorescence, reliable separation and imaging of fluorescent signals in individual channels depends on the spectral properties of the fluorophores. If there is a lot of overlap in the spectra of the fluorophores, analysis of localization of fluorophores will not be possible using the common filter cube microscopic methods. Multispectral fluorescence imaging and linear unmixing allow the separation of fluorescent signals with highly overlapping spectra. A method of multispectral fluorescence excitation imaging is presented. The sample is illuminated with multiple excitation wavelengths, and emission images are detected. This method makes use of the differences in the excitation spectra of different fluorophores or similar fluorophores in different environments. Through the use of a programmable light source such as OneLight (OneLight Corp.), in which the wavelengths of the illumination light can be selected under computer control, it is possible to rapidly illuminate with separate excitation wavelengths without the need for changing filter-cubes. Blind source separation techniques are then applied to the multichannel excitation data to perform linear unmixing without the need for the reference spectra. The method will be demonstrated on lung, oral or cervical tissue samples.

Detecting biomarkers of disease using carbon-fluorine spectroscopy

F. Menaa, B. Menaa, O. N. Sharts, Fluorotronics, Inc. (United States)

An ex-vivo protein detection by our patented fluoro-Raman spectroscopy (FRS) technology is described in the present study. FRS is known to be able to reliably detect any fluoro-labeled molecule, compound or material. The goal of our study was to detect rapidly and in a reliable fashion important biomarkers of disease using an innovative approach and FRS. Indeed, we have developed a method which only required the incorporation of a single fluoro-aminoacid into cells and the enrichment of a target protein by immuneprecipitation. The sample containing the protein of interest was then analysed by FRS using a 532 nm DPSS Qswitched laser with 20 kHz repetition rate, 10 ns pulse with a delivery power as small as 10 mW. In this respect, the cell cycle regulator and onco-suppressor protein, p21, was clearly selected once fluorinated. Thus, we propose, for the first time, that fluoro-Raman spectroscopy can be considered and used as a promising technology to specifically, sensitively and rapidly detect fluorinated biomarkers of disease. Eventually, the detection, screening, imaging of biomarkers of disease shall help to further unravel molecular mechanisms involved in a given pathology since FRS, along with its associated devices, is offering a new fluoro-diagnostic approach.
7902-80, Poster Session

**Bioluminescence tomography with a combined BLT/DOT/CT system**

H. Yan, Univ. of California, Irvine (United States); O. Nalcıoğlu, Univ. of California, Irvine (United States) and Pusan National Univ. (Korea, Republic of); G. Gulsen, Univ. of California, Irvine (United States)

Bioluminescence imaging has become an essential in vivo small animal imaging tool in recent years. A problem associated with this technique is that the location, size and concentration of deeply buried sources cannot be obtained accurately. Bioluminescence tomography (BLT), on the other hand, is capable of generating cross-sectional bioluminescence source distribution map. However BLT prerequisites knowledge of tissue optical properties everywhere in the region under investigation, which is generally unavailable. It is then natural to combine the BLT with another imaging modality that can provide the background optical heterogeneity information. Diffuse Optical Tomography (DOT) can be combined with BLT for this purpose. An advantage of such a combination is that both BLT and DOT can be achieved in the same setting.

The quantitative accuracy of both DOT and BLT is limited due to the ill-posed nature of the inverse problem and can be greatly improved by incorporating a third anatomical imaging modality that provides structural information. The available choices include but are not limited to MRI, ultrasound, and x-ray computed tomography (XCT). In this work, we have chosen XCT due to its lower cost and high-throughput nature. All three systems are installed on the same gantry to achieve natural co-registration in space. The performance of the combined system is evaluated using multi-modality phantoms. In particular, a cylindrical 25.4 mm-diameter heterogeneous phantom is constructed. It has been shown that a 1.5 mm diameter source can be accurately localized only if all the available a priori information is utilized.

7902-55, Session 12

**Raman mapping of biological tissue using clustering analysis based on the Pearson correlation coefficient**

F. Festy, King's College London (United Kingdom)

Recent advances in Raman spectroscopy have generated new interests in biomedical research, in particular in the field of optical diagnosis and the characterization of biological tissue. In few cases, differentiation between cancerous and benign tumors from human patients was shown to be possible using principal component analysis on the collected Raman spectra. However, this simple approach has been limited by a number of factors such as the need of controls and the lack of images such as the ones found in conventional histology. Using the rapid Streamline Raman imaging capabilities of Renishaw's inVia Reflex spectrometer, we have mapped with high resolution the chemical signature of a number of human tissue sections. To extract meaningful chemical information from such large datasets (> 300,000 spectra), we have developed an automated clustering approach based on the Pearson correlation coefficient. Concentration maps were obtained from fitting each pixel with a set of basic spectra derived directly from the results of the cluster analysis. Such “hands-off” approach ensures high quality fitting which is not possible with spectra obtained from chemicals originating from other sources.

7902-56, Session 12

**Clustering and discrimination of pediatric patients undergoing open heart surgery with and without methylprednisolone treatment by cellular and humoral immune parameters**

J. Bocsi, A. Mittag, A. Pierzhalski, Univ. Leipzig (Germany); P.
Osmanicik, Charles Univ. in Prague (Czech Republic); I. Dähnert, A. Tarnok, Univ. Leipzig (Germany)

Introduction: Methylprednisolone (MP) is frequently preoperatively administered in children undergoing open heart surgery. Aim of this medication is to inhibit overshooting immune responses. Cellular and humoral immunological changes in pediatric patients were compared for heart surgeries with and without prior MP administration. Pre and postoperative values were compared. Cluster analysis was applied for identification of suitable parameters characterizing both groups. Aim was to identify applicable parameters for prediction of operation outcome and as decision criterion for MP administration.

Methods: Blood samples were analysed from two aged matched groups with surgical correction of septum defects. Group without MP treatment consisted of 10 patients; MP was administered on 23 patients (median dose: 11mg/kg) before cardiopulmonary bypass (CPB). Blood was taken 24h preoperatively, at the end of CPB and 4h postoperatively and analysed by clinical chemistry and flow cytometry. Humoral response as well as changes in differential blood count, lymphocyte subsets and cellular activation was monitored.

Results & conclusion: More than 200 parameters were obtained from analysis. Cross-validation revealed several parameters able to discriminate between MP groups and identify immune modulation by CPB. MP administration resulted in a delayed activation of monocytes, reduced CD4+ and CD8+ T-lymphocytes and increased B-cell counts. Cluster analysis by geneexpression demonstrated that classification of patients is possible based on the identified cellular and humoral parameters. An analysis of these parameters prior to surgery might be taken as decision criterion for MP administration.

7902-57, Session 12

Computational efficient segmentation of cell nuclei in 2D and 3D fluorescent micrographs

J. de Vylder, W. R. Philips, Univ. Gent (Belgium)

Recent research has led to the development of a wide range of new imaging instruments such as Scanned Light Sheet Microscopy and high-throughput screening. These innovations have led to increasing insight, but comes with a bottleneck: they produce huge amounts of data. This data contains valuable information, but has to be interpreted before being able to use this information. In this paper we propose a new technique to automatically segment cell nuclei in fluorescent micrographs.

In a first step, we build an energy map. This energy map has a high value near edges and slowly decreases in function from the distance of an edge. By defining the energy this way, less problems occur near blurred edges, since edge evidence from nearby high contrast edges is propagated. Note that this work does not start form a binary edge map, making it also interesting for the detection of touching nuclei, for which edge detectors often fail. In a second step, each energy valley is detected and classified as a nucleus or background. The energy map is calculated using a dual scan line algorithm, resulting in a memory and speed efficient technique, which makes it interesting for the analysis of images from 3D-scanners or high throughput systems.

The algorithm is tested on the Synthetic 1 image set available at the Broad bioimage Benchmark Collection. The proposed algorithm resulted in a precision of 0.9960 and a recall of 0.9957. The algorithm will further be evaluated on real datasets both in 2D as in 3D.

7902-58, Session 13

A novel method for multiparameter physiological phenotype characterization at the single-cell level


Non-genetic intercellular heterogeneity has been increasingly recognized as one of the key factors in a variety of core cellular processes including proliferation, stimulus response, carcinogenesis and drug resistance. Many diseases, including cancer, originate in a single cell or a few cells. Early detection and characterization of these abnormal cells can provide new insights into the pathogenesis and serve as a tool for better disease diagnostics and treatment. We report on a novel technology for multiparameter physiological phenotype characterization at the single-cell level. It is based on real-time measurements of concentrations of several metabolites in microchambers of sub-nL volume containing single cells by means of extracellular optical sensors. In its current configuration, the measurement platform features the capability to detect oxygen consumption rate and pH changes under normoxic and hypoxic conditions at the single-cell level. We have conceived, designed and developed a semi-automated method for single-cell manipulation and loading into microwells utilizing custom, high-precision fluid handling approach at the nL scale.

We present the results of a series of measurements of oxygen consumption rates (OCRs) of single human epithelial cells representing different stages of pre-neoplastic progression. In addition, to assess the effects of cell-to-cell interactions, we have measured OCRs of two and three cells placed in a single well.

The major advantages of the approach are a) multiplexed characterization of cell phenotype at the single-cell level, b) minimal invasiveness due to the distant positioning of sensors, c) flexibility in terms of accommodating measurements of other metabolites and/or biomolecules of interest.

7902-59, Session 13

Determination of the PSI/PSII ratio in living plant cells at room temperature by spectrally and temporally resolved fluorescence spectroscopy

K. Elgass, Eberhard Karls Univ. Tübingen (Germany); Z. Martina, V. G. Maurino, Univ. zu Köln (Germany); F. Schleifenbaum, Eberhard Karls Univ. Tübingen (Germany)

Leaf cells of living plants exhibit strong fluorescence from chloroplasts, the reaction centers of photosynthesis. Mutations in the photosystems change their structure and thus can be monitored by recording the fluorescence spectra of the emitted chlorophyll light. These measurements have up to now mostly been carried out at low temperatures (77 K) as these conditions enable the differentiation between the fluorescence of PSI and PSII. In contrast, at room temperature, energy transfer processes between the various photosynthetic complexes result in very similar fluorescence emission which mainly consists of fluorescence photons emitted by PSII hindering a discrimination based on spectral ROIs (regions of interest). However, by statistical analysis of high resolution fluorescence spectra recorded at room temperature, it is possible to draw conclusions about the relative PSI/PSII ratio.

Here, the possibility to determine the relative PSI/PSII ratio by spectrally and temporally resolved fluorescence spectroscopy is demonstrated in living maize plants, which are known to exhibit mainly grana thylakoids and therewith mainly PSI in mesophyl cells. On the other hand, bundle sheath cells exhibit agranal thylakoid membranes with mainly PSI. Due to these characteristics, maize plants are an appropriate system for the demonstration of the applicability of this method.

In the following, the method is transferred to several carbon deficient mutants to analyse carbon deficit effects on the composition of plant’s chloroplasts. As a carbon deficit has to be compensated by the plant in some way, an adequate response would be e.g. changes in the composition of the photosystems to increase their efficiency.
Multiphoton microscopy and multiplex, multimodal imaging: impact on 21st century healthcare

P. N. Prasad, Univ. at Buffalo (United States)

This talk will present the important roles played by multimodal and multispectral, multiphoton microscopy using CARS, two-photon excitation, second harmonic and sum frequency generation as well as sequential multiphoton absorption. Combining this optical modality of imaging with other medical imaging techniques such as MRI and PET creates a powerful new direction in medical imaging and disease diagnostics by providing a wealth of information, from molecular to morphological, for disease profiling and real time monitoring of therapy. The talk will conclude with a discussion of exciting multidisciplinary opportunities in this field and their impact on 21st century healthcare.

Spectral optical imaging using a combination of nonlinear optical phenomena such as two-photon absorption, coherent anti-stokes Raman scattering (CARS), and second harmonic and sum frequency generation can provide chemically selective imaging and probing of the local macromolecular content in biological specimens, in order to provide molecular information on diseases as well as aiding in drug discovery. Usage of the rare-earth ion doped up-converting nanoparticles utilizing multiphoton processes allowed us to demonstrate 3D imaging in vitro and produce high contrast imaging in vivo with nearinfrared-to-near-infrared up-conversion nanoparticles. Subsequent nanoprobes combining multiphoton microscopy with MRI and PET imaging will also be presented. Control of excitation dynamics of multiphoton processes provides approaches for light activated and optically monitored therapy. Examples provided are photodynamics therapy and gene therapy; their impact on a broad range of health care challenges covering cancer, neurological diseases, infection diseases, addiction, obesity and depression will be discussed.

Multiphoton microscopy with gold nanoparticles as contrast agents

C. J. R. Sheppard, N. K. Balla, Singapore MIT Alliance (Singapore) and National Univ. of Singapore (Singapore); P. T. C. So, Singapore MIT Alliance (Singapore) and Massachusetts Institute of Technology (United States)

Recent years have seen increasing use of gold nanoparticles as contrast agents for two photon luminescence (TPL) microscopy. One of the early experimental demonstrations of TPL from silver was reported by Chen. Later the same group showed that multiphoton luminescence arises from rough metal surfaces due to electron hole excitation followed by recombination. The first demonstration of gold nanospheres as a contrast agent for TPL microscopy appeared after a long gap. Gold nanorods, due to their stronger near field enhancement factor and tunable longitudinal plasmon resonance, were shown to be superior contrast agents for TPL microscopy. Gold nanoparticles show very low levels of cytotoxicity and they are resistant to photobleaching. All these properties have made gold nanorods one of the most appealing contrast agents for TPL microscopy in recent years. A number of other applications like photo-thermal therapy, gene delivery, optical data storage, etc have been developed based on these novel contrast agents.

Another less known second order optical response of gold nanoparticles is second harmonic generation (SHG). Second harmonic scattering from noble metal nanoparticles is stronger than that from many known nonlinear molecules. Clusters of gold nanoparticles tend to show stronger SHG than single nanoparticles. Since second harmonic scattered light is coherent in nature, careful arrangement of these nanoparticles in a cluster can give rise to strong SHG. Such effects are easy to predict by means of simulations. Analytical models of SHG from metal nanoparticles have also been reported earlier. These models have been validated by experimentally but their applications are restricted to simple shapes of the particles. The discrete dipole approximation (DDA) has been extensively used to simulate linear scattering by metal nanoparticles. Recently, DDA has been extended to predict SHG from small particles of different shapes and under complex illumination conditions. This method makes it possible to look at SHG from a cluster of metal nanoparticles or composite nanoparticles, and can possibly be used to design clusters with desired optical properties.

Effective lung cancer medical diagnostics utilizing multiphoton microscopy

W. W. Webb, I. P. Pavlova, Cornell Univ. (United States)

Lung cancer, the deadliest human malignancy, invokes the need for new diagnostic imaging approaches. Here, we report evaluations of multiphoton microscopy (MPM) for optical interrogation of early nodules in transgenic murine and veterinary examples of lung adenocarcinoma. MPM fluorescence and second harmonic generation diagnostics of lung cancers including preliminary evaluations in larger animals indicate effective diagnostics! For MPM diagnostics within humans, we have modified Gradient Refractive Index lens (GRIN lens), to be guided to cancer nodules by CT or x-ray. Finding lung cancer diagnostics consistently promising, compared to pathologists’ H&E stains, we are ready for first applications to human patients.

Connecting the atomic scale dynamics to the macro-world by second-harmonic generation microscopy

F. S. Pavone, Univ. degli Studi di Firenze (Italy)

Second Harmonic Generation Microscopy nowadays has been
developed for applications in many fields, such as cell imaging or tissue analysis, both for lab or clinical use.

Mature applications have been demonstrated for cell physiology studies up to skin pathologies diagnosis or muscle diseases. Here, we will show the features and the capabilities of SHG ranging from electrical activity detection up to physiology measurements in living cells and tissue diagnostic. In particular, we will demonstrate how atomic scale conformation dynamics knowledge will be useful to predict bulk system functional behavior by means of SHG signal analysis. This allows the connection of the molecular structural dynamics with physiological behavior and order/symmetry of biological structures in more complex systems such as tissues. New capabilities in clinical practice or cell biology can be in this contest developed by SHG.

7903-05, Session 1

Determination of collagen nanostructure from nonlinear microscopy

W. Chen, P. Su, Y. Chen, C. Dong, National Taiwan Univ. (Taiwan)

In this work, we propose a two-component model of molecule for describing the excitation polarization resolved second harmonic generation from type I collagen fibrils. The experiment of sum frequency vibrational spectrum has confirmed that the molecular origins of collagen second-order susceptibility are from peptide groups in the backbone and methylene groups in the pyrrolidine rings. In our model SHG experiment, data are fitted with the model for the determination of peptide pitch angle. In addition, pitch angle of methylene groups and its tilt from the axis perpendicular to collagen axis can be determined. Our results demonstrate that the two-component model can be used to describe the excitation polarization resolved SHG signal produced by collagen.

7903-06, Session 1

Holographic microscopy of second-harmonic generation in biological specimens

P. Schlup, O. Masihzadeh, R. A. Bartels, Colorado State Univ. (United States)

We apply holography to second harmonic generation (SHG) microscopy to obtain three-dimensional images of biological specimens including starch, corn seed, and muscle tissue with integration times as low as 10 ms.

Nonlinear scanning microscopy techniques like SHG have proven to be a powerful technique for imaging spatially structured biological samples such as collagen, myosin, and muscle fibrils, but have limited acquisition speeds, making video rate imaging difficult. In nonlinear holography, full 3D images can be numerically reconstructed from one interference image (hologram) recorded between the endogenous SHG and an independent reference. Our source is a femtosecond Yb:KGW laser oscillator, whose low power and 1-um emission wavelength, within a tissue transparency window, prevents damage to our samples, even for prolonged exposures. Our imaging resolution is limited by the NA of our collection objective, which gives a point-spread function (PSF) of 0.52(\Lambda SHG)/NA = 0.53 um under optimal conditions, in good agreement with the value measured using nanospheres.

We recorded various images with 500-ms integration times, to maximize the signal-to-noise ratio (SNR) of the data and minimize readout artifacts in the camera. Using a mechanical shutter to reduce the camera illumination time, we observed an approximately linear decrease in SNR with shorter integration times, to the limiting 10 ms shutter switching speed. Extrapolating the trend measured above this limit, we anticipate a 10-dB SNR at 40 us integration time. Such high 3D image frame rates would yield a great deal of information of chemical waves, neurological networks, and other biological systems.

7903-07, Session 1

Characterization of stem cell-derived cardiomyocytes using second-harmonic generation (SHG) microscopy

S. Awasthi, NSF Ctr. for Biophotonics Science and Technology (United States); D. K. Liu, N. Chiamvimonvat, UC Davis Medical Ctr. (United States); D. L. Matthews, J. W. Chan, NSF Ctr. for Biophotonics Science and Technology (United States)

Cardiomyocytes have been derived from human embryonic stem cells (hESCs) for their potential use in regenerative therapeutics and in drug discovery for the screening of cardiac drug candidates. An important area of research in this field is the development of new technologies for identifying hESC-derived cardiomyocytes (hESC-CMs) and characterizing their intracellular contractile architecture and kinetics. Ideally, such technologies should be non-invasive and non-destructive to enable real-time, continuous cellular analysis and to allow cells to be recovered post-analysis. Unfortunately, these capabilities are lacking in existing technology. Given accumulated evidence that myosin rod domains in cardiomyocytes can undergo second harmonic generation (SHG) intrinsically, we explored the utility of the SHG signal in characterizing hESC-CMs. Using an ultrashort pulsed laser (930 nm, 140 fs, 80 MHz), we observed SHG at 465 nm from the sarcomeric myosin of hESC-CMs in beating and fixed cardiophores. Fluorescent staining for alpha-actinin confirmed the sarcomeric origin of the signal. The average sarcomeric distance in hESC-CMs was 2nm, which is comparable to that of the adult cardiomyocytes reported in the literature. In this study, SHG microscopy was evaluated for its ability to discriminate hESC-CMs from hESCs, assess sarcomere development and contractile kinetics during the differentiation and maturation of hESCs into CMs, and characterize myofilament organization during the plating of hESC-CMs as single cells. Our results suggest that SHG microscopy is a potentially powerful single cell analytical tool for accurately identifying hESC-CMs and characterizing their maturity, based on myosin content, in an entirely label-free manner.

7903-08, Session 1

Molecular third-harmonic generation imaging of melanin with real-level resonance enhancement

S. Chen, M. Tsai, C. Tsai, National Taiwan Univ. (Taiwan); Y. Liao, National Taiwan Univ. Hospital (Taiwan); C. Sun, National Taiwan Univ. (Taiwan)

In dermatology, melanin plays an important role in various skin diseases including vitiligo, lentigines, and melanoma, and an imaging tool capable for molecular imaging of melanin is strongly desired for skin disease diagnoses and treatment. In this study, melanin is proved through in vivo human skin imaging to provide strong imaging contrasts for 1230nm-based third harmonic generation (THG) microscopy. Based on the strong absorption of melanin within the visible range, the strong THG contrasts are contributed by melanin through the mechanism of real-level resonance enhancement. With the melanin-induced THG enhancement, not only the distributions of melanin in human skin tissues can be easily revealed by the 1230nm-based THG imaging; but also different concentrations of melanin can be analyzed from the brightness of the THG signals. Combined with the advantages of low photodamages, high spatial resolution, and high penetrability, 1230nm-based THG microscopy is shown to have the capability for performing noninvasive virtual skin biopsy and molecular imaging of melanin and is a desirable diagnostic tool for skin diseases.
Second-harmonic generation and multiphoton microscopy for automatic texture analysis of human of elastic fibers and collagen distribution in human thoracic aorta

G. Vieira, V. B. Pelegati, A. A. Thomaz, D. Peixoto Ferro, R. L. Adam, C. Lenz Cesar, K. Metze, Univ. Estadual de Campinas (Brazil)

Aging and diseases of the aorta are usually accompanied by remodeling of the architecture of the elastic fibers and by changes of the collagen content. We describe a method of simultaneous detection of collagen and elastic fibers using a multiphoton and second harmonic generation microscope together with computerized image analysis system. Elastic fibers can be seen in eosin-stained sections by two-photon excited fluorescence while collagen fibers are observed with second harmonic generation microscopy in a laser scanning confocal system consisting of an Olympus FV300 microscope and a MaiTai Ti:Sapphire laser.

To observe the whole aorta cross section individual images were joined together to a single patchwork image with a computer controlled translation stage. For quantification, a gliding step running in 1-pixel steps from the intima to the adventitia, collected texture features which are plotted in diagrams according to their topographic position in the vessel wall. The collagen content increases at the external parts of the media. Architectural disturbances of the elastic fiber network can be observed, predominantly, at the transition between the first and second third of the media in normotensive patients.

Visualization of the first hyperpolarizability tensor elements with second-harmonic generation microscopy in biological spherocrystals

V. Barzda, R. Cisek, A. E. Tuer, Univ. of Toronto Mississauga (Canada)

Direct visualization of the tensor elements of the first hyperpolarizability can be achieved in radially arranged crystals using second harmonic generation (SHG) microscopy. Two representative biological spherocrystals, starch and otoconia, have been investigated. Both structures are very important for biological function, former providing storage of the chemical energy in form of starch granules in plants, and later sensing a linear acceleration and gravity in animals. Despite a very different physiological function, the radial structure of both biological spherocrystals carries organizational similarities. We used nonlinear differential multicontrast microscopy to study polarization dependent SHG imaging of freshly dissected otoconia from mouse ear. The detected features were compared with experimental examination of starch granules, and principles of growing radial spherocrystals were investigated. Imaging SHG of otoconia and starch granules has been performed with a home built multicontrast laser scanning microscope coupled to a femtosecond Yb:KGW laser radiating at 1030 nm with a pulse repetition rate of 15 MHz. We could directly visualize the hyperpolarizability tensor elements by imaging with linearly polarized light and appropriately arranged analyzer in front of the detector. Information about the susceptibility tensor elements was obtained with single scan using differential microscopy, a technique where two beams with alternating polarizations are simultaneously deployed to image the same structure. SHG microscopy is sensitive to small variations in the structure revealing growth rings in starch granules, and heterogeneities in otoconia. Therefore SHG microscopy is highly beneficial for numerous applications such as starch quality control and medical diagnosis of otoconia related disorders.

Second-harmonic generation imaging of collagen matrix remodeling in a stimulated 3D cellular environment: forward versus backward scattering

T. Abraham, A. Scott, J. Carthy, B. McManus, The Univ. of British Columbia (Canada)

The structural remodeling of collagen is important in several biological processes such as wound healing, tendon repair, fibrosis and developmental morphogenesis. Multiphoton microscopy, which uses ultra-short femto-second laser pulses as an excitation source, is efficient in non-linear multiphoton excitation fluorescence (MPEF) of exogenous fluorescent labels tagged to various cellular macromolecular objects and the induction of highly specific second harmonic generation (SHG) signal from non-centrosymmetric macromolecules such as fibrillar collagens. SHG and MPEF signals have been successfully utilized to probe the comprehensive cell-mediated structural remodeling of extracellular collagen matrix as well as the related cellular morphologies in 3D space (Abraham et al, 2010). Although the non-descanned detectors in the reflection geometry have normally been employed for capturing the backward scattered SHG as well as the MPEF signals considering the wide range of engineered thick tissue imaging applications (Abraham et al, 2010, Zoumi, et al. 2002), there are still un-answered questions about the generated 3D collagen structures because of the directional pattern of SHG signals. The present study dealt with an in vitro collagen-fibroblast raft model where the stimulation of fibroblast cells induced the lateral orientation of collagen molecules. The SHG signals originating from 3D collagen matrix were captured simultaneously in both forward and backward scattering directions to understand the collagen structural
Second-harmonic phase microscopy

E. Shaffer, C. Moratal, P. Marquet, C. D. Depeursinge, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

In the past decade, quantitative phase imaging gave a new dimension to optical microscopy, and the recent extension of digital holography techniques to nonlinear microscopy appears very promising, for the phase of nonlinear signal provides additional information, inaccessible to incoherent imaging schemes.

Here, we present our work on second harmonic generation (SHG) phase microscopy. While originally developed almost independently of coherent nonlinear microscopy (multiphoton excitation fluorescence), SHG microscopy contributed to the emergence of all forms of coherent nonlinear microscopy (e.g. higher harmonic generation, sum- or difference frequency generation, wave mixing, coherent anti-Stokes Raman scattering). The signals detected in coherent nonlinear microscopy origin from instantaneous nonlinear interaction of incident electromagnetic fields with the specimen, making selective and ultrafast imaging possible. Yet, we think that one of the most important advantages of coherent nonlinear microscopy techniques lies in their coherence properties that makes possible simultaneous retrieval of the both the amplitude and the phase of the nonlinear signal, by means of digital holography.

We have already reported how the SHG phase makes possible real-time nanometric 3D-tracking of SHG emitters, such as nanoparticles. In this work, we present the technique and look into its possible applications to biological and life sciences, by presenting some applications of label-free SHG phase microscopy to biological specimens.

Three-dimensional polarization second-harmonic generation (3D-PSHG) imaging of tissues: the effect of the tilted-off the plane SHG active structures

S. Psilodimitrakopoulos, I. Amat-Roldan, ICFO - Instituto de Ciencias Fotónicas (Spain); D. Artigas-García, ICFO - Instituto de Ciencias Fotónicas (Spain) and Univ. Politécnica de Catalunya (Spain); P. Loza-Alvarez, ICFO - Instituto de Ciencias Fotónicas (Spain)

In high resolution optical imaging of tissues, the minimally invasive second harmonic generation (SHG) microscopy is one of the most promising modalities to be applied in future clinical applications. The nowadays widespread common intensity based SHG imaging, provides information on the morphology of the biological sample. Nevertheless, imaging based on morphological characteristics can be misleading. Thus, there is need for better interpretation and quantification of the available information based on the nonlinear light matter interaction. Polarization sensitive SHG (PSHG) has lately proved to provide new insights in the elementary SHG molecular structures. The structurally specific information is derived from the ratio of two independent elements of the 2 tensor, which is usually experimentally extracted by rotating the excitation linear polarization and by fitting pixel by pixel a 2D model to the obtained PSHG images. However, this simplified 2D model might lead to a wrong understanding on the geometry of the SHG structures, if those are not lying within or parallel to the plane. Here we propose and develop a novel generalized 3D model that further accounts for the tilted-off the plane SHG active structures. In particular, we correlate the dispersion of the experimentally retrieved 2 elements ratio, to the off the plane angle of the SHG structures. In this way, a quantitative optically retrieved bio-signature was defined. We applied the above to collagen and we retrieved its 3D local organization. We believe that our 3D-PSHG model possesses great potential for diagnosing and monitoring structure specific pathologies of collagen (cornea, skin, connective tissue, etc), microtubules (neurons’ axons, mitotic spindles) and myosin in muscles.
Dependence of third-harmonic generation on melanin concentration in skin
T. Su, C. Liao, C. Yang, Z. Zhuo, S. Chen, S. Chu, National Taiwan Univ. (Taiwan)

Third harmonic generation (THG), as an intrinsic optical contrast, has been applied for tissue imaging with advantages including noninvasiveness and optical sectioning. Due to Gouy phase shift of a focused Gaussian beam, THG signals are routinely observed at interfaces. Recently, THG microscopy has been adopted for deep-tissue observation in various kinds of human skin, and it’s found that in the samples with lower melanin concentration, THG signals did generate at interfaces such as cell membranes. Nevertheless, for samples with higher melanin concentration, THG seemed to be produced in the cytoplasm, and diminished at interface. Since THG is sensitive to local variation of linear and nonlinear susceptibilities, we may use THG to determine the electric susceptibilities of melanin solutions with different concentrations. To examine the assumption, we measured THG at an interface formed by a cover glass and melanin solution. As expected from theoretical calculation, a THG minimum was observed at a specific melanin concentration, whose effective refractive index (linear susceptibility) corresponds to that of the cover glass. By fitting the curve of THG intensity versus melanin concentration, the nonlinear susceptibility of melanin solution can be deduced. Compare to other methods, such as self-focusing damage or self-induced polarization change, for measuring third-order nonlinear susceptibility, this THG-based procedure may provide a valuable tool for noninvasive determination of third-order nonlinear susceptibility of melanin in biological tissues.

Quantitative analysis of diseased horse tendons using Fourier-transform-second-harmonic generation imaging
S. Mayandi, S. Durgam, R. Ambekar Ramachandra Rao, D. Luethke, G. A. Fried, A. Stewart, K. C. Toussaint, Jr., Univ. of Illinois at Urbana-Champaign (United States)

Fourier transform-second-harmonic generation (FT-SHG) imaging is used to quantitatively assess the structural organization of collagen fibers in tendinitis-induced horse tendons. Fiber orientation, isotropy, and the ratio of forward to backward SHG signal (F/B ratio) are used to differentiate the fiber organization between the normal and diseased horse tendons. Each second-harmonic generation (SHG) image is divided into several smaller regions of interest (ROI) and the aforementioned quantitative metrics are calculated across the whole grid. ROIs are further labeled as dark (no or minimal presence of fibers), isotropic (random fiber organization), or anisotropic (regular fiber organization) regions. Results show that the normal tendon possesses minimal isotropic regions and small standard deviations in the histograms of orientation and F/B ratio, indicating an intact and highly regular fiber organization. However, the tendinitis-induced horse tendons possess higher number of dark and isotropic regions, and larger standard deviations of the measured parameters, suggesting significantly disoriented and disorganized collagen fibers. This type of quantification would be highly beneficial in diagnosing and determining the stage of tendinitis in clinical settings. Not limited to tendinitis, the technique could also be applied to other diseases that structurally affect collagen fibers. The advantage of FT-SHG over the conventional polarization microscopy is also discussed.

Examining the feasibility of using multiphoton excited tissue autofluorescence for in vivo human clinical imaging
J. M. Dela Cruz, J. D. McMullen, R. M. Williams, W. R. Zipfel, Cornell Univ. (United States)

Rapid and direct imaging of microscopic tissue morphology, pathology and metabolic state can be achieved using nonlinear imaging of intrinsic tissue fluorophores and second harmonic signals in intact tissue. To design instruments targeted for this type of application, several critical engineering parameters need to be elucidated. Two important questions are what excitation levels and collection efficiencies are required to obtain useable images from different tissue types and whether these levels are mutagenic. Tissue autofluorescences are weak two-photon fluorophores and very often high laser powers are required for imaging. Here we provide data on the typical average powers required for high signal-to-noise in vivo tissue imaging in several different epithelial tissue types, and assess the risk potential of these intensity levels using a mammalian cell gene mutation assay. With 760 nm, 200 fs raster-scanned laser irradiation delivered through a 0.75 NA objective we found negligible mutagenicity at powers less than ~25 mW, while higher laser powers initiated a significant increase in genetic lesions.
Two-photon light sheet microscopy
T. V. Truong, W. Supatto, D. Koos, J. M. Choi, S. E. Fraser, California Institute of Technology (United States)

Fluorescence light sheet microscopy (LISM) has gained widespread recognition in recent years, due to its distinct advantages for the 3-dimensional imaging over time (4D imaging) of living biological samples. These advantages come from the orthogonal geometry between the illumination and detection pathways, which enables: (i) high imaging speed due to parallelization in both illumination and detection; (ii) optical and physical access to 3D samples in ways that are impossible in the confocal geometry of standard microscopes; (iii) low photodamage quality, due to single-plane illumination and lower laser peak intensity (since lower numerical aperture focusing is used for reaching the same resolution). To combine these advantages with high depth penetration, we implemented LISM with 2-photon excitation. We compared between 2p-LISM, 1p-LISM, and conventional 2p laser scanning microscopy (2p-LSM), in imaging live Drosophila and zebrafish embryos. We found that 2p-LISM achieves 2-fold improvement in depth penetration compared to 1p-LISM, rivaling that of 2p-LSM. Also, though the signal rates of 2p-LISM and conventional 2p-LSM are about the same, we found that 2p-LISM is much less photodamaging than 2p-LSM, therefore allowing use of higher laser excitation power, yielding at least an order of magnitude improvement in imaging speed. We further demonstrated that 2p excitation could be used to carry out second-harmonic scattering LISM, in analogy to the well known imaging modality of second-harmonic generation LSM. Thus, the unique combination of high depth penetration, high speed, low photodamage, and multi-modality, should make 2p-LISM an important addition to the repertoire of techniques for 4D biological imaging.

Intensity normalization of two-photon microscopy images for liver fibrosis analysis
V. R. Singh, Singapore-MIT Alliance for Research and Technology (Singapore); J. C. Rajapakse, Nanyang Technological Univ. (Singapore); P. T. C. So, Massachusetts Institute of Technology (United States)

This paper presents the analysis of liver tissue images acquired at different stages of fibrosis using the two-photon microscopy method. Image informatics methods require precise intensity segmentation for analysis of collagen, vessel and cellular structures. Intensities of the images recorded at different time intervals corresponding to the progression of fibrosis could vary spatially and temporally depending on the experimental conditions. These variations in intensities significantly affect the image segmentation process and thus the final image analysis, especially when automatic computer-based methods are used for diagnostic parameters quantification. We propose an adaptive intensity normalization method that facilitates spatial and temporal intensity variations of the images before the segmentation process. The images are first portioned into a tessellation of regions with relatively uniform background pixels intensities and then the normalization is performed to make sure the intensity range is unified throughout the whole set of image data. This approach is further extended for montage of images acquired from multianode photomultiplier tube based multifocal multiphoton microscope (MMM) system. The proposed approach significantly improves the automated analysis of images with varying intensities without any user intervention.

Combined high-speed two-photon microscopy and optical coherence microscopy for in-vivo tissue imaging
B. Lee, K. H. Kim, B. Jeong, H. Nam, S. J. Yoon, M. S. Jang, J. Doh, B. G. Yang, M. H. Jang, Pohang Univ. of Science and Technology (Korea, Republic of)

Two-photon microscopy (TPM) is a 3D fluorescent imaging technique suitable for studying live cells within tissue. Although TPM has good contrast in visualizing fluorescent cells, information of surrounding tissue such as non-fluorescent cells and tissue structure is not shown. We developed a combined system of TPM and optical coherence microscopy (OCM) to overcome this limitation. OCM is a high-resolution version of optical coherence tomography (OCT) which is a 3D imaging technique based on back reflection. OCM can visualize unlabeled cells, tissue structure, and vasculature based on Doppler effect, down to 1.0 mm deep from the surface by using 1.3 um wavelength and coherence detection. Therefore, the combination of TPM and OCM will be a good tissue imaging modality by providing complementary information of tissue covering molecular, cellular, and tissue structure and physiology. TPM is designed to study kinematics of live cells within tissue, and runs at 40 frames/s by using a resonant scanner for fast axis scanning. OCM is based on optical frequency domain imaging using a wavelength-swept source running at 50 K depth-scans/s and can do both intensity and Doppler imaging. The combination of TPM and OCM was incorporated into a modified upright microscopy setup for in vivo or explanted tissue imaging. We will present detail procedure of system design, implementation, and various tissue imaging.

Label-free ex vivo imaging of human breast tissue using coherent anti-Stokes Raman scattering microscopy
Y. Yang, Methodist Hospital Research Institute (United States)

Breast cancer is a common disease in women. In order to make a final diagnosis, breast biopsy is usually necessary. However, current breast biopsy method confronts several limitations such as time-consumingness, invasiveness, and high costs. Alternative strategies are in high demand to alleviate the patient trauma and medical costs. Coherent anti-Stokes Raman scattering (CARS) imaging technique offers many advantages, such as label-free, sub-wavelength spatial resolution and video-rate imaging speed, and has been demonstrated as a powerful tool for various biomedical applications. This study aims at determining the feasibility of using CARS to differentiate tumors from normal breast tissues. Diagnosed human infiltrating breast cancer tissues, including infiltrating ductal carcinoma (IDC) and infiltrating lobular carcinoma (ILC) tissues, were imaged ex vivo using a CARS microscope, and compared to their corresponding results from hematoxylin and eosin (H&E) staining. Our data have shown that the CARS technique is capable of identifying cellular features in a similar way as H&E staining, and these features can be used to distinguish cancer areas from normal tissues. In addition, different subtypes of infiltrating carcinomas can also be separated from each other using their unique pathological features captured by CARS. Our experiments suggest that the CARS imaging technique is capable of identifying pathological relevant information in breast tissues with cellular
resolution, and it thus holds great promise of in vivo, real-time breast cancer detection and diagnosis.

7903-86, Poster Session

Photo-induced cell damage analysis for multifocus CARS microscopy

T. Minamikawa, Y. Murakami, N. Matsumura, H. Nioka, S. Fukushima, T. Araki, M. Hashimoto, Osaka Univ. (Japan)

We investigated photo-induced cell damage for multi-focus CARS (coherent anti-Stokes Raman scattering) microscopy. In general, using a near-infrared pulse light source, photo-induced damage is dominantly caused via multi-photon induced phenomena, and the peak intensity of the excitation light source is limited for the non-invasive imaging. The multi-focus excitation prolongs image exposure time proportionally to number of the foci, and the high-speed CARS imaging is performed without the increase of excitation laser intensities. However, the total power of light source in multi-focus excitation is higher than single-focus excitation. We obtained cell viability images during single- or multi-focus (7 foci) exposure of which the wavelength and the pulse duration were 709 nm and 5 ps. The laser intensities were respectively set at 27.8 mW and 14.5 mW for single- and multi-focus excitation, in which the corresponding signal intensity of CARS is as same as each other. The cell viability was observed using DAPI fluorophores that mainly stained DNAs of dead cells. The single- and multi-focus excitation were performed using nonresonant/resonant galvano mirror pair with a frame rate of 125 frames/s and a microlens array scanner with a frame rate of 1000 frames/s. As a result, we found that the fluorescence of DAPI in the nucleus of a HeLa cell on single-focus excitation was 2.9 times stronger than that on multi-focus excitation for 20 min laser irradiation. The result indicates that the photodamage will be a serious problem on the single-focus excitation, and the multi-focus excitation method is preferable for CARS imaging.

7903-87, Poster Session

Raman spectroscopy: a powerful tool for the non-contact discrimination of bone-marrow mesenchymal stem cells and fibroblasts

M. Pudlas, S. Koch, Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik (Germany); C. Bolwien, Fraunhofer-Institut für Photonische Mikrosysteme (Germany); T. Hirth, H. Walles, K. Schenke-Layland, Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik (Germany)

Bone-marrow mesenchymal stem cells (BM-MSCs) are a promising cell source for regenerative medicine applications, which can be easily isolated and expanded ex vivo. BM-MSCs have the potential to differentiate into osteoblasts and chondrocytes in vitro and in vivo; an important feature when aiming to treat bone and cartilage defects. However, due to the lack of specific cell surface markers, it is difficult to identify whether ex vivo isolated BM-MSC populations are free of stromal fibroblasts. Bright-field microscopical analyses are insufficient to determine fibroblastic contaminations since these two cell types have similar cell morphologies. Here, we employed traditional flow cytometric (FACS) analyses and in vitro differentiation assays as well as Raman spectroscopy to distinguish BM-MSCs and fibroblasts. We found that FACS analysis utilizing previously described fibroblast-identifying antibodies was inadequate to separate stromal fibroblasts from BM-MSCs as over 75% of the BM-MSCs shared these antigens. In vitro differentiation assays revealed that, in contrast to fibroblasts, BM-MSCs successfully differentiated into osteoblasts, chondrocytes and adipocytes; therefore this method allowed for the discrimination between BM-MSCs and fibroblasts. However, the need for prolonged in vitro culture periods of up to 4 weeks appears to be the major disadvantage of this test method. Raman spectroscopy, a non-contact technique measuring the wavelength and intensity of inelastic scattered light from molecules by employing high-power near-infrared lasers, distinguished ultra-fast between BM-MSCs and fibroblasts (integration time of 100 seconds/ cell). In conclusion, we showed that Raman spectroscopy is a suitable tool for the rapid detection of fibroblastic contaminations in BM-MSC cultures.

7903-88, Poster Session

Broadband coherent Raman imaging for colocalisation studies

B. Littleton, F. Festy, S. M. Ameer-Beg, D. R. Richards, King’s College London (United Kingdom)

Fluorescence microscopy suffers from a number of inherent problems, such as photobleaching, sample autofluorescence, crosstalk and bleedthrough. Also, when used for simultaneously localising different tagged proteins within a cell, it is necessary that the chromophores employed have distinct excitation and emission bands, which limits the number of tags that be investigated in parallel. The Raman spectrum of a fluorescent chromophore typically has many spectral features, which differ markedly between dyes even if their electronic spectra are similar. This high information content makes it possible to use techniques such as principal component analysis to determine the relative contribution of different biomarkers to a measured spectrum. Raman spectra may therefore allow for the simultaneous measurement of more biomarkers than is possible with fluorescent imaging; however, spontaneous Raman scattering is very weak, and has to compete with sample autofluorescence. Coherent anti-Stokes Raman scattering (CARS) is a better alternative, as it is a parametric process that leaves no energy in the molecule (and therefore avoids photobleaching), has orders of magnitude higher cross-section, and generates signals spectrally separated from autofluorescence. To perform colocalisation studies via CARS multiplexing we have built a broadband CARS microspectrometer. CARS microscopy systems to date have tended to use expensive and complex laser systems, so to make the technique more accessible the system was designed around a low-cost, commercial supercontinuum laser source. We will report on the capabilities of this system as a CARS microscope, present proof-of-concept results of CARS multiplexing, and examine the application of the system to studies of biological samples.

7903-89, Poster Session

SRS microscopy in the fingerprint region

X. Zhang, M. B. Roelffaers, S. Basu, C. W. Freudiger, B. G. Saar, W. Min, S. X. Xie, Harvard Univ. (United States)

Label free imaging is an important technique both in biological research and in medical practices because of its noninvasiveness. Spontaneous Raman scattering provides us an effective way to detect specific chemical bonds in compounds, but it is not ideal as a microscopy technique because of its limited imaging speed. Coherent Raman scattering makes faster imaging possible up to videorate. We recently demonstrated stimulated Raman scattering (SRS) microscopy with high sensitivity under biocompatible excitation conditions due to implementation of a high-frequency phase-sensitive detection scheme. Compared to CARS microscopy, SRS is free of the unwanted nonresonant background and spectral interference from neighboring vibrational resonances, which distort the Raman excitation spectra and limit sensitivity. This is particularly important for imaging in the crowded fingerprint region, which contains many specific Raman vibrations. Here we show the SRS fingerprint imaging of distributions of DNA and proteins in the model organism drosophila. The distribution map of these components in cells will facilitate further study of different cellular processes such as cell division and apoptosis. We also show some SRS images of different vibrational modes in the fingerprint region of several food samples with high spatial and spectral resolution.
In-vivo coherent Raman scattering imaging with a periodically poled crystal OPO

D. Zhang, M. N. Slipchenko, Y. Shi, J. Cheng, Purdue Univ. (United States)

We demonstrate a new microscope for highly sensitive coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) imaging. By using femtosecond pulse excitation and a high-power Stokes beam provided by a periodically poled crystal OPO tunable from 1.0 to 1.6 micron, our setup provides CARS and SRS signals that are more than 10 times larger than current systems using picosecond pulses. Meanwhile, the high tunability of the compact OPO allows chemical imaging at a large range of Raman shift, capable for C-H, O-H and C-D vibration. The high signal levels enable high-quality CARS and SRS imaging of live animals with a fast acquisition rate.

Implementing label-free microscopy and spectroscopy to study a new mouse model of non-alcoholic steatohepatitis

I. W. Schie, UC Davis Medical Ctr. (United States)

Methods: Mice were fed a modified AIN 93G diet or the same diet containing 0.5% CLA for 8 weeks. Changes of lipid accumulations in liver were visualized by CARS microscopy, analyzed by Raman spectroscopy and compared to liver histology.

Results: Liver histology showed marked macrosteatosis (oil red-O-staining) with focal hepatocellular death and minimal pericellular fibrosis (trichrome staining) in mice fed diets containing CLA, but only minimal steatosis was visualized in mice fed control diets. These observations were confirmed by CARS microscopy of lipid modes. Raman spectral analysis of the lipid droplets showed that although conjugated linoleic acid has two double bonds, the average number of unsaturated fatty acid bonds in the droplets is (1.29±0.15) bonds, indicating that CLA is not a major component of the droplets. Furthermore, the presence of the peak at 7142cm⁻¹ indicates that the fatty acids found in the lipid droplets are esterified.

Conclusion: Mice fed diet containing CLA developed steatohepatitis, which is similar to the concurrent occurrence of both in human NASH subjects. CARS microscopy and Raman spectroscopy could visualize and analyze the lipid accumulations.

Monitoring the lipid metabolism in living Drosophila larvae by coherent anti-Stokes Raman scattering (CARS) and two-photon excitation (TPE) microscopy

C. Chien, W. Chen, National Yang-Ming Univ. (Taiwan) and Academia Sinica (Taiwan); J. Wu, National Taiwan Univ. (Taiwan) and National Taiwan Univ. Hospital (Taiwan); T. Chang, Academia Sinica (Taiwan) and National Yang-Ming Univ. (Taiwan)

Recently, Drosophila has been exploited in the studies of lipid metabolism under starvation. It is known that the larvae release lipids from the fat body and provide the energy needed. Similar to hepatocytes in mammals, oenocytes in Drosophila play a role in processing and regulating the released lipids. Here we use the coherent anti-Stokes Raman scattering (CARS) and two-photon excitation (TPE) microscopy to monitor this process in living Drosophila larvae. Without any labeling, we can identify oenocytes by their intrinsic TPE fluorescence and visualize lipid droplets and the fat body by CARS signals at ~2845 cm⁻¹. During the period of starvation, we observe that the lipid droplets accumulate in oenocytes and aggregate in the fat body, accompanied with the change of lipid contents in other organs. Compared with traditional tissue staining, this in vivo imaging can provide more information of the dynamic process to improve our understanding of lipid metabolism in Drosophila.

Two-photon selective plane illumination microscopy (2p-SPIM) in living biological samples

J. A. Palero, S. I. C. O. Santos, ICFO - Instituto de Ciencias Fotónicas (Spain); D. Artigas-Garcia, Univ. Politècnica de Catalunya (Spain); P. Loza-Alvarez, ICFO - Instituto de Ciencias Fotónicas (Spain)

The past two decades saw the emergence of two photon (2p) laser-scanning microscopy as a powerful imaging tool for thick tissue imaging. Owing to its inherent optical sectioning capability, deep penetration, and its capability to excite multiple fluorophores, 2p laser-scanning microscopy has been used in a wide range of imaging applications. The drawback, however, of this imaging technique is its relatively slow image acquisition rate. In this study, we demonstrate a novel and simple scanless two-photon imaging technique based on selective plane illumination microscopy (SPIM). Light-sheet-based microscopes such as SPIM use a wide-field detection microscope. For excitation, illumination plane, coinciding with the sample plane of the microscope is used. This configuration restricts the excitation to the fluorophores in the volume around the plane of illumination, in effect provides instantaneous (as opposed to raster scanning) optical sectioning. This allows for a fast two-dimensional high resolution imaging of the specimen. Since in SPIM, fluorophores outside the illumination are not excited, photobleaching and phototoxic effects are dramatically reduced. SPIM is also used for imaging relatively large samples which require objective lenses with low NA and low magnifications. We show the relative simplicity of the experimental setup which mainly consists of two cylindrical lenses forming a thin sheet of light, and an objective lens forming the optically sectioned image on to an imaging detector. We present results on the characterization of the light sheet and finally its application to depth-resolved in vivo biological imaging.
single-mode fiber at that wavelength. Because of the large core area, this fiber initially supported two guidance modes and became a single-mode transmission line by the help of the mode filter.

7903-96, Poster Session

A cationic 1,4-Bis(styryl)benzene derivative and its cyclodextrin inclusion complexes for two-photon biological imaging

O. K. Nag, P. Jeong, H. Y. Woo, Pusan National Univ. (Korea, Republic of)

Recently, the development of efficient water-soluble two-photon (TP) materials has attracted considerable interest because of their various applications such as optical power limiting, 3D optical data storage, photodynamic therapy, and particularly biological imaging by using two-photon microscopy (TPM). We synthesized and studied the TP absorption properties of 1,4-bis(4-[N,N-bis(6''-trimethylammoniumhexyl)amino]styryl)benzene (C1) and its inclusion complexes (ICs) with cyclodextrins (CDs). Upon complexation with CDs, the absorption spectra of C1 showed a slight red shift, whereas the emission spectra showed a blue shift with concomitant increase in the fluorescence quantum efficiency in the presence of CDs. Comparison of the spectroscopic results reveals that C1 forms increasingly more stable ICs in the order C1/γ-CD < C1/β-CD < C1/α-CD (C1/CD 3:1, molar ratio). The two-photon action cross section (γ) of C1 increased from 200 GM for C1 to 400 GM for C1/γ-CD, 460 GM for C1/β-CD, and 650 GM for C1/α-CD, respectively. Moreover, the γ value of C1/(3/β)-CD in water is close to the maximum possible γ value of 710 GM (n = 1.39). Furthermore, the TPM images of HeLa cells stained with C1 emitted strong two-photon excited fluorescence in the plasma membrane that persisted for more than 1 h. When the cells were stained with C1/α-CD, however, the TPEF intensity decreased rapidly with time, probably because of the destruction of the lipid rafts by CDs. These results indicate that C1 is a useful TP fluorescent tag for the plasma membrane, whereas C1/CD may be useful for applications that require large γ in water.

7903-97, Poster Session

Quantifying the surface chemistry of porous biomaterials by two-photon microscopy

D. S. Tzeranis, I. V. Yannas, P. T. C. So, Massachusetts Institute of Technology (United States)

A limited number of biomaterials have been shown to be able to change the outcome of the wound healing process in injured adult organs and induce regeneration. This ability has been shown to depend strongly on certain structural and chemical properties of the biomaterial. The surface chemistry of a biomaterial (type and density of ligands recognized by particular cell adhesion receptors) is expected to be one of the major determinants of this activity. This work presents a new optical-based method for quantifying in situ the surface density of ligands of particular cell adhesion receptors on the surface of porous biomaterials. The ligands for a particular receptor are labeled by a fluorescent biomarker that imitates the binding characteristics of the corresponding receptor. The distribution of bound biomarkers is imaged in situ via multiphoton microscopy. The surface density of the labeled ligands is then estimated by processing the acquired 3D images via Bayesian image processing. The method is demonstrated by measuring the surface density of ligands for collagen-binding integrins on the surface of porous collagen-based scaffolds, similar to products used clinically to induce regeneration in injured skin and peripheral nerves. The proposed method can be used to quantify the density of ligands for a wide variety of adhesion receptors on the surface of biomaterials or native tissue extracellular matrix, providing a novel tool for quantifying the effect of matrix surface chemistry on the phenotypes of the interacting cells.

7903-98, Poster Session

Novel nanocarriers for topical drug delivery: investigating delivery efficiency and distribution in skin using two-photon microscopy

V. Kirejev, S. Gulbrand, B. Bauer, M. H. Smedh, M. B. Ericson, Göteborg Univ. (Sweden)

The complex structure of skin represents an effective barrier against external environmental factors, as for example, different chemical and biochemical compounds, yeast, bacterial and viral infections. However, this impermeability prevents efficient transdermal drug delivery which limits the number of drugs that are able to penetrate the skin efficiently. Current trends in drug application through skin focus on design and use of nanocarriers for transport of active compounds. The transport systems applied so far have several drawbacks, as they often have low payload, high toxicity, a limited variability of inclusion molecules, or long degradation times. The aim of the current studies is to investigate novel topical drug delivery systems, e.g. nanocarriers based on cyclic oligosaccharides - cyclodextrins (CD) or iron (III)-based metal-organic frameworks (MOF). Earlier studies on cell cultures imply that these drug nanocarriers show promising characteristics compared to other drug delivery systems.

In our studies, we use two-photon microscopy to investigate the ability of the nanocarriers to deliver compounds through ex-vivo skin samples. Using near infrared light for excitation in the so called optical window of skin allows deep-tissue visualization of drug distribution and localization. In addition, we employ two-photon based fluorescence correlation spectroscopy for quantitative analysis of drug distribution and concentration in different cell layers.

7903-99, Poster Session

Compact ultrafast semiconductor disk laser for nonlinear imaging in living organisms

R. A. Aviles-Espinosa, IFCO - Instituto de Ciencias Fotónicas (Spain); G. Filippidis, Foundation for Research and Technology-Hellas (Greece); C. Hamilton, Solus Technologies Ltd. (United Kingdom); G. Malcolm, M Squared Lasers Ltd. (United Kingdom); T. Sändig, T. Südmeyer, Y. Barbarin, U. Keller, ETH Zurich (Switzerland); D. Artigas-Garcia, Univ. Politècnica de Catalunya (Spain); P. Loza-Alvarez, IFCO - Instituto de Ciencias Fotónicas (Spain)

Ultrashort pulsed laser systems (such as Ti:Sapphire) have been used in nonlinear microscopy devices during the last years. However, their implementation is not straightforward because they are maintenance-intensive, bulky and expensive. These limitations have prevented their wide-spread use for nonlinear imaging, especially in "real-life" biomedical applications.

In this work we present the suitability of a compact ultrafast semiconductor disk laser source, with a footprint of only 140x240x70 mm, to be used for nonlinear microscopy applications. The mode-locking mechanism of the laser is based on a quantum-dot semiconductor saturable absorber mirror (SESAM). The laser delivers an average output power of 287 mW with 1.5 ps pulses at 500 MHz, corresponding to a peak power of 0.5 kW. The laser center wavelength is 965 nm. Given the fact that the widely used Green Fluorescent Protein (GFP) marker has an absorption maximum at 470 nm, this laser is ideally suited for two-photon excitation of such protein.

Based on the capabilities of our laser source we reveal that it is possible to obtain, in a living C. elegans nematode, two photon excited fluorescence (TPEF) images of GFP labeled neurons and second-harmonic generation (SHG) images of pharynx and body wall muscles. Our results also demonstrate that this compact laser is well suited for long-term time-lapse imaging of living samples. Importantly this non expensive, turn-key, compact laser system could be used as a platform...
to develop portable nonlinear bio-imaging devices, facilitating its widespread adoption in "real-life" applications.

7903-100, Poster Session
**Single-particle imaging by two-photon microscopy in vivo**
L. Krapf, Univ. zu Lübeck (Germany); J. Dimitrijevic, Univ. Hamburg (Germany); A. Schütz, Univ. zu Lübeck (Germany); T. Vossmeyer, Univ. Hamburg (Germany); A. Gebert, Univ. zu Lübeck (Germany); H. Weller, Univ. Hamburg (Germany); G. Hüttmann, Univ. zu Lübeck (Germany)

Due to the excellent penetration of NIR light in biological tissue, and only minimal photodamage, two-photon excitation laser scanning microscopy is an ideal tool to study the morphology and dynamic processes in living tissue. Here, this technique is employed to investigate the interaction of nanoparticles with cells of the small intestine, i.e., the gut epithelium and subepithelial cells of the immune system. Tracking of single nanoparticles in vivo is hampered by the strong autofluorescence background of the tissue. Therefore, highly luminescent nanoparticles with high efficiency for two-photon excitation are required. In this study we characterized several fluorescent particle types (dye-loaded latex beads, quantum dots (QDs) and quantum dots/quantum rods (QDs/QRs)) for use in intravital imaging. Measurements of two-photon action cross sections were performed which showed that the values for commercial dye-loaded latex beads are approximately proportional to their volume. QDs had cross sections of about $10^3$-10$^4$ GM and QDs/QRs of about $10^5$ GM. We show that, at our detection sensitivity, two-photon action cross sections of 10$^4$ GM or higher are needed to detect single nanoparticles. This was fulfilled by latex beads with diameters of 100 nm or more and by QDs/QRs with dimensions below 100 nm. Blinking of QD/QRs was observed indicating the presence of single, non-aggregated particles. This was further shown by detecting the linear polarization of emitted fluorescence. Both particle types were successfully used for studying nanoparticle uptake by the small intestinal mucosa of mice.

7903-101, Poster Session
**Two-photon excitation STED-CW microscopy**
P. Bianchini, S. Gallani, A. Diaspro, Istituto Italiano di Tecnologia (Italy)

Two-photon excitation laser scanning microscopy (2PELSM) has allowed exceptional fluorescence imaging of structure and function within thick tissues. However, the resolution of this approach decrease going deep in the tissue and in general is poorer than conventional confocal microscopy.

Here, we report sub-diffraction resolution in two-photon excitation (TPE) fluorescence microscopy achieved by merging this technique with continuous-wave (CW) stimulated-emission depletion (STED). We show an easy-to-implement and promising laser combination based on Ti:Sapphire ultrafast laser source for two-photon excitation and a commercial Leica STED-CW microscope for resolution enhancement. Images of fluorescent nanoparticles produce comparable similar resolution to the one photon excitation. Two-photon excitation STED microscopy achieves approximately 3-fold improvement in resolution in the radial direction over conventional 2PELSM. Further improvements in resolution are theoretically achievable, suggesting that 2PE STED microscopy will permit nanoscale imaging, for instance, of neuronal structures located in relatively intact brain tissue.

7903-102, Poster Session
**Two-photon fluorescence excitation within a light-sheet-based microscopy architecture**
F. Cella Zanacchi, Z. Lavagnino, E. Ronzitti, A. Diaspro, Istituto Italiano di Tecnologia (Italy)

Light-sheet based microscopy, such as single plane illumination microscopy (SPIM) and digital scanned laser microscopy (DSLM), represent a useful tool for biological investigations of thick samples. Such techniques have been found particularly useful in developmental biology applications since it provides the capability to perform fast imaging of living samples reducing photobleaching effects. The high signal to noise ratio and the intrinsic optical sectioning capability provided by SPIM suggest this technique to be the best choice for imaging of thick scattering samples. Nevertheless imaging in depth of large samples suffers from a decreasing in the image quality due to scattering effects. Two photon excitation microscopy became a popular tool to perform imaging in turbid media since it allows to improve the penetration depth capability and to reduce the image quality degradation due to scattering and light matter interactions. Therefore, two photon excitation within the light sheet illumination scheme has been exploited in order to reduce scattering effects due to light-sample interactions. Scattering effects have been characterized and two photon excitation imaging in SPIM scheme has been performed in order to achieve an improvement in the penetration depth while imaging large biological samples.

7903-103, Poster Session
**Spectral decomposition of multicolor imaging in multifocal multiphoton microscopy**
J. Cha, J. L. Chen, E. Nedivi, P. T. C. So, Massachusetts Institute of Technology (United States)

The study of synapse formation and dendritic remodeling is critical in understanding the mechanisms underlying brain plasticity, and it often requires imaging over a volume of several cubic millimeters. For this large volume, high imaging speed becomes critical for minimizing animal stress due to prolonged anesthesia, overcoming motion artifacts, and improving imaging throughput. Multifocal multiphoton microscopy (MFM) is known as one of the best methods for the high throughput neurobiological imaging. In addition to improving imaging speed, spectral resolved imaging is further required to study both structural and synaptic dynamics during plasticity. To monitor synapse and dendritic arbors dynamics in interneurons simultaneously, we have developed spectral resolved MFM for imaging interneurons with labeling the synapses and dendrites with different colors of fluorescent proteins. The spectral resolved MMM uses one axis for the spatially resolved multiplexing and the other axis for the spectrally resolved multi-color detection. However, due to the spectral overlap of the emission spectrums, computational spectral unmixing is often required. The success of spectral unmixing is often limited by the inherent Poisson noise in the image data. We propose Poisson noise removal before the spectral unmixing to improve the outcome of spectrally overlap signals.

7903-104, Poster Session
**Characterization of third-degree burned skin by nonlinear microscopy technique**
M. O. dos Santos, Instituto de Pesquisas Energéticas e Nucleares (Brazil); C. Lenz Cesar, V. B. Pelegati, Univ. Estadual de Campinas (Brazil); T. M. T. Zorn, Univ. de São Paulo (Brazil); D. M. Zezzel, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

The assessment of burn wound depth and/or its healing capability are the most important determinants of the proper burn management
and of the residual morbidity or scarring. The objective of this study is to characterize demis of third-degree burned skin by two-photon excited fluorescence microscopy (TPEFM) and second harmonic generation (SHG) technique. It was used a mode-locked laser (Mai tai, Spectra Physics) source with pulse width of approximately 100 fs at 80 MHz directed into a multiphoton microscope using a laser scanning unit (Fluoview 300, Olympus), mounted on an inverted confocal system microscope (IX81, Olympus). The skin samples were obtained from Wistar rats, male, adult. Two dorsal areas were submitted to burns caused by vapour exposure. The skin biopsies obtained were cryosectioned in slices of 20 µm width. Selected areas of interface between the injured and healthy subdermal burned skin were imaged by TPEFM and SHG technique. Two autofluorescence signals were observed as a function of excitation wavelength. The autofluorescence observed at 760 nm and 690 nm suggest elastic fibers at different depths. In SHG images, collagen fibers were visible and elastic fibers do not produce a SHG signals. According to the images obtained, these methodologies can be used to characterize demis of burned tissue during the healing process with reduced out-of-lane photobleaching and phototoxicity. Acknowledgement: FAPESP/CEPID (05/51689-2), Instituto Nacional de Fotônica/CNPq (573.916/2008-0), and FAPEAM-RH-POSGRAD Program.

7903-105, Poster Session

Diagnosing hepatocellular carcinoma with the intensity and the lifetime of two-photon red autofluorescences

T. Liu, C. Hsieh, Y. Chen, F. Huang, National Taiwan Univ. (Taiwan); H. Huang, W. Lee, National Taiwan Univ. Hospital (Taiwan); C. Sun, National Taiwan Univ. (Taiwan)

We demonstrated that the intensity levels and lifetimes of two-photon autofluorescences (2PAF) in human liver tissues can be exploited to diagnose hepatocellular carcinoma (HCC). Excited by an infrared femtosecond laser, we suppressed the two-photon autofluorescences of most endogenous fluorophores and made red autofluorescences more specific to particular molecules in the cryo-sectioned human livers. We found the levels of 2PAF in HCC tissues reduced to one-fourth of those in non-tumor ones. Besides, the averaged fluorescence lifetime will be increased from 400ps (non-tumor part) to 800ps (HCC part). According to these contrast on 2PAF intensity and lifetime, without a staining, the HCC tissues can thus be differentiated from non-tumor ones on the same patient (p<0.001).

7903-106, Poster Session

New methods for high-sensitivity and high-thoughput spectral FLIM

F. Köberling, B. Krämer, M. Wahl, PicoQuant GmbH (Germany); S. Fore, PicoQuant Photonics North America, Inc. (United States); R. Erdmann, PicoQuant GmbH (Germany)

Modern biological research applying Confocal Laser Scanning Microscopy (CLSM) requires often a highly parallelized detection of many different cell constituents in order to unravel e.g. complex signal pathways. The combination of simultaneous spectral detection together with Fluorescence Lifetime Imaging (sFLIM) allows to collect the complete information inherent to the fluorescence signal in order to unambiguously identify fluorescent labels according to their fingerprint of time resolved and spectral properties. Multiple labels can be investigated in parallel and separated from inherent autofluorescence of the sample. In addition, spectral FLIM FRET has the prospect to allow for a simultaneous detection of multiple FRET signals with quantitative analysis of FRET-efficiency and degree of binding. Spectral FLIM measurements generate a huge amount of data. Suitable analysis procedures must be found to condense the inherent information to answer the scientific questions in a straightforward way. Different analysis techniques have been evaluated for a diversity of applications as multiplex labelling, quantitative determination of environmental parameters and distance measurements via FLIM FRET.

In order to reach highest sensitivity in single photon detection, different detector types are investigated and developed. SPAD arrays equipped with micro-lenses promise a superior detection efficiency while the integration of a spectrograph with a PMT array is easier to realize and allows for a higher number of detection channels.

High detection speed can be realized through parallel TCSPC channels. In order to overcome the limits of the USB 2.0 interface, new interface solutions have been realized for the multichannel TCSPC unit HydraHarp 400.

7903-107, Poster Session

Proposal of a new method to measure FRET quantitatively in living or fixed biomedical specimens on a laser microscope

P. J. Helm, O. P. Ottersen, Univ. of Oslo (Norway)

" Förster Resonance Energy Transfer", abbreviated "FRET", is a fluorescence phenomenon, which can be used to study and map co-localizations and dynamics of co-localizations at nanometer precision on a light microscope. FRET has been described as a "spectroscopic ruler". The efficiency of the radiationless energy transfer from an excited chromophore, the "donor", to another chromophore, the "acceptor", the excitation energy of which approximately matches the energy to be released by the donor, is dependent on the sixth power of the mutual distance between the two molecules in space. We propose a new, non-destructive technique for measuring FRET quantitatively and at high spatial and temporal resolution on a laser scanning microscope. Two laser beams of wavelengths suitable for the mutually exclusive excitation of the donor and the acceptor, the "donor beam" and the "acceptor beam", respectively, are intensity modulated by means of two electro optical modulators (EOM). The modulation patterns are rectangular at duty cycle ½. The modulation frequencies differ slightly. The acceptor beam is saturating the acceptor so that it cannot accept energy from the donor. The saturation is modulated in the same way as the acceptor beam. Since the donor beam also is modulated, though at a frequency slightly different from that of the acceptor beam, the intensity of the released donor fluorescence is modulated with the beat frequency of the frequencies of the two laser beam modulations and can be detected and interpreted in quantitative terms by means of a lock in amplifier.

7903-108, Poster Session

Assessing changes in collagen levels of castrated rat prostates using second-harmonic generation and two-photon fluorescence

B. Favetta, V. B. Pelegati, A. A. Thomaz, T. M. Augusto, H. F. Carvalho, C. Lenz Cesar, Univ. Estadual de Campinas (Brazil)

The simultaneous detection of nonlinear optical processes, such as higher harmonic generation and multiphoton fluorescence microscopy, has increasing biological applications. While second harmonic generation (SHG) is detected in areas with lack of symmetry, two photon excitation fluorescence (TPEF) is usually induced using fluorescent labels.

In this study, SHG and TPF imaging were performed to characterize differences in amount of collagen in the extracellular matrix of prostate glands after castration (hormonal ablation). The confocal system used for imaging is composed of an inverted IX-81 microscope and FV300 scanner, both made by Olympus. A femtosecond Ti:Sapphire laser, with tunable wavelength from 690 nm to 1040 nm,
The increase of NADH fluorescence lifetime is associated with the metabolic change during osteogenic differentiation of human mesenchymal stem cells (hMSCs)

H. Guo, J. Yu, S. Hsu, Y. Wei, O. K. Lee, H. Wang, National Yang-Ming Univ. (Taiwan)

Fluorescence lifetime of NADH had been used as an optical marker for monitoring cellular metabolism. In our previous studies, we have demonstrated that NADH lifetime of hMSCs increase gradually with time of osteogenic differentiation. We hypothesized that this change of NADH lifetime was associated with the metabolic shift from anaerobic glycolysis to oxidative phosphorylation during stem cell differentiation. We also hypothesized that this increase of NADH lifetime was possibly due to increased interactions of NADH and Complex proteins, particularly Complex I, in mitochondria. In this study, we measured NADH lifetime of hMSCs from a different donor as well as the corresponding metabolic indices such as ATP level, oxygen consumption and lactate release. We also measured the relative protein level of Complex I, III, IV and V. The results show that during differentiation more oxygen consumed, higher ATP level expressed and less lactate released, and the increase of NADH lifetime was associated with ATP level. Higher expression of the total Complex protein was observed at 3 and 4 weeks after differentiation than controls. However, Complex I expression did not show significant correlation with the increase of NADH fluorescence lifetime. In summary, we demonstrated that the change of NADH lifetime was associated with the metabolic change during osteogenic differentiation of hMSCs. The increase of NADH lifetime was in part due to the increased Complex protein interaction in mitochondria after differentiation.

Spectrally resolved CARS microscopy of condensed carbohydrate systems

A. D. Slepkov, A. Ridsdale, A. F. Pegoraro, D. J. Moffatt, A. Stolow, National Research Council Canada (Canada)

Coherent anti-Stokes Raman scattering (CARS) microscopy has garnered widespread interest as a label-free and chemically-specific nonlinear optical imaging modality. In particular, newer schemes that seek to utilise femtosecond laser-source based CARS imaging are allowing for integration with established nonlinear optical microscopes, and provide for true multimodal imaging. Because CARS signal intensities are independent of the concentration, CARS microscopy is best suited to condensed and aggregated phases. As such, while ever-growing in utility in the biomedical imaging community, CARS microscopy is garnering a reputation as a widely-used imaging modality. We use a single-source femtosecond laser-based multimodal CARS microscope to demonstrate bright CARS and second-harmonic generation (SHG) signals from biologically-relevant condensed carbohydrate-polymer systems such as starch, cellulose, and glycogen. Because SHG is sensitive to local crystallinity and CARS is sensitive to the local density of resonant bonds in the sugar moieties, the two modalities provide complimentary information. We demonstrate this via studies of starch grain swelling during heat-moisture treatments, and show that the crystalline structure of the starch grains is affected before the bulk dilution of the starch. By implementing spectral focussing, we are also able to rapidly obtain CARS spectra, further allowing for spatially-resolved spectroscopic imaging of these systems. Starch and cellulose display distinct CARS spectra, thus opening the door for new and wide-reaching CARS microscopy applications.
system for a commercial multiphoton microscope. By using phase-array analysis, we calculate second harmonic pattern distribution generated by an arrangement of scatterers during transient population of pores for different locations at the cell.

Hippocampal neurons were extracellularly labeled with Synaptored C1 (Biotium) and placed in a home-made chamber designed to electrically excite the cells during imaging. The experiment was performed in a Leica TCS SP5 MP microscope adapted for pr-SHG Microscopy. The electrical signal was generated by a commercial electroporator and with Ag/C1Ag electrodes (gap = 5 mm), immersed in the cell medium.

7903-114, Poster Session
Nonlinear miniaturized microscope with spectral detection for in-vivo tissue imaging
J. van Voskuilen, J. van Weelden, O. Nadiarnykh, Utrecht Univ. (Netherlands); G. Thomas, H. J. C. M. Sterenborg, Erasmus MC (Netherlands); H. C. Gerritsen, Utrecht Univ. (Netherlands)

A nonlinear miniaturized microscope is presented designed for nonlinear optical biopsies. The microscope utilizes a miniaturized scanner that resonantly drives a fiber tip with an objective lens attached to it. The scanner is fitted into a 3mm diameter tube and can be operated at a 1Hz frame rate. The focal spot is scanned in a circular pattern in the xy-plane and z-scanning is accomplished by a piezo drive. The scanner is fiber coupled to the rest of the microscope using a double clad photonic crystal fiber. This facilitates signal guiding of both single-mode infrared excitation and broadband multimode visible emission. The group velocity dispersion of the excitation light by the fiber is pre-compensated with a grating pair. Emission is detected using a custom built spectrograph with a sensitive EM (electron multiplication) CCD for fast (10 kHz spectral rate) spectral detection. The nonlinear excitation provides contrast without applying stains in living tissue. Signals are detected from, amongst others, auto-fluorescence of NADH, FAD, melain, and second harmonic generation of collagen. Tests and results on tissue are shown.

7903-115, Poster Session
Visualization of heat propagation in biological tissue with two-photon fluorescence microscopy
C. Yang, C. Liao, S. Chu, National Taiwan Univ. (Taiwan)

Understanding heat transfer scheme in biological tissues is fundamental and plays a key role not only for characterization of molecular functioning/metabolism but also for clarification of temperature-related biophysical mechanism, such as cooking and thermal therapy. Conventionally, the distribution of heat may be mapped by infrared thermography, but the spatial resolution and sensitivity is limited by the detection of infrared radiation. Here we propose to adopt time-lapsed two-photon fluorescence microscopy (2PFM) to monitor the heat-induced fluorescence change of endogenous molecules in biological tissues. It is known that many native biological fluorophores, such as myosin and chlorophyll, exhibit temperature-dependent fluorescence response. Compared to infrared thermography, 2PFM provides sub-micrometer spatial resolution. In addition, the nonlinear nature of 2PFM offers novel optical sectioning capability and deep-tissue observation that infrared thermography lacks.

As a proof of principle, 2PFM was used to monitor the fluorescence variation in a leaf of Eucalyptus Robusta Smith, which is placed on a home-made heater. Significant change of fluorescence was observed in the temperature range of 30оС to 60оС, and 0.5оС thermal resolution was demonstrated. By heating the leaf asymmetrically, the dynamics of heat conduction was visualized. Moreover, the latent heat of chlorophyll phase transition could be quantified by analyzing the temporal dependence of fluorescence variation. Such technique will be useful for the characterization of heat-related biophysical mechanisms with high spatial and temperature resolution in a thick tissue.

7903-117, Poster Session
Two-photon excitation in life sciences: neurotransmitter and fluorescence uncaging
F. Bolze, J. Nicoud, S. Gug, S. Charon, A. Specht, M. Goeldner, D. Warther, W. Sun, Univ. de Strasbourg (France); P. Kesler, Y. Lutz, J. Vonesch, Institut de Genetique et Biologie Moleculaire et Cellulaire (France); A. Losonczy, Columbia Univ. (United States)

The growing interest in two-photon excitation of organic chromophores is mainly due to the growing emphasis of few of its applications, particularly in material sciences and more recently in life sciences. In the biological field, two-photon excitation found important applications such as two-photon excited microscopy, photodynamic therapy or photorelease of active substances. They take advantages of the two-photon process: strong penetration in living tissues, spatio-temporal localization of the excitation, low damage induced by the exciting light... Nevertheless, in the case of the photorelease of biologically active compounds, the classical photolabile protecting groups were designed for one-photon process and exhibit low two-photon response. The lack of efficient molecules, specifically designed for two-photon applications, led us to the design of new photolabile protecting groups with increased sensitivity to two-photon excitation. In addition we specifically worked to improve the water solubility. We will describe the conception and synthesis of such new compounds for two-photon photorelease of biologically active substances such as neurotransmitters and fluorescent compounds (also known as uncaging).

We will first describe the molecular engineering of new highly efficient two-photon cages, based on disymmetrical systems (electron donor-acceptor couple) and symmetrical systems (bis-electron acceptor systems). Complete characterizations of these new cages, based on a biphrenyl, a fluoreryl or oligothiophenyl central core will be presented.
In vitro photochemistry will be described (uncaging quantum yield, two-photon uncaging action cross-section, kinetics...) and we will then focus on results obtained in cell culture and on acute brain slices (with glutamate, GABA and fluorescence uncaging).

7903-119, Poster Session
Structural analysis of articular cartilage using multiphoton microscopy: input for biomechanical modelling
M. B. Lilledahl, Norwegian Univ. of Science and Technology (Norway); D. M. Pierce, G. Holzapfel, Technische Univ. Graz (Austria); C. de Lange Davies, Univ. of Duisburg-Essen (Germany)

Cartilage diseases in articular joints is a growing medical problem in the industrialized world, as the population becomes older and the incidence of obesity increases. Biomechanical modeling of cartilage is a valuable tool in the management of cartilage pathologies. We have developped a method for the quantitative characterization of articular cartilage using Fourier image analysis techniques on multiphoton images. Specifically, the primary direction and degree of dispersion of the collagen fibers has been quantified. These parameters can be used directly in a novel constitutive model describing the biomechanical properties of cartilage.
As a proof of concept study, we imaged articular cartilage from the knee of chicken, sectioned in three orthogonal planes, to quantify the direction and dispersion of the collagen fibers in three dimensions throughout the cartilage volume. A better description of the collagen fibers, yielding a higher fidelity biomechanical model will increase our understanding of the biomechanics of cartilage which will open up new opportunities for improving clinical management of cartilage diseases.
Delivery and characterization of sub-8fs laser pulses at the imaging plane of a two-photon microscope

M. M. Dantus, D. Pestov, Michigan State Univ. (United States); B. Xu, H. Li, Biophotonic Solutions, Inc. (United States)

We report on a modular and versatile experimental setup that enables straightforward compression (and then shaping) of ultrashort laser pulses at the imaging plane of a two-photon microscope. The laser system is comprised by commercially available broadband Ti:Sapphire oscillator (VENTEON Laser Technologies GmbH, Germany) and 4f shaper (MIIPS Box 640PA, Biophotonic Solutions, Inc). After the shaper, the laser beam is directly coupled into a high-numerical-aperture microscope objective. No other dispersion compensation means are used. Snapshots of the system dispersion for various objectives are obtained by shaper-assisted scanning of the linear chirp (parabolic phase). For high-finesse pulse compression, we use multiphoton intrapulse interference phase scan (MIIPS), which is an adaptive routine that measures the spectral phase to be compensated through active pulse shaping and monitoring of the nonlinear response. To account for chromatic aberrations and pulse temporal distortion (PTD) effects, the pulse compression is verified by in situ interferometric autocorrelation. For the laser spectrum supporting 5.6fs transform-limited pulse duration, we routinely obtain sub-8fs pulse duration at the focus of both Nikon Plan Fluor ELWD 40x/0.60 and water-immersion Zeiss LD C-APOCHROMAT 40x/1.1 objectives. Two-photon excited fluorescence image of a mouse kidney slide is obtained to demonstrate that microscopic sectioning capabilities are preserved. The results presented indicate that nonlinear microscopy can now contemplate the use of ultrashort pulses with bandwidths exceeding 300 nm, with no moving parts and no cumbersome or tailored pulse compression schemes. A calibrated shaper offers desirable flexibility and empowers the user to apply advanced pulse shaping techniques.

Chemical release from single-PMMA microparticles monitored by CARS microscopy

A. M. Enejder, F. Svedberg, L. Nordstierna, M. Nydén, Chalmers Univ. of Technology (Sweden)

Microparticles loaded with antigens, proteins, DNA, fungicides, and other functional agents emerge as ideal vehicles for vaccine, drug delivery, genetic therapy, surface- and crop protection. The microscopic size of the particles and their collective large specific surface area enables highly active and localized release of the functional substance. In order to develop designs with release profiles optimized for the specific application, it is desirable to map the distribution of the active substance within the particle and how parameters such as size, material and morphology affect release rates at single particle level. Current imaging techniques are limited in resolution, sensitivity, image acquisition time, or sample treatment, excluding dynamic studies of active agents in microparticles. Here, we demonstrate that the combination of CARS and THG microscopy can successfully be used, by mapping the spatial distribution and release rates of the fungicide and food preservative IPBC from different designs of PMMA microparticles at single-particle level. By fitting the experimental data to a radial diffusion model, single particle diffusion coefficients were determined. We show that release rates are highly dependent on the size and morphology of the particles and differ from average assembly values determined by conventional bulk analysis. Hence, CARS and THG microscopy provides adequate sensitivity and spatial resolution for quantitative studies on how single-particle properties affect the diffusion of active agents at microscopic level. This will aid the design of innovative microencapsulating systems for controlled release.

Phase-cycling coherent anti-Stokes Raman scattering using shaped femtosecond laser pulses

B. Li, W. S. Warren, Sr., M. C. Fischer, Duke Univ. (United States)

The vibrational level structure of bio-molecules provides the potential for highly specific structural, metabolic and functional contrast in tissue. Because of its nonlinear origin, coherent anti-Stokes Raman scattering (CARS) imaging offers inherent optical sectioning, and the generated higher-energy anti-Stokes radiation is distinct from possible linear background fluorescence. However, non-resonant four-wave mixing processes generally produce a strong, non-specific background at the anti-Stokes frequency, which results in distorted line shapes and a loss of imaging contrast. Techniques such as polarization-sensitive CARS or time-delay CARS have been demonstrated to reduce or suppress this ubiquitous background, but these techniques suppress the non-resonant components at the expense of a reduction of the generally already much smaller resonant components. A more efficient approach to distinguish resonant from non-resonant polarization is to take advantage of the difference in their phase properties: the non-resonant susceptibility is purely real, while the resonant susceptibility is complex. A reference anti-Stokes field of a determined phase (the local oscillator) can extract the imaginary part of the resonant contribution background-free by homodyning the signal. Here we demonstrate a new pulse shaping approach to cleanly and efficiently detect CARS signals. Our approach uses a femtosecond pulse shaping technique with rapid update rates to generate both a static non-resonant LO and a phase-rotating resonant contribution at the focus within the sample. These two contributions result in a stable interference that can be recorded with a detector and a lock-in amplifier without the need for a high-resolution spectrometer, whose performance is inevitably degraded by scattering.
7903-26, Session 4

Mechanism for epi-detected stimulated Raman scattering

P. Wang, M. N. Slipchenko, J. Cheng, Purdue Univ. (United States)

Forward- and epi-detected stimulated Raman scattering (SRS) signals from phantoms of controlled thickness are studied. It was found that both forward and epi-detected SRS intensities are linearly proportional to the number of the local oscillator photons at the photodiode detector, in spite of the pathway that the photons pass through. The ballistic and diffused photons contribute equally to the SRS signal. Epi-detected SRS imaging of biological tissues is demonstrated by maximizing the back-scattering photons collected at the detector. This study provides the understandings of Epi-SRS mechanism that enables the in vivo SRS imaging acquisition and future SRS endoscopy development.

7903-27, Session 4

Ordered water in biopolymers studied by coherent Raman microscopy

E. O. Potma, R. Younger, Univ. of California, Irvine (United States)

Water plays an important role in the structure and function of biopolymers. In this contribution we explore the degree of ordering of water that is coordinated in a various polysugars. Coherent Raman microscopy, supplemented with Raman microspectroscopy and second harmonic generation is used to identify ordered water in cellulose and starch.

7903-28, Session 5

High-energy picosecond fiber lasers for coherent Raman microscopy

F. W. Wise, L. Kong, S. Lefrancois, D. Ouzounov, Cornell Univ. (United States); C. Yang, Tsinghua Univ. (China)

Proliferation of Coherent Raman Microscopy (CRM) will benefit greatly from the development of convenient, inexpensive, compact sources of the required picosecond light pulses. Fiber lasers and amplifiers offer major practical advantages, but the control of adverse nonlinear effects in picosecond fiber sources is a challenge. We will report recent developments in such sources, including the demonstration of a single-mode fiber amplifier that matches the performance of currently-employed solid-state lasers. Work toward the development of all-fiber sources of wavelength-tunable pulses will be described.

7903-29, Session 5

All fiber, 1064-nm time-lens source for coherent anti-Stokes Raman scattering and stimulated Raman scattering microscopy

K. Wang, Cornell Univ. (United States); C. W. Freudiger, B. G. Saar, Harvard Univ. (United States); J. Lee, Cornell Univ. (United States); S. X. Xie, Harvard Univ. (United States); C. Xu, Cornell Univ. (United States)

We use the time-lens concept to demonstrate a new scheme for synchronization of two pulsed light sources for biological imaging. An all fiber, 1064 nm time-lens source is synchronized to a picosecond solid-state Ti: Saphire mode-locked laser by using the mode-locked laser pulses as the clock. Because the pulses are generated through active electro-optic phase modulation, the time-lens source does not require modelocking or a cavity, enabling it to synchronize to arbitrary repetition rates and pulse patterns. The 1.7 ps, 240 mW output of the time-lens source at a repetition rate of 76 MHz is ideally suited for chemically specific imaging techniques such as coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopy. The rms timing jitter between the two synchronized sources is 120 fs. Radio frequency (RF) delay scanning enables tuning of the optical delay without any adjustment of the optical elements, eliminating the tuning of an optical delay line that may give rise to spatial misalignment. Furthermore, the time-lens source is naturally compatible with modulation schemes such as intensity modulation and phase modulation. Modulated pulse trains (e.g., at 10 MHz or 20 MHz) for SRS microscopy can be directly generated from the time-lens source. We demonstrate the application of this synchronized source for CARS and SRS imaging by imaging mouse tissues. Synchronized two wavelength pulsed source is a major technical difficulty for CARS and SRS imaging. The time-lens source demonstrated here may provide an all fiber, user friendly alternative for future SRS imaging.

7903-30, Session 5

Coherent anti-Stokes Raman scattering microspectroscopy based on a compact Er:fiber laser

R. Selm, M. Winterhalder, A. M. Nagy, A. Zumbusch, G. Krauss, T. Hanke, A. Sell, A. Leitenstorfer, Univ. Konstanz (Germany)

Coherent anti-Stokes Raman scattering (CARS) microspectroscopy is a novel type of vibrational microscopy. Molecular selective contrast is created without the need for external labeling and therefore it has attracted considerable attention for biological investigations. To improve the performance and ease of use of existing systems a two-color picosecond Er:fiber laser has been developed. This is a compact, cost effective, and highly stable light source for CARS microscopy. A mode-locked Er:fiber oscillator provides seed pulses for two parallel femtosecond amplifier stages. Because the pulses of both amplifiers originate from the same seed source, they are synchronized with attosecond precision. The pump pulse in the first branch is generated by frequency doubling of the fundamental inside a periodically poled lithium niobate crystal (PPLN). In the second branch a solitonic wave around 2 µm is generated using a highly nonlinear fiber, followed by frequency doubling inside a fan-out PPLN crystal. A tuning range from 850 nm to 1100 nm is achieved in this manner giving access to vibrational resonances from 115 cm⁻¹ to 3800 cm⁻¹. The output pulses are coupled into a commercial microscope system. This enables rapid scanning at a pixel dwell time of 0.5 µs resulting in fast image acquisition. Here we present images of a variety of living cells, for example yeast and adipocytes. Minor changes to the laser system allow for broadband background-free CARS signal generation.

7903-31, Session 5

CARS module for multimodal microscopy

R. Zadoyan, T. Baldacchini, C. Kuo, J. L. Carter, D. Ocepek, Newport Corp. (United States)

We describe a stand alone CARS module allowing upgrade of a two-photon microscope with CARS modality. The Stokes beam is generated in a commercially available PCF using fraction of the power of femtosecond excitation laser. The output of the fiber is optimized for broadband CARS at Stokes shifts around 2900 cm⁻¹. The spectral resolution in CARS signal is 30 cm⁻¹. It is achieved by introducing a bandpass filter in the pump beam. The timing between the pump and Stokes pulses is matched inside the module. We demonstrate functionality of the device on examples of multimodal images of several biological and non-biological samples. We also present results of studies where we used CARS modality to monitor in real time the process of fabrication of nanostructures by two-photon polymerization (CARS movie will be presented). We show that intensity of the CARS signal can
Synchronized picosecond pulses at two different wavelengths from a compact fiber laser source for Raman microscopy

K. Q. Kieu, N. Peyghambarian, College of Optical Sciences, The Univ. of Arizona (United States)

We report on the development of a fiber laser system that supplies synchronized picosecond pulses at two different wavelengths suitable for Raman microscopy. The starting point of the laser system is a compact low cost femtosecond fiber laser mode-locked by use of a fiber taper carbon nanotube saturable absorber (FTCNT SA). Tunable narrow linewidth picosecond pulses around 1550 nm are generated from this source by appropriate spectral filtering and amplification. Picosecond pulses at around 775 nm could be then generated by frequency doubling of the narrow linewidth pulses around 1550 nm. Next, a stable and coherent supercontinuum (SC) could also be generated in a short piece (~ 3 cm) of highly nonlinear fiber using the same femtosecond fiber laser as the pump source. This SC extends more than an octave from 1000 nm to beyond 2000 nm. Tunable narrow linewidth pulses could be formed in this wavelength range again by spectral filtering of the SC. We choose the portion of the SC near 1000 nm for this purpose since these pulses could be then amplified to high power level using existing highly efficient Yb-doped gain fibers. Thus, this scheme allows us to get synchronized picosecond pulses at around 1550 nm, 775 nm and 1000 nm. Detailed laser construction and its performance will be presented.

Label-free biomedical imaging by listening to molecular vibration

J. Cheng, Purdue Univ. (United States)

Signals from molecular vibration permit chemical imaging without labeling. However, the limited penetration depth of c.a. 100 micron in linear and nonlinear vibrational microscopy prevents deep-tissue molecular imaging in clinical settings. To overcome this limitation, we demonstrate vibrational photoacoustic microscopy based on excitation of molecular overtone vibration and acoustic detection of the resultant pressure transients in tissues. In this new approach overtone excitation with a near-infrared nanosecond laser provides label-free chemical selectivity and undetectable photodamage to tissues. Acoustic detection eliminates the tissue scattering problem encountered in near-infrared spectroscopy and enables depth-resolved signal collection. The second overtone of the CH bond stretch around 8300 cm⁻¹, where blood interference is minimal, is excited. We demonstrate three-dimensional vibrational photoacoustic imaging of lipid-rich atherosclerotic plaque from the artery lumen with a penetration depth of 1.5 mm, and of intramuscular fat with a penetration depth of over 1 mm into the muscle tissue.

Broadband coherent Raman microscopy: noninvasive chemical imaging for biology

M. T. Cicerone, Y. Lee, S. H. Parekh, K. Aamer, National Institute of Standards and Technology (United States)

Coherent Raman scattering methods such as coherent anti-Stokes Raman (CARS) and stimulate Raman gain or loss (SRG / SRL) are promising as noninvasive contrast mechanisms for microscopy of chemically complex materials as well as biological cells and tissues. These coherent processes possess several desirable characteristics for biological imaging, including high spatial resolution, high sensitivity, and potential for spectral sensitivity over the “fingerprint” frequency range of (500 to 1800) cm⁻¹. We have recently described instrumental [1, 2] and data analysis [3] approaches that allow us to perform high-resolution imaging using full spectra as contrast in biological samples, with 50 ms pixel, precisely account for the nonresonant signal component of CARS, and extract the resonant signal of interest.

I will discuss CARS signal generation and methods of extracting quantitative chemical information from broadband CARS and SRG spectral images. I will also discuss application of these methods to label-free, noninvasive chemical imaging of cells and tissues.

References

Wavelength-swept CARS spectroscopy

S. Begin, Ctr. de Recherche de l’Univ. Laval Robert-Giffard (Canada); B. Burgoyne, Genia Photonics Inc. (Canada); D. Cote, Ctr. de Recherche de l’Univ. Laval Robert-Giffard (Canada)

We have built a CARS spectroscopy system where the Raman lines are excited sequentially by synchronizing a master oscillator power amplifier (MOPA) Pump laser with a rapidly tunable programmable laser (PL). Using our first prototype, we have acquired lipid CARS spectra over a significant fraction of the high wavenumber region (2700-2900 cm⁻¹) at 500 points per second, or about 4 orders of magnitude faster than current tunable CARS systems.

The PL is based on a dispersion-tuned actively mode-locked fiber laser with 4 chirped fiber Bragg gratings as dispersive elements. This dispersion generates different round-trip times for each wavelength in the cavity. Thus, the wavelength can be tuned by electronically changing the frequency driving the active mode-locker. The synchronization is obtained by triggering the MOPA from the PL. A wavelength-dependent delay is added to compensate for dispersion. Careful calibration ensures that the pulses are synchronized at the sample for any wavelength, enabling the acquisition of CARS spectra over most of the high wavenumber region.
7903-37, Session 6

**Integrated multiplex CARS and two-photon fluorescence microscopy for imaging biological systems**

D. Li, W. Zheng, J. Y. Qu, Hong Kong Univ. of Science and Technology (Hong Kong, China)

The endogenous nonlinear optical (NLO) signals of two-photon excitation fluorescence (TPEF), second harmonic generation (SHG), and coherent anti-stokes Raman scattering (CARS) have been widely used to image a variety of biological samples. Different nonlinear optical signals could convey different structural and biomolecular information. Therefore, it is desirable to combine multiple nonlinear optical signals together for biomedical imaging. However, the simplification of the sophisticated, high cost laser source and the simultaneous excitation and detection of multiple NLO signals are the challenges for the multimodal NLO microscopy. In this work, we instrument a multimodal nonlinear optical microscopy system which integrates the multiplex CARS module with the TPEF, SHG microscopy. The excitation source is the combination of femtosecond Ti:sapphire laser and the broadband near infrared supercontinuum light from a photonic crystal fiber. The multiplex CARS measurements, covering the vibrational frequency from 2400 to 3300 cm\(^{-1}\), allowed us to detect the pure non-resonant background (NRB) signals and the CARS signals of aliphatic C-H and O-H bonds simultaneously. The relatively large NRB in the femtosecond laser excited CARS images could be efficiently suppressed by simple subtraction operation. The TCSPC detection system records the spectral and temporal characteristics of the TPEF signals and spectrally resolves the CARS signals from different molecular vibrational bonds. We demonstrate the multimodal imaging capability of the system using C. elegans and 3T3-L1 cells as the living biological samples. The changes of metabolic status associated with the 3T3-L1 cell differentiation process from fibroblast to adipocyte are analyzed by C-H CARS and NADH TPEF signals.

7903-38, Session 6

**Label-free histopathology images with SRS/TPA lipid-protein-blood contrast provide comparable diagnostic content to permanent H&E stained sections**

G. S. Young, Brigham and Women's Hospital (United States); C. W. Freudiger, B. G. Saar, Harvard Univ. (United States); R. Pfannl, Brigham and Women's Hospital (United States); S. X. Xie, Harvard Univ. (United States)

We present progress in development, application, validation, and preclinical translation of a label free histopathology method for improving the safety, efficacy and cost-effectiveness of pre-operative and intraoperative pathological diagnosis, particularly in neurosurgical resection and treatment of mass-like brain lesions. The method comprises two recently developed label-free microscopy techniques, stimulated Raman scattering (SRS) and two-color two-photon absorption (TPA) microscopy that produce chemical contrast based on intrinsic molecular vibration and absorption and allow optical sectioning by nonlinear excitation. We present three color lipid-protein-blood (LPB) images derived from CH2- and CH3- vibrations and hemoglobin absorption in fresh unstained tissue from various organs and from brain pathologies including primary brain cancer, metastasis, demyelination and strokel. The LPB images will subcellular resolution and clearly depict the same diagnostic features as conventional histologic gold standard hematoxylin & eosin (H&E) stained sections, enabling clinical pathologists to confidently interpret the images with minimal training in a blind test.

7903-39, Session 6

**Fiber optic endomicroscope for CARS imaging**

Y. Zhang, The Johns Hopkins Univ. (United States); R. Zadoyan, Newport Corp. (United States); X. Li, The Johns Hopkins Univ. (United States)

Coherent Anti-Stokes Raman Scattering (CARS) microscopy permits label-free, video-rate vibrational optical imaging. Combined with a flexible fiber-optic scanning endomicroscope, CARS would be a powerful tool for many clinical applications such as in detecting/characterizing lipid rich atherosclerotic plaques and even cancer. A miniature fiber-optic scanning endomicroscope with a broadband CARS imaging system is demonstrated for the first time. The endomicroscope consists of a customized double-clad fiber (DCF) for the delivery of the single-mode pump and the Stokes beam through the core and for the collection of multimode anti-stokes signal through a large inner-cladding of 200 µm diameter. A tubular PZT actuator was used to drive a fiber cantilever to achieve high-speed, two-dimensional spiral beam scanning. A compound aspheric lens with a high numerical aperture of 0.8 and low chromatic aberration allowed for tight focus for the excitation beam and efficient collection of the CARS signal. A 800 nm Ti:Sapphire laser was applied as the pump beam and a broadband supercontinuum generated from a photonic crystal fiber was used as the Stokes beam. The compact system facilitated the broadband multiplex CARS in the spectral range from 500 to 3500 cm\(^{-1}\). Preliminary imaging of 3um polystyrene beads and lipid/water mixture were performed with the fiber-optic scanning endomicroscopy system, and the results demonstrated the fiber-optic CARS endomicroscope could easily detect the CARS signal from the CH stretch with a micron scale lateral resolution. Fiber-optic CARS endomicroscopy imaging of biological tissues is underway to investigate the potential of the technology for in vivo clinical applications.

7903-40, Session 6

**Imaging luminal atherosclerosis by femtosecond CARS to determine plaque burden**

L. B. Mostaço-Guidolin, A. Ridsdale, M. S. D. Smith, M. Hewko, A. F. Pegoraro, E. M. Kohlenberg, B. J. Schattka, National Research Council Canada (Canada); M. Shiomi, Kobe Univ. School of Medicine (Japan); A. Stolow, M. G. Sowa, A. C. T. Ko, National Research Council Canada (Canada)

In North America, atherosclerotic coronary heart disease is the leading cause of death in both men and women. It is estimated that more than 7 million North Americans will experience atherosclerosis-related symptoms during their lifetime. Current clinical imaging modalities to visualize vulnerability within the atherosclerotic plaque include angiography, intravascular ultrasound (IVUS), multi-detector CT (MDCT) and more recently cardiac MRI, intravascular OCT and PET. Each of these techniques may provide some unique insight in determining plaque burden and associated vulnerability however none can provide high resolution compositional information within lesions in understanding plaque development and predicting the risk of plaque rupture. Recently arterial/atherosclerosis imaging was successfully demonstrated by multimodal CARS-based microscopy. [1-4] These studies showed the power of label-free CARS imaging in differentiating plaques from...
Conference 7903: Multiphoton Microscopy in the Biomedical Sciences XI

Nonlinear microscopy, IR, and Raman microspectroscopy for brain tumour analysis

B. Dietzek, Friedrich-Schiller-Univ. Jena (Germany); T. Meyer, N. Bergner, C. Bielecki, C. Krafft, Institut für Photonische Technologien e.V. (Germany); B. F. M. Romeike, R. Reichart, R. Kauff, Friedrich-Schiller-Univ. Jena (Germany); J. Popp, Institut für Photonische Technologien e.V. (Germany)

Vibrational microspectroscopy bears enormous potential to impact medical diagnosis and clinical practice. This is due to the non-invasiveness of the techniques, the intrinsically high chemical specificity intrinsic to the spectroscopic techniques, which allows for detection of changes in the chemical composition during the early stages of disease formation and classification of diseases and the high spatial resolution allowing to access information of a sample on a sub-cellular level.

We focus on applying an all-optical multimodal imaging approach to investigate brain tissue focusing on two central contemporary issues of brain tumour research: First, tumour typing and grading by analyzing excised tissue is of utmost importance for detailing a particular therapy plan. Secondly for prognostication the tumour has to be removed as completely as possible. While nowadays histopathology of excised tissue using haematoxylin-eosin staining is the golden standard for the definitive diagnosis of surgical pathology specimens, this technique is not applicable in vivo. Furthermore, it does not allow for precise tumour typing in those cases when only non-representative specimens are procured. To address these issues and to show how multimodal imaging is used to resolve them, we apply IR and Raman spectroscopy in concert with CARS, SHG and two-photon fluorescence (TPF) microscopy, IR and Raman spectroscopy, allow for very precise cancer analysis due to their molecular specificity, while nonlinear microscopy is a suitable tool for rapid imaging of large tissue sections. Our data indicate that vibrational microspectroscopic imaging is a promising tool for fast and precise in vivo diagnostics of brain tumours.

Nonlinear Raman imaging through turbid medium

V. V. Yakovlev, Univ. of Wisconsin–Milwaukee (United States)

Nonlinear Raman microspectroscopy imaging is an emerging technique for non-invasive, chemically specific optical imaging, which can be probabilistically used to analyze the chemical composition and its distribution in biological tissues. Under proper excitation conditions, coherent anti-Stokes Raman scattering (CARS) microspectroscopy is capable of providing much stronger signal than a conventional spontaneous Raman scattering and has the potential for real-time chemical analysis of cells and tissues. To make a quantitative chemical analysis possible, it is often necessary to collect the extended vibrational spectrum from a selected cell or tissue volume and use sophisticated mathematical algorithms to extract the required information. While most of the research efforts are focused on optimizing experimental conditions for excitation and signal collection, we point that the efforts of light scattering on signal formation and chemical information retrieval have been left aside, despite of the significance and importance of these effects for analysis of molecular structures in vivo.

One of the least explored advantages of CARS microspectroscopy is its ability to utilize long wavelength excitation, which allows deep tissue imaging. We used that fact that CARS microspectroscopy tremendously benefits from the use of near-infrared excitation wavelengths, which allow the use of high-intensity laser sources capable of dramatically increasing the CARS signal strength. Using the earlier developed set-up, we evaluated the imaging capabilities of CARS microspectroscopy through turbid media. Using several model systems, we have explored the capabilities of CARS microspectroscopy to chemically identify microscopic objects hidden in turbid media. This type of systems is important not only for the growing needs of biomedical imaging, but also for identifying potential biohazards and terrorist’s treats, such as anthrax spores, which can be potentially delivered through an ordinary mail.

We have found that imaging of objects, which possess strong isolated Raman lines, is generally not a problem even for a complex texture of scattering medium and large depth of an object. However, imaging objects, which have relatively weak Raman lines, overlapping with major Raman bands of a surrounding medium, might be a problem. We have also found that non-resonant CARS signal might be also used as an additional image contrast mechanism.

In conclusion, we will provide give some recommendations, which might be useful in consideration of using CARS or any other nonlinear optical Raman microspectroscopy in deep tissue imaging, and will outline some of the future work in this area [1-2].
Stokes beams are focused by the small diameter (~ 1.8 mm) multiple of 500 µm that scans the excitation light in a Lissajous pattern. A strong beam) This is coupled using free space optics onto the surface of a beam) and a supercontinuum generated in a nonlinear PCF (Stokes microscopy in a benchtop setup with the miniaturized optics and MEMS We demonstrate CARS and two photon excitation fluorescence collection of the nonlinear optical signal. The basic design concept, major signals. The miniaturized microscope design includes light delivery using biaxial microelectromechanical system (MEMS) mirror for scanning and single living animal. We demonstrate for the first time, the use of a coherent anti-Stokes Raman Scattering (CARS) miniaturized CARS instrument to illustrate the high flexibility of our system. Confocal and multiphoton microscopy are powerful fluorescence techniques for morphological and dynamics studies of labeled elements. For non-fluorescent components, CARS (Coherent Anti-Stokes Raman Scattering) microscopy can be used for imaging various elements of cells such as lipids, proteins, DNA, etc. This technique is based on the intrinsic vibrational properties of the molecules. Leica Microsystems has combined CARS technology with its TCS SPS II confocal microscope to provide several advantages for CARS imaging. The Leica TCS CARS combines two technologies in one system: a conventional scanner for maximum resolution and a resonant scanner for highly time resolved imaging. For CARS microscopy, two picosecond near-infrared lasers are tightly overlapped spatially and temporally and sent directly into the confocal system. The conventional scanner can be used for morphological studies and the resonant scanner for following dynamic processes of unstained living cells. The fast scanner has several advantages over other solutions. First, the sectioning is truly confocal and does not suffer from spatial leakage. Second, the high speed (29 images/sec @ 512x512 pixels) provides fast data acquisition at video rates, allowing studies at the sub-cellular level. In summary, CARS microscopy combined with the tandem scanner makes the Leica TCS CARS a powerful tool for multi-modal and three-dimensional imaging of chemical and biological samples. We will present our solution and show results from recent studies with the Leica CARS instrument to illustrate the high flexibility of our system. We discuss the design and implementation of a novel multimodal coherent anti-Stokes Raman scattering (CARS) miniaturized microscope for imaging of injured and recovering spinal cords in a single living animal. We demonstrate for the first time, the use of a biaxial microelectromechanical system (MEMS) mirror for scanning and diffraction limited multiple lens miniaturized objective for exciting a CARS signal. The miniaturized microscope design includes light delivery using a large mode area photonic crystal fiber (PCF), and multimode fiber for collection of the nonlinear optical signal. The basic design concept, major engineering challenges, solutions, and preliminary results are presented. We demonstrate CARS and two photon excitation fluorescence microscopy in a benchtop setup with the miniaturized optics and MEMS scanning. The light source is based on a single femtosecond laser (pump beam) and a supercontinuum generated in a nonlinear PCF (Stokes beam). This is coupled using free space optics onto the surface of a resonantly driven two dimensional scanning MEMS mirror with a diameter of 500 µm that scans the excitation light in a Lissajous pattern. A strong CARS signal is obtained from lipid rich samples when the pump and Stokes beams are focused by the small diameter (~ 1.8 mm) multiple lens objective corrected for chromatic aberration at the pump and Stokes wavelengths. The novel design of the miniaturized microscope is expected to provide significant new information on the pathogenesis of demyelinating diseases such as Multiple Sclerosis and Spinal Cord Injury. We have developed a polarization mode controllable CARS microscope with compact polarization mode converters. A converter consists of two liquid-crystal spatial-light-modulators and a quarter wave plate, and a modulate has eight electrodes. By control the applied voltage to the electrodes, the mode of the polarization of the CARS excitation beams such as linear polarization, radial polarization and azimuth polarization. We observed the three-dimensional molecular orientation of liquid crystal (8CB) using the developed system. The CARS signal around 1608 cm-1 was used for detection of the molecular orientation because the band highly sensitive to the molecular orientation. We observed two type of liquid crystal cells. Liquid crystal molecule is aligned parallel to the window in one cell, and that is aligned normal to the window in the other cell. The former gave the largest CARS signal with linear polarization, but the latter gave the largest CARS signal with radial polarization. The detection of the three dimensional molecular orientation and its imaging were demonstrated. The biomedical application of the developed system will also be demonstrated. We discuss the design and implementation of a novel multimodal coherent anti-Stokes Raman scattering microscopy using tightly focused cylindrical vector beams Coherent anti-Stoke Raman scattering (CARS) microscopy is a label-free imaging technique based on the vibrations of Raman active molecules in tissues and cells. The major drawback of this technique is the existence of a nonresonant background that limits the sensitivity and applications. In this study, an annular aperture-detection scheme was introduced to significantly suppress the nonresonant solvent background in CARS imaging. By using cylindrical vector polarized beams as excitation fields, several orders of magnitude of improvements in signal to background ratios can be achieved in annular aperture-detected CARS microscopy, and the three-dimensional molecule orientations of samples can also be probed using the cylindrical vector polarized CARS microscopy. We discuss the design and implementation of a novel multimodal coherent anti-Stokes Raman scattering (MCARS) with biological applications Coherent anti-Stoke Raman scattering (CARS) microscopy is a label-free imaging technique based on the vibrations of Raman active molecules in tissues and cells. The major drawback of this technique is the existence of a nonresonant background that limits the sensitivity and applications. In this study, an annular aperture-detection scheme was introduced to significantly suppress the nonresonant solvent background in CARS imaging. By using cylindrical vector polarized beams as excitation fields, several orders of magnitude of improvements in signal to background ratios can be achieved in annular aperture-detected CARS microscopy, and the three-dimensional molecule orientations of samples can also be probed using the cylindrical vector polarized CARS microscopy.
non-specific binding, and cellular toxicity. In this work, we present the first multiparameter label-free flow cytometer that observes the elastically forward-scattered light (FSC) and probes the intrinsic Raman vibrations of passing samples using multiplex coherent anti-Stokes Raman scattering (MCARS). MCARS, as a broadband technique, probes a large region of the Raman spectrum; thus, leading to rich molecularly-sensitive information. In our work, samples are fed into a glass microfluidic chip with a syringe pump (sample velocity of ~1 - 40 mm/s) and a sheath flow within the chip hydrodynamically focuses the sample into a confined stream. An MCARS microscope, focused on this stream, records spectra covering approximately 1450 - 3300 cm⁻¹. The MCARS microscope uses a femtosecond laser as the pump source and to seed a photonic crystal fiber that produces a supercontinuum Stokes source. The resultant spectrum of the sample susceptibility. Furthermore, their relationship is not necessarily trivial, especially if the illuminating pulse is broadband and spectrally shaped. A forward problem can be formulated that takes in to account interferometric measurement of the anti-Stokes field for a given susceptibility, and yields a forward operator that maps the sensitivity of the spectrum to the anti-Stokes signal. In this presentation, we show the construction of the inverse operator to that problem, by which the third order susceptibility of the sample is retrieved from the anti-Stokes field in a broadband, interferometric CARS system. We analyze the stability of the inverse operator for different pulse-shaping schemes and construct regularized versions of it. We expect these results to lead to retrieval of high-resolution background-free vibrational spectra in the next generation of broadband CARS systems.

7903-50, Session 7

Differential-CARS microscopy with linearly chirped femtosecond laser pulses

W. Langbein, I. Rocha-Mendoza, P. Watson, R. Borri, Cardiff Univ. (United Kingdom)

Coherent Anti-Stokes Raman Scattering (CARS) microscopy has recently emerged as a new technique for imaging in cell biology, offering chemical specificity without the need of staining and fluorescence-tagging. However, a background from non-resonant CARS contributions limits its sensitivity and image contrast. We demonstrate a method to reject the nonresonant background via frequency differential CARS (D-CARS) that utilizes a simple and efficient design based on femtosecond laser pulses linearly chirped by glass elements of high group-velocity dispersion. By replicating the exciting Pump-Stokes pulse pairs to create a pulse train at twice the laser repetition rate, and controlling the instantaneous frequency difference of each pair by glass dispersion, we can adjust the Raman frequency probed by each pair in an intrinsically stable and cost-effective way. The resulting CARS intensities are detected simultaneously by a single photomultiplier as sum and difference using phase-sensitive frequency filtering. We demonstrate imaging of polymer beads and living cells with suppressed non-resonant CARS background and improved chemical sensitivity. The method can be extended to more than two pairs by cascading the setup, and we show four-pair D-CARS.

We also demonstrate D-CARS using a single femtosecond laser source, appealing for the realisation of a cost-effective multimodal multiphoton microscope.

7903-51, Session 7

Stimulated Raman scattering for chemical-specific analysis of cellular response to thermal insult

H. T. Beier, Air Force Research Lab. (United States); G. D. Noojin, TASC, Inc. (United States); B. A. Rockwell, Air Force Research Lab. (United States)

A stimulated Raman scattering microscopy system, based on supercontinuum generation from a photonic crystal fiber, has been developed to monitor the cellular mechanisms resulting from thermal insult. Stimulated Raman scattering has been demonstrated to allow label-free, background-free imaging of cells with chemical specificity. The method is used here for rapid spectral detection of protein denaturation, a characteristic of thermal damage, as well as shift-specific imaging of intracellular protein distribution. Understanding the extent of this denaturation and possible recovery is important for various therapeutic measures, as well as for establishing safety standards. For high temperature insults damage may be immediate, but for more moderate temperature increases the damage is often cumulative and may not result in cell death until long after exposure. Cell viability as well as possible protein renaturation is monitored after exposure. These results are compared to previous research on cell death from thermal damage due to laser exposure.

7903-52, Session 7

Novel implementation of a widefield CARS microscope

A. Jesacher, G. Thalhammer, S. Bernet, M. A. Ritsch-Marte, Innsbruck Medical Univ. (Austria)

We present a novel implementation of a Coherent Anti-Stokes Raman Spectroscopy (CARS) microscope where an extended area of the sample is exposed simultaneously by nanosecond Pump-, Stokes-, and Probe-beams. Optimal wave matching can be obtained by tuning the angles of incidence of the Pump- and Probe-beams which are brought into the sample plane by a condenser lens of high numerical aperture. The Stokes beam is coupled in through the microscope objective. This beam geometry ensures that the generated Anti-Stokes-Raman signal is mainly emitted towards the objective lens (counter-propagating to the Stokes beam). Compared to the more widely used scanning CARS, our widefield approach has specific advantages and drawbacks, which will be addressed in the presentation. Ways of optimizing efficiency and flexibility of the widefield setup will be discussed.

7903-53, Session 7

Coherent anti-Stokes Raman scattering (CARS) holography

K. Shi, P. S. Edwards, H. Li, Q. Xu, The Pennsylvania State Univ. (United States); D. Psaltis, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Z. Liu, The Pennsylvania State Univ. (United States)

We present a non-scanning three-dimensional (3D) holographic coherent anti-Stokes Raman scattering (CARS) imaging technique, which combines the unique capabilities of both chemically selective CARS and 3D holography. Briefly, due to the intrinsic coherent nature of the CARS process a CARS image generated by a pair of nanosecond pump/probe
and Stokes pulses under a weakly focused scheme can interfere with a reference pulse to create a CARS hologram. The complex CARS field, which contains both the amplitude and the phase information, can be retrieved digitally from the recorded digital hologram. Three-dimensional imaging is then realized by digital propagation of the field. We have recently demonstrated experimentally single-shot three-dimensional CARS holographic imaging of polystyrene microspheres suspended in water. We are currently applying the CARS holographic technique to imaging live biological cells (e.g., HeLa cell) and the most recent results are presented.

7903-54, Session 8

Sub-100-nm material processing with sub-15-femtosecond picojoule near-infrared laser pulses

K. Koenig, A. A. Uchugonova, M. H. Straub, H. Zhang, M. Afshar, D. Feili, H. Seidel, Univ. des Saarlandes (Germany)

Ultrabroad band in situ 12 femtosecond near infrared laser pulses at transient TW/cm² intensities and low picojoule pulse energies (mean powers < 20 mW at 85 MHz repetition rate) have been used to perform 3D material nanomanipulation based on multiphoton ionization and plasma formation. Cut sizes of sub-wavelength, sub-100 nm which is far beyond the Abbe diffraction has been realized without any collateral damage effect in silicon wafers, photoreists, glass, metals, and biological targets.

7903-55, Session 8

Nanosurgery with near-infrared femtosecond and picosecond laser pulses

A. A. Uchugonova, H. Zhang, K. Koenig, Univ. des Saarlandes (Germany)

Laser-assisted surgery based on multiphoton absorption of NIR light has great potential for high precision surgery at various depths within the cells and tissues. Especially such non-contact method supports contamination-free cell surgery. Here we apply femtosecond laser scanning microscopes for sub-100 nm surgery of human cells and metaphase chromosomes. A mode-locked 85 MHz Ti:Sapphire laser with an M-shaped ultrabroad band spectrum (maxima: 770 nm/830 nm) with an in situ pulse duration at the target of 12 femtoseconds up to 3 picoseconds due to the introduction of chirped mirrors, flint glass wedges, and glass blocks was employed.

The results of laser nanomanipulation in cell/chromosome structures have been quantified by atomic force microscopy (AFM) and electron microscopy. These studies demonstrate the potential of extreme ultrashort femtosecond laser pulses at low mean milliwatt powers for sub-100 nm surgery.

7903-56, Session 8

Wide-field two-photon microscopy with spatio-temporal focusing and HiLo background rejection

E. Y. Yew, Singapore-MIT Alliance for Research and Technology (Singapore); H. Choi, D. Kim, P. T. C. So, Massachusetts Institute of Technology (United States)

Scanningless depth-resolved microscopy is achieved through spatial-temporal focusing has been demonstrated by Oron et al (2005), and Zhu (2005) et al. The advantage of this method is that a large area may be imaged without scanning resulting in higher throughput and reduced complexity of the imaging system. On the other hand imaging in biological samples is often depth-limited due to scattered photons from the fluorescence signal resulting in ‘ghost’ images. It is possible to post-process the images by reassigned these scattered photons but the process is computationally intensive. We propose to perform wide-field two-photon microscopy based on spatio-temporal focusing while employing background rejection based on the HiLo microscope principle. In HiLo microscopy (Lim et al, 2008), two images are sequentially acquired with uniform and non-uniform illumination. The mixed images provide background rejection and reduce the computational overhead of the wide-field two-photon imaging.

7903-57, Session 8

Sequential photon absorption induced luminescence from gold nanoparticles

A. Ben-Yakar, N. J. Durr, The Univ. of Texas at Austin (United States)

Plasmonic gold nanoparticles have attracted significant interest as nonlinear contrast agents, due to their large cross sections, chemical stability, ease of synthesis, and biocompatibility. We will present the properties of multiphoton luminescence (MPL) from colloidal gold nanospheres and nanorods in response to femtosecond and picosecond near infrared excitation light. As the excitation pulse duration is changed, we find that unlike band-gap fluorophores, the luminescence does not scale with the inverse of the pulse duration in the femtosecond regime. At picosecond pulse durations, MPL does scale with the inverse of the pulse duration. This result supports the hypothesis that MPL depends on a sequential rather than simultaneous absorption process, with an intermediate state lifetime of approximately one picosecond. We also find a deviation from a strict quadratic dependence of MPL on excitation fluence at long pulse durations. We quantify the effective two-photon action cross sections of gold nanospheres and nanorods using florescence as a reference, and find them to exhibit several orders of magnitude larger cross sections that the brightest quantum dots.

7903-58, Session 8

Two-photon autofluorescence spectroscopy of oral mucoza tissue

K. Edward, T. Shilagard, S. Qiu, V. Resto, S. McCammon, G. Vargas, The Univ. of Texas Medical Branch (United States)

It is well established that the survival rate for individual diagnosed with oral cancer is positively correlated with the stage of detection. Thus the development of novel techniques for the earliest possible detection of malignancies is of critical importance. Single photon autofluorescence spectroscopy has proven to be a powerful diagnostic tool in this regard, but 2P (two photon) spectroscopy remains essentially unexplored. In this investigation, a custom built spectroscopic system was incorporated into a commercial 2P laser scanning microscope. Oral cancer was induced in the buccal pouch of Syrian Golden hamsters by tri-weekly topical application of 9,10-dimethyl-1,2-benzanthracene (DMBA). Three separated sites where investigated in each hamster at 780 nm, 800 nm, 840 nm and 890 nm. A Total of 7 hamsters were investigated (3 normal and 4 DMBA). A novel two photon autofluorescence imaging, marked for biopsy, processed for histology and H&E staining, and graded by a pathologist. The investigated in vivo sites represented normal, hyperkeratosis, dysplasia and squamous cell carcinoma oral mucosa tissue.

Our results indicate the discrimination of the constituent layers of the oral mucosa (i.e. keratin layer, superficial and basal epithelium, and stroma) from an analysis of the 2-photon auto-fluorescence spectra only. Furthermore, it is shown that spectral interrogation allows for the early detection, monitoring and staging of the DMBA induced carcinoma. The implementation of this approach into a fiber-based endoscopic system will also be demonstrated.
Femtosecond pump-probe imaging reveals chemical and architectural changes in human melanoma

T. E. Matthews, I. Pletic, M. A. Selim, M. J. Simpson, W. S. Warren, Sr., Duke Univ. (United States)

Malignant melanoma is the most lethal form of skin cancer, and was the fifth most common new cancer diagnosis in men in the U.S. in 2009. Five year survival rates fall from 98% for local cancers to 16% for metastatic melanomas. Therefore, early diagnosis of melanoma is critical to the successful treatment of melanomas. However, there is no satisfactory non-invasive screening method available. Melanin, the main pigment in melanocytes and a biomarker for their biochemistry, occurs in two forms: eumelanin and pheomelanin. We have developed a nonlinear pump-probe technique to discriminate between eumelanin and pheomelanin. When pumped at 720 nm and probed at 810 nm, eumelanin exhibits excited state absorption whereas pheomelanin exhibits ground state depletion and/or stimulated emission. We adapted this nonlinear spectroscopy to scanning laser microscopy. This allows us, for the first time, to microscopically image eumelanin and pheomelanin directly in tissue. We imaged eumelanin and pheomelanin in samples of excised pigmented lesions including benign and dysplastic nevi, melanomas, basal cell carcinomas and seborrheic keratoses. Both forms of skin cancer had a significantly higher eumelanin to pheomelanin ratio than benign lesions, increasing towards the center of the lesion and away from surrounding normal tissue. It was also found that dysplastic nevi had increased pheomelanin to eumelanin ratios compared to normal tissue. We have been able to extend this technique to H&E stained slices, allowing its use with current clinical samples and the co-localization of melanin with other structural features.
diabetes, hyperdyslipidemia, insulin resistance and/or excessive alcohol abuse, while liver fibrosis is the excessive accumulation of extracellular matrix proteins such as collagen that occurs in most types of chronic liver diseases. The two diseases represent the complex conditions and major symptoms for many liver diseases. Carbon tetrachloride (CCl4) is a frequently used chemical to experimentally induce hepatic steatosis, fibrosis, hepatocellular death, and carcinogenicity in small animal models, depending on the dose and duration. Recently nonlinear optical (NLO) microscopy has emerged as a powerful tool for label-free tissue imaging with high sensitivity and chemical specificity for major biochemical compounds. In this paper, the four nonlinear microscopy imaging modalities are implemented on the sectioned tissues from CCl4 induced steatosis/fibrosis model of rat livers with different dose and durations. Specifically, second harmonic generation (SHG) imaging quantifies the growing of the collagens, the two-photon excited fluorescence (TPEF) imaging reveals the morphology of hepatic cells, coherent anti-Stokes Raman scattering (CARS) imaging maps the distributions of fats or lipids quantitatively across the tissue, and third harmonic generation (THG) imaging is used to visualize the microstructures and morphology changes of the tissue. Our results demonstrate that the liver steatosis represents the early stage of disease progression, while liver fibrosis occurs at a late stage. A certain correlation between the two diseases in the same model can also be revealed. This study may provide new insights into the understanding of the mechanisms of liver disease (steatosis/fibrosis) transformations at the cellular and molecular levels.

7903-64, Session 9

Time-resolved fluorescence microscopy to study protein interactions in cellellar and model media

L. L. Chandler, HORIBA Scientific, Inc. (United States); G. Hungerford, M. Touny, D. McCluskey, HORIBA Jobin Yvon IBH Ltd. (United Kingdom); A. S. Smith, Glasgow Caledonian Univ. (United Kingdom)

Fluorescence microscopy provides a non-invasive means for visualising dynamic protein interactions. As well as allowing the calculation of kinetic processes via the use of time-resolved fluorescence, localisation of the protein within cells or model systems can be monitored. These fluorescence lifetime images (FLIM) images have become the preferred technique for elucidating protein dynamics due to the fact that the fluorescence lifetime is an absolute measure, in the main independent of fluorophore concentration and intensity fluctuations caused by factors such as photobleaching.

In this work we demonstrate the use fluorescence techniques, such as Förster resonance energy transfer and the influence of a metal surface on fluorescence tagged proteins. These were studied in a cellular environment and in a model system based on a sol-gel derived material, in which silver nanostructures were formed in situ using irradiation from a semiconductor laser in CW mode incorporated on a compact time-resolved fluorescence microscope (HORIBA Scientific DeltaDiode and DynaMyc). The results obtained are discussed in relation to their significance to biology and potential biosensor applications.

7903-65, Session 9

Enhanced-eumelanin fluorescence by stepwise three-photon excitation

J. Kerimo, Northeastern Univ. (United States); M. Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States); C. A. DiMarzio, Northeastern Univ. (United States)

The fluorescence of eumelans from Sepia officinalis and black human hair was activated and enhanced by almost three orders of magnitude by exposure to near-infrared radiation. A step-wise excited state absorption (ESA) process with a third order dependence was present and could be observed for either continuous wave (CW) or femtosecond pulsed laser excitation. The ESA process is very efficient and does not depend on the peak intensity of the laser but on the average power as evidenced by the almost same fluorescence level for both CW and femtosecond laser excitation. The activation was likely by an excited state process since it could not be reproduced when the eumelanin sample was heated up to 100 degrees Celcius. The near-infrared irradiation caused photodamage to the eumelanin granules and could be seen on the images. The enhanced emission originated from the damaged regions. Based on the excitation wavelength and power it was possible to distinguish two different components. One component could be excited efficiently with wavelengths in the visible region with linear power dependence while the other component could be excited efficiently in the near-infrared with third order dependence; the third order power dependence is explained by the ESA process. The emission from one of the components bleached quickly in air and lasted only a few milliseconds but lasted almost a minute in nitrogen atmosphere and could be studied. The new method for photoactivating the eumelanin fluorescence was used to accurately map the melanin content in human hair.

7903-66, Session 9

Subcellular recording of action potentials in cardiac myocytes with random access two-photon microscopy

L. Sacconi, R. Coppini, C. Ferrantini, J. Lotti, C. Tesi, E. Cerbai, C. Poggesi, F. S. Pavone, Univ. degli Studi di Firenze (Italy)

In cardiac cells many membrane currents are heterogeneously distributed between the surface and t-tubule membranes. Though delubulation has been shown to occur in many cardiac disease conditions, the electrophysiological properties of the t-tubules remain poorly known. Simultaneous recording of membrane potential across different subcellular portions of sarcolemma can elucidate the electrophysiology of t-tubules and the functional consequences of loss of t-tubular currents. Unfortunately, this is not feasible with traditional electrophysiological techniques. Here, we developed an ultrafast random access two-photon microscope capable to optical record fast membrane potential transients in multiple positions of the cell membrane with a µm spatial resolution. The random accesses microscope, in combination with a voltage sensitive dye was used to simultaneously record electrical activity from multiple non-contiguous membrane sites in isolated cardiac myocytes in real time with ms time resolution. Optical recording of AP waveforms were performed simultaneously in two different membrane sites: the surface and t-tubule membrane. Preliminary measurements indicate that APs from t-tubular membranes are likely regenerative and not electrotonically conducted, as indicated by the fast upstroke phase, whose raising rate did not differ from the surface sarcolemna. Simultaneous APs recording in surface and t-tubule membrane was also performed in delubulated cardiac myocyte highlighting a significant reduction of the voltage transient in the T-tubule membrane. The high accuracy and temporal resolution of our newly developed method allowed identification of small changes in the dynamics of APs among different subcellular membrane sites.
regions in biological sample has not been shown. Here we present a microscopy setup that combines beam shaping with temporal focusing of amplified pulses (10 microjoules/pulse) for calcium dynamics imaging in neurons from hippocampus acute slices and cultured cells. Multi-photon video-rate (30 fps) imaging of areas as wide as 8100 microns squared with an optical sectioning under 10 microns at 800nm is achievable with our setup. To choose regions of interest in the field of view without any mechanical parts, we use a spatial light modulator (SLM). Because the grating surface is imaged onto the sample, the SLM can easily be integrated into such imaging system by shaping the illumination pattern on the grating. Coupling wide-field temporal focusing with a spatial light modulator for patterned illumination is straightforward and results in an imaging tool very well adapted to functionally probe biological samples over a wide area without delays associated with beam scanning.

7903-68, Session 10

Simultaneous fluorescence and phosphorescence lifetime imaging

W. Becker, B. Su, Becker & Hickl GmbH (Germany)

We present a lifetime imaging technique that simultaneously records fluorescence and phosphorescence lifetime images in laser scanning systems. It is based on modulating a high-frequency pulsed laser by a signal synchronous with the pixel clock, and recording the fluorescence and phosphorescence signals by multi-dimensional TCSPC. Fluorescence is recorded during the on-phase of the laser, phosphorescence during the off-phase. The technique does not require reduction of the pulse repetition rate by a pulse picker. It thus eliminates a general problem of phosphorescence decay measurement: Low duty cycle either leads to extremely low average excitation power, or to extremely high peak power. By controlling the commonly used AOMs, the technique can easily be implemented in standard confocal or multiphoton laser scanning microscopes. Potential applications are oxygen concentration measurements with simultaneous monitoring of cell metabolism, and the migration of nanoparticles of sunscreens and cosmetical products into deep skin layers or inner organs.

7903-69, Session 10

Multiwavelength FLIM: new applications and algorithms

A. C. Rueck, Univ. Ulm (Germany)

The combination of time-resolved and spectral resolved techniques as achieved by SLIM [1,2] (spectrally resolved fluorescence lifetime imaging) improves the analysis of complex situations, when different fluorophores have to be distinguished. This could be the case when endogenous fluorophores of living cells and tissues are observed to identify the redox state and oxidative metabolic changes of the mitochondria (reviewed in [3]). Other examples are FRET (resonant energy transfer) measurements, when different donor/acceptor pairs are observed simultaneously. SLIM is working in the time domain employing excitation with short light pulses and detection of the fluorescence intensity decay in many cases with time-correlated single photon counting (TCSPC). Spectral resolved detection is achieved by a polychromator in the detection path and a 16-channel multianode photomultiplier tube with the appropriate routing electronics.

Within this presentation special attention will be focused on new diagnostic systems involving autofluorescence, photosensitizers and FRET measurements with respect to protein interactions in Alzheimers disease. Using global analysis as the phasor plot approach or integration of the kinetic equations taking into account the multidimensional datasets in every spectral channel we could demonstrate considerable improvement of our calculations.


7903-71, Session 10

Determination of calcium concentrations in cells and tissue with fluorescence lifetime imaging - from neurons to smooth muscle cells.

T. Gensch, Forschungszentrum Jülich GmbH (Germany)

The determination of ion concentrations in cells - in particular in neurons - is very important for understanding cell function and life. Calcium is an ubiquitous messenger in almost all cell types. Fluorescence lifetime imaging (FLIM) can be of advantage over intensity based fluorescence microscopy, when comparisons between micro-domains of one cell or between different cells of one cell type are performed. Several (organic chromophores and genetically-encoded calcium sensors) have been tested in culture cells and cell tissue with respect to their applicability in FLIM studies. The calcium concentration (changes) in several cell types (including smooth muscle cells, retinal and auditory brain stem neurons and cardiomyocytes) were investigated by FLIM with two-photon excitation. The advantages/disadvantages of intensity- and lifetime-based determination of calcium concentrations as well as different organic chromophor or genetically-encoded calcium sensors will be discussed.

7903-72, Session 10

Fluorescence lifetime imaging (FLIM) and time-resolved fluorescence anisotropy imaging (TR-FAIM) of molecular rotors in living cells

P. Chung, J. A. Levitt, King’s College London (United Kingdom); M. K. Kuimova, Imperial College London (United Kingdom); G. Yahioglu, PhotoBiotics Ltd. (United States); K. Suhling, King’s College London (United Kingdom)

We perform fluorescence lifetime imaging (FLIM) and time-resolved fluorescence anisotropy imaging (TR-FAIM) of molecular rotors in living cells via time-correlated single photon counting (TCSPC). The fluorescence properties of the molecular rotors, a meso-substituted Boron-Dipyrrin (BODIPY), are a function of the viscosity of their environment according to the Förster-Hoffmann equation. We observe that both the fluorescence lifetime and the rotational correlation time of BODIPY in homogenous solutions are a function of the viscosity of the solvent. Using this characteristic, the fluorescence lifetime and rotational correlation time of the dye in live HeLa cells at 37°C can be imaged to yield viscosity maps of the cells.

7903-73, Session 10

Determination of the stoichiometry, structure, and distribution in living cells of protein complexes from analysis of single-molecular-complexes FRET

D. R. Singh, M. R. Stoneman, V. Raicu, Univ. of Wisconsin-Milwaukee (United States)
Advances in two-photon microscopy with spectral resolution (TPM-SR) and the development of a simple theory of Förster Resonance Energy Transfer (FRET) for single molecular complexes recently lead to the development of a novel method for the determination of structure and localization in living cells of membrane protein complexes (Raicu et al., Nature Photonics, 3, 2009). An appealing feature of this method is its ability to provide such important information while being unaffected by spurious signals originating from stochastic FRET (Singh and Raicu, Biophys. J., 98, 2010). This presentation will review the results obtained from our recent studies of oligomeric complexes of several cytoplasmic and membrane proteins in living cells. Emphasis will be placed on the measurement and analysis of single-molecular-complex FRET data for determination of the quaternary structure of some proteins (or the protein complex structure).

Bayesian analysis of fluorescence lifetime imaging data

M. I. Rowley, King’s College London (United Kingdom); P. R. Barber, Univ. of Oxford (United Kingdom); A. Coolen, King’s College London (United Kingdom); B. Vojnovic, Univ. of Oxford (United Kingdom)

Fluorescence Lifetime Imaging (FLIM) is an intensity independent and sensitive optical technique for studying the cellular environment and can be used in Förster Resonance Energy Transfer (FRET) experiments enabling protein-protein interactions to be located within living or fixed cells. Careful analysis of fluorescence lifetime data, usually comprising multi-exponential kinetics, is crucial to conducting sensitive experiments via FLIM. There is a need for more accurate lifetime fitting of data with lower photon counts to allow greater acquisition speeds.

We have employed the Bayesian framework to develop a photon-by-photon analysis that obviates the need to choose a specific noise model and respects the truly multinomial nature of the data. The effects of detector instrument deadtime, which can cause data to be non-Poissonian, can be incorporated into our model, offering an opportunity to score an advantage over maximum likelihood methods which are considered to be the current gold standard. Using both real and synthetic data, parameter estimates obtained with our mono-exponential Bayesian analysis compared favourably with those using maximum likelihood and least squares, offering robust estimation with greater precision at low total photon counts and in the presence of significant background levels. Applied to burst integrated fluorescence lifetime (BIFL) data, our Bayesian analysis was implemented in a discrete framework to speed up analysis to perform a simple model selection task, demonstrating single Bayesian analysis was implemented in a discrete framework to speed up performance.

Laser-induced photobleaching of NAD(P)H fluorescence components in cardiac cells resolved by spectral unmixing of TCSPC signals

A. Chorvatova, A. Mateasik, D. Chorvat, Jr., International Laser Ctr. (Slovakia)

NAD(P)H fluorescence, naturally occurring endogenous fluorescence of cells offers broad possibility of investigation of mitochondrial metabolic state directly in living cells [1]. However, photobleaching - the loss of fluorescence intensity caused by prolonged exposition to light - is an inherent phenomenon of long-term fluorescence acquisition with negative impact on the observed system, and is therefore an essential limitation of metabolic imaging. NAD(P)H fluorescence was investigated by spectrally-resolved lifetime detection, while individual NAD(P)H fluorescence components were resolved by spectral linear unmixing approach [2]. Our data indicate presence of three individual components in cardiac cell autofluorescence, two correlating with NAD(P)H in organic (“bound”) and inorganic (“free”) solvents, respectively, and one with the residual flavin fluorescence. Photobleaching was induced by excitation of a defocused elliptical spot (20x10 µm) with a 375nm picosecond laser (~1 mW output power) for 30s repeated every 80s for 7min. Our data indicate comparable photobleaching of both NAD(P)H components. In both cases, decrease in photon counts was induced by lowering of the component amplitude; no modification in fluorescence lifetimes was noted. Interestingly, ratio of the two amplitudes remained unchanged during photobleaching. Gathered results point to a decrease in the number of endogenously fluorescing molecules, rather than change in their environment during the photobleaching process. These findings are important for choosing appropriate light excitation and fluorescence acquisition for long-term studies of metabolic state in living cells [3].

References:

New methods for FLIM and FCS for confocal laser scanning microscopy

S. Fore, PicoQuant Photonics North America, Inc. (United States); F. Koberling, B. Krämer, M. König, V. Buschmann, M. Wahl, S. Orthaus, U. Ortmann, R. Erdmann, PicoQuant GmbH (Germany)

Confocal laser scanning microscopes (CLSM) are an essential tool in biological and biomedical research. Their functionality can be further enhanced by adding sensitive time-resolved data acquisition capabilities, enabling Fluorescence Lifetime Imaging (FLIM), lifetime based Förster Resonance Energy Transfer (FRET) and Fluorescence (Lifetime) Correlation Spectroscopy (FLICS) down to the single molecule level. By using the non-descanned (NDD) internal detectors of the laser scanning microscope, also NDD FLIM acquisition becomes easy and powerful. Based on a recently added network interface, the FLIM and FCS data acquisition can now be directly accessed from the CLSM computer. This unique integration enables a seamless work flow.

In order to achieve fast spectral FLIM measurements, up to eight parallel TCSPC detection channels can be employed. To overcome data transfer restrictions of the USB 2.0 interface, we have evaluated and developed new host interface solutions for our multichannel TCSPC device HydraHarF 400.

Förster Resonance Energy Transfer (FRET) studies provide a very powerful tool for a broad range of biological applications since this technique enables to measure intra- and intermolecular distances down to several nanometres. Other than intensity-based FRET measurements, FLIM can further reveal sub-populations; thus, allowing to determine the fraction of free donors compared to associated donor molecules within a complex. The result of such an analysis yields not only the FRET efficiency distribution of FLIM-FRET images, but also the fraction and distribution of complete to incomplete FRET complexes. In addition, so-called lifetime sensitive sensors allow the monitoring of environmental conditions such as pH and ion concentration.
Drug transport mechanism of P-glycoprotein monitored by single-molecule fluorescence resonance energy transfer
S. Ernst, Univ. Stuttgart (Germany); B. Verhalen, SUNY Upstate Medical Univ. (United States); N. Zarrabi, Univ. Stuttgart (Germany); S. Wilkens, SUNY Upstate Medical Univ. (United States); M. Börsch, Univ. Stuttgart (Germany)
We monitor the catalytic mechanism of P-glycoprotein (Pgp) using single-molecule fluorescence resonance energy transfer (FRET). Pgp, a member of the ATP binding cassette family of transport proteins, is found in the plasma membrane of animal cells where it is involved in the ATP hydrolysis-driven export of hydrophobic molecules. When expressed in the plasma membrane of cancer cells, the transport activity of Pgp can lead to the failure of chemotherapy by excluding the mostly hydrophobic drugs from the interior of the cell. Despite ongoing effort, the catalytic mechanism by which Pgp couples MgATP binding and hydrolysis to translocation of drug molecules across the lipid bilayer is poorly understood. Using site-directed mutagenesis, we have introduced cysteine residues for fluorescence labeling into different regions of the nucleotide binding domains of Pgp. Double-labeled single Pgp showed fluctuating FRET efficiencies during ATP-driven transport as expected from the distinct recent X-ray crystallographic structures. Duty cycle-optimized pulsed alternating laser excitation (DCO-ALEX) is applied to minimize FRET artifacts and to select the appropriate transporters. Hidden-Markov-Models (HMM) provide the objective way of analyzing the fluorescence time trajectories of Pgp. Thus we report on the dynamics of individual Pgp as well as the effects of different hydrophobic drugs and inhibitors.

Measuring the diffusion of fluorophores in human skin by two-photon fluorescence correlation spectroscopy combined with measurements of point spread function
S. Gulbrandsen, C. Simonsen, M. Goksöyr, M. H. Smedh, M. B. Ericson, Göteborg Univ. (Sweden)
Two-photon excitation fluorescence correlation spectroscopy (TPFCS) has been used in combination with measurements of the point spread function (PSF), for quantitative analysis of fluorophores, e.g. sulphorhodamine B, applied to excised human skin. The PSF was measured as the full width at half maximum (FWHM) of subresolution beads embedded in the skin samples. The FWHM was measured in the lateral direction and the axial direction. The circular symmetry of the beads was well preserved in the lateral direction but in the axial direction, the beads were elongated (as expected) and in some cases, tilted, probably due to astigmatism. Interestingly the FWHM was not found to depend significantly on the depth down to 40 μm, which was unexpected because the scattering properties of skin. TPFCS measurements were performed on skin exposed to various fluorophores. The diffusion coefficient and the number of molecules were measured in the intracellular as well as the extracellular environment. The number of molecules was found to accumulate close to the surface of the sample and decreased rapidly deep down. The diffusion coefficient was not found to depend on the depth, but more on the location in the lateral plane, whether the measurement was performed in the intracellular or the extracellular area. This study is the first to perform TPFCS on human skin. The results show that TPFCS can be used for quantitative analyses of fluorescent compounds in human skin.

Two-photon phosphorescence lifetime microscopy (2PPLM) for high-resolution imaging of oxygen
S. A. Vinogradov, L. E. Sinks, E. Roussakis, Univ. of Pennsylvania (United States)
Imaging oxygen distributions presents a challenging problem in modern physiology and medicine. We are developing a method for imaging of oxygen in biological tissues with micron-scale resolution and three-dimensional capability. Our technique is based on the combination of the phosphorescence quenching approach with multiphoton laser scanning microscopy. We describe design of two-photon-enhanced porphyrin-based dendritic nanoprobe whose phosphorescence upon two-photon excitation is enhanced via intramolecular Förster-type energy transfer from covalently attached two-photon antennae. The probe’s oxygen sensitivity can be tuned by way of dendritic encapsulation, while peripheral functionalization prevents interactions of the probes with biological macromolecules and ensures their high selectivity for oxygen. Further, we describe modifications to two-photon microscope required for phosphorescence lifetime imaging and address the interplay between the probe photophysics, spatial and temporal imaging resolution. Finally, we demonstrate functionality of the method by performing pilot intracellular oxygen imaging as well as depth-resolved intravascular and tissue high-resolution oxygen measurements in vivo in the brain. The unique properties of the probe allowed simultaneous 3D mapping of pO2 in vasculature and tissue as well as measurements of the rate of blood flow in individual capillaries.

A multispectral FLIM microscope for in-vivo imaging of skin cancer
C. B. Talbot, R. Patalay, I. H. Munro, Imperial College London (United Kingdom); G. Bruning, K. Koenig, JenLab GmbH (Germany); Y. Alexandrov, S. Warren, A. Chu, G. W. Stamp, M. A. Neil, P. M. W. French, C. W. Dunsby, Imperial College London (United Kingdom)
We present a multi-spectral fluorescence lifetime imaging (FLIM) detector and an optically efficient spectrally resolved detector that we have integrated into the commercially available two-photon DermaInspect microscope, which we are utilising for in vivo fluorescence imaging studies of human skin. We also present image segmentation algorithms that facilitate the automatic processing of the large data sets obtained with this system. The combination of multi-dimensional data acquisition and automated processing provides the realistic potential to aid in the classification of skin lesions without the need for biopsy. The multispectral FLIM detector is based on four high quantum efficiency detectors interfaced with time correlated single photon counting electronics. Combined with an efficient optical design, this facilitates the simultaneous in vivo acquisition of shot-noise limited four-channel FLIM images at clinically viable speeds (<30 s). The spectral resolution of the detector system prevents us from directly collecting an instrument response function in each channel from a second harmonic generating sample. Instead, we present the use of reconvolution with a reference fluorophore in order to reliably fit complex exponential decay curves. The hyperspectral detector utilises a spot-to-line fibre bundle to deliver the fluorescence via a dispersing prism onto an EMCCD camera. This approach for in vivo acquisition of depth resolved emission spectra at clinically viable speeds. The multi-dimensional resolution afforded by these detectors, combined with wavelength tuneable excitation, allows for the decomposition of the fluorescence into the signature of the autofluorescent compounds, making the instrument ideal for studying skin and offers substantial diagnostic potential.
Using adaptive optics for deep in-vivo multiphoton FLIM

S. P. Poland, G. O. Fruhwirth, T. C. Ng, S. M. Ameer-Beg, King's College London (United Kingdom)

Multiphoton microscopy (MPM) is a high resolution (sub-μm) 3D optical imaging technique that has seen widespread use for microscopy at moderate depth within biological tissue (~1 mm). MPM enables accurate determination of such parameters as inter-vessel distance, branching ratios and leakage of fluorescent markers. MPM combined with Fluorescence lifetime imaging (FLIM) and Fluorescent resonant energy transfer (FRET) provides the ability to image protein-protein interactions. When applied in-vivo at depth, it will be a key component to identifying and evaluating drug interaction in tumours.

Unfortunately as one images more deeply into biological tissue, depth is restricted due to the specimen induced aberrations, which result in deterioration in both the image quality and resolution. Adaptive optics (AO), a technique first developed for astronomy, has been shown to be successful in overcoming problems associated with imaging in depth in confocal, multiphoton, CARs and SHG microscopy. The principle relies on shaping the wavefront with a wavefront modulator to compensate for the distortions introduced by the biological tissue sample. The success of such a technique relies on being able to correctly determine the wavefront correction required.

In this paper we will discuss the development a dedicated MPM FLIM-FRET microscope incorporating an AO for use in-vivo applications. Using a deformable membrane mirror as a wavefront modulator, a number of alternative strategies for implementation will be examined.

A STED-FLIM microscope applied to imaging the natural killer cell immune synapse

M. O. Lenz, A. Brown, E. Auksorius, D. M. Davis, C. W. Dunsby, M. A. Neil, P. M. W. French, Imperial College London (United Kingdom)

We present a stimulated emission depletion (STED) fluorescence lifetime imaging (FLIM) microscope, excited by a tunable microstructured optical fibre supercontinuum source that is pumped by a femtosecond Ti:Sapphire-laser, which is also used for depletion. Implemented using a piezo-scanning stage on a laser scanning confocal fluorescence microscope system with FLIM realised using time correlated single photon counting (TCSPC), this provides convenient switching between confocal and STED-FLIM with spatial resolution down to below 50 nm. We will present our design considerations to make a robust instrument for biological applications including a comparison between fixed phase plate and spatial light modulator (SLM) approaches to shape the STED beam and the correlation of STED and confocal FLIM microscopy.

Following our previous application of FLIM-FRET to study intercellular signalling at the immunological synapse (IS), we are employing STED microscopy to characterize the spatial distribution of cellular molecules with sub-diffraction resolution at the IS formed between two cells. In particular, we are imaging receptor organisation and cytoskeletal structure at the Natural Killer cell activated immune synapse. We will also present our progress on multilabel STED microscopy to determine how relative spatial molecular organisation, previously undetectable by conventional microscopy techniques, is important for NK cell cytotoxic function.

Intracellular oligomerization of HIV-1 Vpr and interaction with Gag polyprotein: a two-photon FRET-FLIM investigation

Y. Mely, J. Fritz, P. Didier, Univ. de Strasbourg (France); J. Darlix, Ecole Normale Supérieure de Lyon (France); H. de Rocquigny, Univ. de Strasbourg (France)

The viral protein R (Vpr) is a HIV-1 regulatory protein causing G2/M cell cycle arrest and apoptosis. During HIV-1 assembly, Vpr is incorporated into viral particles through interaction with the Pr55(Gag) polyprotein. Since Vpr oligomerizes efficiently in solution, we investigated first whether Vpr oligomerizes also in the cellular context. To this end, we quantified Vpr-Vpr interaction by FRET-FLIM in HeLa cells co-transfected by eGFP- and mCherry-tagged Vpr proteins. Vpr was found to oligomerize in the whole cell and notably, at the nuclear envelope. Moreover, using FCS, Vpr was shown to form dimers and trimers. Point mutations in the helices of Vpr drastically impaired its oligomerization and localization at the nuclear envelope while mutations outside the helical regions had no effect. Interestingly, all point mutants caused cell apoptosis suggesting that Vpr-mediated apoptosis functions independently from Vpr oligomerization. Next, we monitored by FRET-FLIM the interaction between eGFP-Vpr and tetracysteine-tagged Pr55(Gag) in HeLa cells. The Pr55(Gag)-Vpr complexes were observed to accumulate at the plasma membrane. Vpr oligomerization was shown to be crucial for Pr55(Gag) protein to the plasma membrane was necessary for the recruitment of Vpr. Finally, the mechanism of Vpr uptake into cells was investigated using fluorescently labeled Vpr(52-96) peptides. Both non-endocytotic and endocytotic pathways were evidenced, as well as a passive entry of Vpr peptide, associated to its membrane destabilization properties. The efficient uptake of Vpr through multiple routes is consistent with its role on bystander cells.
High-speed high-resolution cross-structured illumination confocal microscopy

M. Ahn, T. Kim, Y. Kim, D. Gweon, KAIST (Korea, Republic of)

Recently, the resolution of conventional microscopy has been improved over the diffraction limits in structured illumination microscopy (SIM). In order to improve the lateral resolution two times than the conventional lateral resolution, three images should be acquired with the phase of the illumination pattern shifted 120° and this procedure should be repeated twice with the orientation of the pattern rotated by 60° and 120°. In SIM, at least a total of nine raw images are necessary for the reconstruction of an image with doubled lateral resolution only or with improved axial as well as lateral resolution. This leads the speed of the image acquisition to be slow and the process of the image reconstruction to be complex. In this paper, we propose the cross structured illumination confocal microscope (CSICM) that improves the lateral resolution and the image acquisition speed. The CSICM is combined with the cross SI pattern generation optics and the line scanning confocal microscope. Performances of the conventional SI and the cross SI are compared by the analysis of the modulation transfer function. The cross SI method shows similar resolution to conventional SI method. Since the cross SI method has no rotation of the grating, fast SI pattern can be generated. The acquisition of a total of six raw images shortens the image acquisition time. Accordingly, The CSICM has the two times enhanced lateral resolution than the conventional microscope, the optical sectioning ability and the fast image acquisition speed.

Design and analysis of confocal-spectral microscopy using wavelength scanning scheme

D. Do, W. Chun, KAIST (Korea, Republic of); H. Jeong, ; D. Gweon, KAIST (Korea, Republic of)

Spectral microscopy has ability to detect broadband fluorescence signals which are useful in case of studying interactions and phenomena between biological samples. Recently, commercial devices are combining with confocal microscope so to enhance lateral resolution and to have axial direction discernment. Also Acousto-Optic Tunable Filter(AOTF) is used instead of using multiple-dichroic mirrors to divide excitation and emission signals to maximize light efficiency. In addition, AOTF used in spectral microscopy have many merits, that are very fast switching speed and high resolution in wavelength selection. However AOTF uses the principle of acousto-optic interactions in a birefringent material, the excitation light interacts with appropriate radio frequency signal so that it is diffracted to 1 or -1 order beam-path. Also, the fluorescence signals from the sample propagate in 0 order beam-path with different angles according to the wavelength and its polarization state.

In this paper, the confocal-spectral microscopy is proposed with the novel spectrometer design having wavelength-scanning galvano mirror. It makes possible to detect broadband(480-750nm) fluorescence signal by single point detector(PMT) instead of CCD pixel arrays. For this purpose, some optical elements are appropriately designed. In order to amplify dispersion angle of fluorescence signal, prism is used. And relay lens are designed to match the signals with diameter of wavelength-scanning galvano mirror. Also a birefringent material, calcite is used to compensate polarization effect.

The proposed spectral confocal microscopy with unique spectrometer body has many advantages in comparison with commercial devices. In terms of detection method, it can be easily applied to other imaging modalities. Hence this system will be adapted in many applications.

Correction of defocused images in full-field optical coherence tomography using digital holography

G. Min, J. W. Kim, W. J. Choi, B. Lee, Gwangju Institute of Science and Technology (Korea, Republic of)

We propose the digital holographic technique that can correct the defocused two dimensional en-face images obtained in the full-field optical coherence tomography (FF-OCT). As a powerful biomedical imaging modality, FF-OCT provides a noninvasive inner microstructure image of a biological sample with a submicron depth resolution. The advantage of the FF-OCT over other OCT techniques is that, since it employs a two-dimensional array sensor such as a charge coupled device (CCD), it requires only depth scanning (C-scan) without any transversal mechanical scanning (B-scan). The FF-OCT system based on a Michelson interferometer is composed of the reference arm and the sample arm. In both arms, water immersion objective lenses are used in order to compensate the dispersion between air and biological samples. However, the presence of the sample having a very high refractive index than the surrounding water medium gives a problem of index mismatching between the reference arm and the sample arm, which results in degradation of OCT image along the depth. In this study, we confirm the existence of the defocusing problem in the FF-OCT and propose the correct method based on the digital holographic technique. The digital holography, with the help of the Fresnel diffraction theory, can freely adjust the focal plane of already taken image. One of the major advantages of the proposed technique is that it does not require any mechanical movement in the measurement system, but the re-focusing process is performed only with numerical calculation. The performance of the digital holographic algorithm is demonstrated with the image of the USAF resolution target, which was taken at out of the focal plane but refocused digitally to get the on focus clear image.

Two dimensional scanning probe using off-axis magnetic force of single solenoid for 3D OCT imaging

E. J. Min, J. G. Shin, Y. Kim, B. Lee, Gwangju Institute of Science and Technology (Korea, Republic of)

We propose a single piece optical fiber-based two-dimensional scanning hand-held probe suitable for three-dimensional optical coherence tomography (OCT). The probe consists of only a single piece of optical fiber loaded with a bead of ferromagnetic material, which acts as a vibrating cantilever. The fiber cantilever is two dimensionally actuated with a single miniaturized solenoid. For effective beam focusing, a fiber lens is formed at the end of the fiber. The inductance and input current of the solenoid were 100 uH and 260 mA, respectively. The iron-bead on the fiber is located at the off-axis of solenoid for two-dimensional scanning. Then, by modulating the input current to the solenoid, it was possible to mechanically oscillate the fiber cantilever in an elliptically spiral pattern. With the proposed probe, 2-dimensional scanning could be experimentally achieved in a rate of 4 s/vortex across a scanning area of approximately 33 mm², which could be controlled with the length of the fiber, and the weight of the iron-bead. Three-dimensional tomographic image of a coin was successfully obtained with the spectral domain optical coherence tomography (SD-OCT) equipped with the proposed scanner. It is expected that the scheme of 2-dimensional scanning with a single actuator might be useful in various real-time imaging applications including OCT owing to the advantages of low cost, low power consumption, simple fabrication process and versatile design.
7904-58, Poster Session

Comparison of resolution in tomographic diffractive microscopy using combinations of sample rotation and illumination rotation

S. Vertu, J. Flügge, Physikalisch-Technische Bundesanstalt (Germany); J. Delaunay, The Univ. of Tokyo (Japan); O. Haebeler, Univ. de Haute Alsace (France)

Tomographic Diffractive Microscopy (TDM) is a technique, which permits to image transparent living specimens without staining. For weakly diffractive samples, the three dimensions distribution of the complex Refractive Index (RI) can be reconstructed from the knowledge of the measured scattered fields sampled under various viewing and illumination angles according to the diffraction tomography theorem. TDM is commonly implemented in two ways, by either rotating the sample illumination keeping the specimen fixed, or by rotating the sample using a fixed illumination. Unfortunately, both methods present limitations. Under the first-order Born approximation, the varying illumination direction method presents a strong anisotropic resolution along the optical axis due to the so-called “missing cone” of non captured frequencies. If the sample rotation method presents a better isotropic resolution, it suffers severely from a reduced maximal extension of the captured frequencies compared to tomography with rotating illumination.

With the purpose of overcoming the limitations of each method, we report first results about various techniques for expanding the Optical Transfer Function with a tomographic microscope by combining in different configurations the sample rotation method with the varying illumination direction method. Here, we aim at obtaining a high and isotropic resolution. Using simulations, we investigate the effects of the missing frequencies of the different configurations on reconstructed objects to estimate the performances of the different configurations.


7904-60, Poster Session

Digital micromirror device (DMD) based confocal fluorescence detection of 3D cell cultures

J. Choi, D. Kim, Yonsei Univ. (Korea, Republic of)

A digital micromirror device (DMD) consists of multiple micromirrors that are individually controllable and can provide extremely fast scanning speed for confocal imaging. The use of a DMD shows promises in various imaging systems that may need non-conventional scanning strategies; for example, multiple pinhole scanning and structured light incidence for reduced acquisition time and to improve image quality. For this reason, we constructed a compact and portable 3D confocal fluorescence detection system based on a DMD for monitoring 3D culture assays. 3D cell cultures are microfluidic cell culture systems that draw tremendous interests recently because they can provide a microenvironment that is close to in vivo conditions and thus are useful to better mimic animal physiology. Compact confocal imaging is required to fully understand cellular activities in a 3D cell culture. The constructed confocal system combines a DMD with an automatic vertical stage to implement three-dimensional optical sectioning measurement at fast scanning speed. The performance of the DMD-based confocal fluorescence optical detection system, initially tested by imaging an USAF target and fluorescent microbeads that model stained cells, was determined to be diffraction-limited. For proof-of-concept, we have further studied cell dynamics in 3D alginate cell culture matrix using Calcein AM as a fluorescence indicator and observed cellular activities such as cell growth and death. The confocal fluorescence detection system is expected to be useful to provide information on specific cell status, for instance, enzymatic activities and cell viability in 3D cell culture systems.

7904-61, Poster Session

An automated wide-field, time-gated, optically sectioning, fluorescence lifetime imaging multiwell plate reader for high-content analysis of protein-protein interactions

S. Kumar, Imperial College London (United Kingdom); D. R. Alibhai, Imperial College London (United Kingdom) and Pfizer Group Ltd. (United Kingdom); C. B. Talbot, J. A. Mcginty, I. H. Munro, Y. Alexandrov, A. Margineanu, Imperial College London (United Kingdom); T. Murray, F. Stuhmeier, Pfizer Group Ltd. (United Kingdom); C. W. Dunsby, M. A. Neil, P. M. W. French, Imperial College London (United Kingdom)

We describe the implementation of optically-sectioned fluorescence lifetime imaging (FLIM) in a multiwell plate reader (IN Cell 1000, GE Healthcare) and its application to high content analysis including Forster Resonant Energy Transfer (FRET). Incorporating wide-field time-gated FLIM with a gated optical intensifier and ultrafast tunable supercontinuum excitation source, and using a Nipkow spinning disc to realise optical sectioning, this automated plate reader acquires sectioned FLIM images in <10 s/well, requiring only <11 minutes to read a 96 well plate. This instrument has been applied to study the formation of immature HIV virus like particles (VLPs) in live cells by monitoring Gag-Gag protein interactions in the late stage HIV-1 lifecycle using FLIM FRET. HIV-1 Gag is the major structural protein within HIV-1 virions and expression of HIV-1 Gag alone within live cells leads to the formation of VLPs. We tagged HIV-1 Gag with either Cyan Fluorescent Protein (CFP) or Yellow Fluorescent Protein (YFP) at its C-terminus. Co-transfection of Gag-CFP and Gag-YFP results in the production of VLPs that undergo FRET from Gag-CFP to Gag-YFP due to the close packing of Gag proteins within newly formed VLPs. Our FLIM analysis includes automatic image segmentation using an in-house algorithm, which permits lifetime fitting in regions of interest. Specific analysis of the cell membranes, where VLPs form, increases the FLIM FRET contrast and enhances this readout of protein clustering. This exemplar assay highlights the potential of automated multiwell plate FLIM to study signalling pathways for drug discovery and basic research.

7904-62, Poster Session

Spectral characterization of a volume holographic imaging system

E. E. de Leon, J. Brownlee, College of Optical Sciences, The Univ. of Arizona (United States); J. M. Castro, R. K. Kostuk, The Univ. of Arizona (United States)

Volume holographic imaging systems are being developed for use in multidimensional imaging of tissue samples. Volume holographic imaging systems show promise as an emerging technology which will allow real-time 3-dimensional imaging of a volume object. We present the optical characterization of a novel volume holographic imaging system (VHIS) under development for future clinical trials in the detection of ovarian cancer. The volume holographic imaging system is a spectral-spatial optical device which eliminates the need for mechanical scanning. The volume holographic imaging system maps the spatial-spectral four-dimensional data set to a two-dimensional image array, allowing simultaneous imaging of multiple projections of the spatial and spectral content from different depths within biological tissue samples. The volume holographic imaging system uses dispersion to increase the lateral field of view. This results in performance limitations unique to volume holographic imaging systems. In this paper we review the principle of operation of the volume holographic imaging system and aberrations due to the dispersive nature of a volume hologram. We report our experimental results of spectral performance present in a volume holographic imaging system.
7904-01, Session 1

**Lensless holographic microscope with high-resolving power and no distortion**

K. Sato, Univ. of Hyogo (Japan)

Conventional optical microscopes have certain limitations. High resolution conventional microscopes are costly and the resolution of such microscopes is limited by the physical imperfections inherent in lenses. Conventional microscope takes only two dimensional images. The focusing time required by conventional microscopes limits how rapidly successive images of a specimen may be taken. Holography offers solutions to some of the inherent problems with conventional microscopy. In this work, a lens-less holographic microscope is developed for recording and reconstructing a microscopic high-resolution 3-D image with no distortion. No imaging lens is used in our optical system. A large off-axis hologram is recorded to get maximum resolution by adopting a point light reference source, and a complex-amplitude in-line hologram is obtained from the off-axis hologram by applying one-shot digital holography. A new method of fast and accurate numerical reconstruction is developed. This is achieved by using exact solutions of the Helmholtz equation to express the object beam. This is followed by an image interpolation scheme to generate an equidistant intensity distribution to witch FFT methods are applied. This is essentially an exact solution of a wave equation. A small complex-amplitude in-line hologram is generated for numerically reconstructing a high-resolution image by dividing the large hologram into a number of small holograms and by superimposing them. A focus-free no-distortion image with a high resolving power is reconstructed from the generated small hologram in real time by using Fast-Fourier-Transform. Resolutions less than 1μm are obtained in the optical experiment, which can be improved up to the wave length.

7904-02, Session 1

**In-line digital holographic microscopy based on intensity measurements at two planes**

B. Das, C. S. Yelleswarapu, D. V. Rao, Univ. of Massachusetts Boston (United States)

A large number of biological processes such as cell membrane fluctuations, cell swelling, neuronal activity, and cytoskeletal dynamics occur at shorter time scales. Visualizing these fast dynamical processes requires a microscopy technique that not only can achieve high acquisition rate but also facilitates quantitative phase and/or three-dimensional information. Digital holographic microscopy (DHM) is becoming increasingly popular due to its ability to provide simultaneous amplitude and quantitative phase information of biological specimens [1, 2]. It is a non-destructive, full-field, and label-free imaging technique. Several methods have been developed to reconstruct the desired positive-order (object wavefront) while suppressing the undesired dc and negative-order diffracted waves. Of them off-axis DHM and phase-shifting DHM (PS-DHM) are widely employed [1-3]. However, both methods have their own drawback. While the off-axis DHM does not make efficient use of whole area of the detector, PS-DHM requires at least three sequential holograms. We present a novel in-line DHM technique which enables simultaneous acquisition of two interferograms in order to increase the acquisition rate [4, 5]. Experimental results of both amplitude and phase objects demonstrate the feasibility of this method. The technique utilizes full spatial bandwidth of the camera and requires only two interferograms recorded at two different planes, which can be recorded simultaneously using two sensor arrays. Thus the hologram acquisition time can be significantly shortened. This increased acquisition rate together with the improved reconstruction capability of the current technique may find applications in biomedical research enabling visualization of rapid dynamic processes at the cellular level.

References


7904-03, Session 1

**Simplified setup for imaging with digital holographic microscopy and enhanced quantitative phase contrast by osmotic stimulation of living cells**

B. Kemper, S. Przibilla, C. E. Rommel, A. Vollmer, S. Kethlhus, J. Schnekenburger, G. von Bally, Westfälische Wilhelms-Univ. Münster (Germany)

Interferometry-based quantitative phase contrast imaging techniques enable high-resolution inspection of reflective surfaces and minimally invasive live cell analysis. However, a drawback of many experimental arrangements is the requirement for a separate reference wave which results in a phase stability decrease and the demand for a precise adjustment of the intensity ratio between object and reference wave. In order to avoid a separately generated reference wave a shearographic digital holographic microscopy approach is presented which only requires a single object illumination wave. In this way, a simplified experimental setup is achieved. First results obtained from time-lapse live cell investigations demonstrate that the proposed method is suitable for quantitative phase contrast imaging.

The visibility of subcellular structures in quantitative phase contrast images of living cells depends on the spatial variation of the intracellular refractive index which is often low. Thus, in further experiments it was analyzed if the intracellular refractive index distribution in living cells can be improved by an adequately chosen osmotic stimulation. Experimental data obtained from investigations from human pancreas tumor cells show that the choice of suitable buffer solutions provides live cell imaging with enhanced phase contrast.

7904-04, Session 1

**Whole-cell-imaging based on wide-field interferometric phase microscopy and its application to cardiomyocytes**

N. T. Shaked, L. L. Satterwhite, N. Bursac, A. Wax, Duke Univ. (United States)

Whole-cell-imaging is a novel technique by which the time-dependent quantitative phase profiles of live unstained biological cells are analyzed numerically to learn on the cell functionality. According to this approach, dynamic phase profiles of the sample are acquired by wide-field digital interferometry, a quantitative holographic approach, without the need for scanning or using exogenous contrast agents. The resulting phase profiles are proportional to the multiplication between the geometrical height profile of the cell and the difference between the integral refractive index profile of the cell and the refractive index of the cell surrounding. Nevertheless, many morphological parameters, which are useful for cell biologists, including cell volume and cell force distribution, are based on the cell geometrical height profile, rather than on its phase profile. Numerous efforts have been performed to decouple geometrical height from refractive index difference using the cell phase profile. However, these approaches typically require more than a single exposure, with the risk of losing transient acquisition, or alternatively, assume homogenous refractive-index in the cell cytoplasm. We show that the phase profiles are useful for numerically analyzing biological cells even in cases where decoupling of geometric height and refractive index is not possible or desired. To obtain this goal, we define new numerical phase-profile-
Spatial-spectral volume holographic imaging systems (VHIS) have been developed and applied to the multidimensional imaging of tissue samples. Enhancement of holographic elements allows for spectral information about a sample to be acquired simultaneously. Critical to the performance of these systems is the optimization of a multiplexed holographic optical element that acts as a Bragg filter, selecting for the desired positions within a volumetric object. Moreover, the dispersive qualities of the hologram allow for spectral information about a sample to be collected in transmission, reflectance or fluorescent imaging modes. Unlike competing techniques such as OCT, no scanning is required to acquire 3D imagery. Enhancement of the hologram was achieved by optimizing the composition of the 9,10-phenanthrenequinone-doped poly(methyl methacrylate) ("PQ-PMMA") as well the choice of recording wavelength, geometry and exposure times. These improved holographic elements yield greater efficiency and angular selectivity, resulting in higher spatial resolution and greater potential depth penetration when used in a VHIS for diagnostic tissue imaging. In this work we describe the techniques used to improve the performance of the holograms in these systems, experimental validation of greater effective thickness, and results of enhanced image quality using the NIH-OVCA3 cell line.

Full 3D tomography of biological cells by DHM

C. D. Depeursinge, I. Bergöönd, C. Arfire, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

A multi-hologram procedure has been developed to sample the Ewald sphere in the 3D reciprocal space according to improved strategies. Transmission phase images with high phase accuracies (related to OPL) are numerically reconstructed from holograms acquired for different orientations of the illumination beam. Reconstructing the recorded collection of holograms for extraction of true 3D information achieves full 3D reconstruction. We present new results on biological objects: living cells in particular have been imaged in full 3D. The 3D refractive index spatial distribution is achievable with precisions as high as 0.01 for the refractive index estimation and a spatial resolution below one micrometer. Digital Holographic Microscopy (DHM) provides quantitative measurement of the optical path lengths (OPL). Although diffraction-limited in the transverse direction, the axial resolution has been shown to be scaled down to a few hundred of nanometers also[1]. A significant advantage of DHM for complex diffracted wave retrieval is that only a single hologram is needed for each orientation of the specimen. Some studies have established the theoretical basis of reconstructing the 3D distribution of the scattering potential of weakly scattering objects, by recording the waves scattered from the different directions of parallel illumination. An experimental setup has been developed by us, which uses moving illumination beam together with a rotating specimens, as well as variable wavelengths. Full 3D tomography have been achieved. It is shown that standard optical diffraction tomography (ODT) techniques can be efficiently applied to reveal internal structures of cells and to measure 3D RI spatial distributions.


Enhancement of holographic elements in PQ-PMMA for spatial-spectral volume holographic tissue imaging systems

J. Brownlee, College of Optical Sciences, The Univ. of Arizona (United States); J. M. Castro, The Univ. of Arizona (United States); E. de Leon, College of Optical Sciences, The Univ. of Arizona (United States); R. K. Kostuk, The Univ. of Arizona (United States)

Spatial-spectral volume holographic imaging systems ("VHIS’s") have been developed and applied to the multidimensional imaging of tissue samples. These 3D microscopy systems allow images at several depths and wavelengths to be acquired simultaneously. Critical to the performance of these systems is the optimization of a multiplexed holographic optical element that acts as a Bragg filter, selecting for the desired positions within a volumetric object. Moreover the dispersive qualities of the hologram allow for spectral information about a sample to be collected in transmission, reflectance or fluorescent imaging modes. Unlike competing techniques such as OCT, no scanning is required to acquire 3D imagery. Enhancement of the hologram was achieved by optimizing the composition of the 9,10-phenanthrenequinone-doped poly(methyl methacrylate) ("PQ-PMMA") as well the choice of recording wavelength, geometry and exposure times. These improved holographic elements yield greater efficiency and angular selectivity, resulting in higher spatial resolution and greater potential depth penetration when used in a VHIS for diagnostic tissue imaging. In this work we describe the techniques used to improve the performance of the holograms in these systems, experimental validation of greater effective thickness, and results of enhanced image quality using the NIH-OVCA3 cell line.

Development of a digital holographic microscopy system integrated with atomic force microscope

N. Cardenas, N. D. Ingle, S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Though Atomic Force Microscope (AFM) imaging provides high resolution topographical information, digital holographic microscopy (DHM) yields quantitative phase properties of the sample, and therefore sensitive to changes in refractive index along with physical thickness. DHM in off axis configuration was implemented to encode the phase and intensity in a single intensity record. This allowed recording of the hologram in video rate which could be numerically analyzed to reveal surface of the sample with axial resolution at the nanometer scale as well as intensity maps with resolution similar to conventional optical microscope. Co-registration of AFM image provided higher transverse resolution at nanometer scale. However, AFM imaging required orders of magnitude higher recording time for examination of similar sample area. Due to the inherent nature and recent configuration of the Nanonics AFM optical fiber based cantilever, very minute interference was observed during simultaneous AFM and DHM recording. Integration of both the
systems led to realization of a powerful platform for nanoscale imaging. The integrated AFM-DHM system was built on an inverted fluorescence Nikon microscope and employed to analyze two-photon polymerized microstructures and red blood cells. We will present the design and development of the integrated system and its imaging capabilities.

7904-09, Session 2

**3D optical force trapping calibration and optical micromanipulation using 915-nm diode-laser bar**

M. C. Potcoava, Univ. of Colorado (United States) and Colorado School of Mines (United States) and JILA (United States) and National Institute of Standards and Technology (United States); L. G. Krzewina, Univ. of South Florida (United States); E. E. Hoover, Colorado School of Mines (United States); M. K. Kim, Univ. of South Florida (United States); J. A. Squier, D. W. Marr, Colorado School of Mines (United States); R. Jimenez, JILA (United States) and National Institute of Standards and Technology (United States) and Univ. of Colorado (United States)

It has recently been demonstrated that diode laser bars can be used to not only optically trap red blood cells in flowing microfluidic systems but also, stretch, bend, and rotate them. To predict the complex cell behavior at different locations along a linear trap, 3D optical force characterization is required. The driving force for cells or colloidal particles within an optical trap is the thermal Brownian force where particle fluctuations can be considered a stochastic process. For optical force quantification, we combine diode laser bar optical trapping with Gabor digital holography imaging to perform subpixel resolution measurements of micron-sized particles positions along the laser bar. Here, diffraction patterns produced by trapped particles illuminated by a He-Ne laser are recorded with a CCD at 1000 fps where particle beam position reconstruction is performed using the angular spectrum method and centroid position detection. 3D optical forces are then calculated by three calibration methods: the equipartition theorem, Boltzmann distribution, and power spectral density for each particle in the trap at various laser diode currents. This simple approach for 3D tracking and optical control can be implemented on any transmission microscope, by adding a laser beam as the illumination source instead of a white light source.

7904-10, Session 3

**Adaptive selective plane illumination microscope with image synchronization**

J. M. Taylor, C. D. Saunter, Durham Univ. (United Kingdom); B. Chaudhry, D. J. Henderson, Newcastle Univ. (United Kingdom); J. M. Girkin, G. D. Love, Durham Univ. (United Kingdom)

We describe work on producing a selective plane illumination microscope for cardiac imaging in zebrafish embryos. The system has a novel synchronization system for imaging oscillating structures (e.g. the heart) and will have adaptive optics for image optimization.

There is significant interest and progress on using adaptive optics in microscopy in general. There are a number of specific advantages of using adaptive optics with selective plane illumination microscopy - which we outline here.

7904-11, Session 3

**High-speed focal modulation microscopy using acousto-optical modulators**

S. P. Chong, N. Chen, C. Wong, C. J. R. Sheppard, K. F. Wong, National Univ. of Singapore (Singapore)

In recent development of fluorescence microscopy, the out-of-focus fluorescence background that arises when imaging deep inside biological tissues is critical in determining the achievable penetration depth. Focal Modulation Microscopy (FMM) is an emerging single-photon excitation fluorescence microscopy technique that can provide sub-micron spatial resolution imaging of biological tissues at large penetration depths mainly by preserving the signal-to-background ratio. In FMM, two background suppression schemes (focal modulation and confocal pinhole) are combined effectively to achieve a much-improved imaging depth than Confocal Microscopy. An intensity modulation at focal point is being induced by interference of two periodically phase modulated (or frequency-shifted) excitation beams, which are spatially separated except when brought to the focal point by the objective lens. Ballistic photons contribute mainly to the oscillatory excitation confined exclusively at the focal point as they have well defined phase and polarization compared to scattered photons, though both of them could reach the focal point. The fluorescence emission signal from the sample is modulated at the same frequency as excitation, which then collected by the pinhole detector and demodulated by lock-in technique in order to remove the fluorescence signal excited by diffusive photons. Real-time imaging can be achieved with the implementation of FMM using acousto-optic modulator (AOM) to improve the modulation speed. We also discuss the issues of inherent Poisson statistical noise that gradually becoming significant in the case of low intensity signal related to deep imaging of biological tissues and further improvement of the image quality with the inclusion of Poisson noise removal.

7904-12, Session 3

**Simulating structured-illumination microscopy imaging in the presence of spherical aberrations**

A. Mukherjee, C. Preza, The Univ. of Memphis (United States)

We have created a simulated environment to study the effect of spherical aberrations in the context of structured illumination microscopy (SIM). The optical sectioning ability in three-dimensional (3D) microscopy is greatly enhanced by SIM. In this paper, we investigate the performance of the SIM technique in a simulated and real world imaging system as a function of the thickness of the specimen. For the simulations, we have implemented a depth-variant imaging stratum-based model for SIM using multiple depth-variant PSFs. Our 2-D preliminary results show that the SIM result is less accurate in the presence of spherical aberrations. Different levels of spherical aberration were modeled by making the test object thicker while keeping the refractive index (RI) mismatch fixed and similar to the RI mismatch expected in many live cell specimen. In our study we quantify the resolution in the SIM images as a function of increasing spherical aberrations. In our simulations we have also included noise to study simulated SIM results that compare better with the real measured SIM images. The noise was added to the grid raw images and the standard SIM result was computed using a subtraction algorithm as in the case of the noiseless simulations. Our results show that even a small amount of noise gives rise to a very low SNR in the final SIM result demonstrating the sensitivity of the SIM system to noise.

7904-13, Session 3

**Frequency-domain spatially modulated single detector imaging**

R. A. Bartels, G. Futia, Colorado State Univ. (United States)

Optical image capture is commonly performed by recording the intensity of an image formed by an optical imaging system. The most common imaging techniques use either segmented detectors or time domain scanning. Time domain scanning techniques, such as scanning confocal microscopy and laser scanning microscopy, build up images by illuminating a small volume of the sample object, recording an optical
intensity, then shift to a new sample volume. While many methods have been developed for improving the speed of microscopy techniques, the speed is limited by the read out rate of the segmented detector, or alternatively the scan rate of scanning methods.

We have recently developed a microscopy technique that makes use of a single element detector to form optical images in the frequency domain. Applying a time varying spatial modulation across the illumination beam maps the spatial information of both beam and sample into the frequency domain of the detector. The modulated beam and object are imaged onto the area of a single photodiode, where the time-sampled data can be directly transformed to acquire the spatial information. We demonstrate the use of the technique to capture images of absorbing objects. Theory for this imaging modality has been developed and will be discussed to explain the performance and trade offs in terms of imaging rates and spatial resolution.

7904-14, Session 3

Single-plane illumination Raman imaging of biological samples

I. Barman, Massachusetts Institute of Technology (United States); K. E. Tan, Univ. of St. Andrews (United Kingdom); N. C. Dingari, Massachusetts Institute of Technology (United States); G. P. Singh, Univ. of St. Andrews (United Kingdom)

Characterization of the structure and distribution of components in heterogeneous systems impacts key applications in the areas of nanotechnology, material science, catalysis, and bio-sciences. Specifically, modern biological problems often necessitate multidimensional imaging as well as bio-chemical characterization of live cells and tissue samples. To this end, Raman spectroscopy has shown substantial promise due to its ability to provide spatially resolved molecular information in a non-invasive manner, without the use of additional contrast agents such as dyes or molecular probes. Additionally, confocal Raman microscopy has been pursued to obtain optically sectioned images of chemical and morphological composition. However, confocal systems suffer from poor axial resolution (as compared to lateral resolution), photodamage due to uniform sample illumination, long acquisition time and limited penetration depth in heterogeneous samples. Recently, investigators have developed light sheet based selective plane illumination microscopy to generate multidimensional images of a few millimeter thick samples. Incorporating this approach into a Raman spectroscopic framework, we provide first results of Raman images where the optical sectioning effect is achieved by exciting the sample along a separate optical path orthogonal to the detection axis. In this talk, we focus on the rapid imaging of micro-calculations embedded in biological tissue (in less than 1/30th the time required for confocal imaging). Furthermore, the proposed approach is sufficiently broad and general to potentially address other major disease classes including atherosclerotic plaques and malignancies in mucosal tissues. The present result can also be directly utilized in compositional analysis of thin films and pharmaceutical tablets.

7904-15, Session 4

Positioning systems for high-resolution tissue imaging

T. M. Haylock, A. T. Cenko, Univ. of Waterloo (Canada); P. B. Christensen, Tornado Medical Systems (Canada); J. T. Meade, F. Kazemzadeh, L. M. Chifman, A. R. Hajan, Univ. of Waterloo (Canada); J. Hendriksen, Tornado Medical Systems (Canada)

Tissue handling systems position ex vivo samples to a required accuracy that depends on the features to be imaged. For example, to resolve cellular structure, micron pixel spacing is needed. 3D tissue scanning at cellular resolution allows for more complete histology to be obtained and more accurate diagnosis to be made. However, accurate positioning of a light beam on the sample is a significant challenge, especially when fine spacing between scan steps is desired or large, inconsistently shaped samples need to be imaged. Optical coherence tomography (OCT) is an application where accurate positioning systems are required to reap the full benefit of the technology. By simultaneously manipulating the light beam position and sample location, a 3D image is reconstructed from a series of depth profiles produced. To automate image acquisition, a fully integrated and synchronised system is necessary. A tissue handling and light delivery system for free-space optical devices will be presented. Performance characteristics such as resolution, uncertainty, and repeatability are evaluated for novel hardware configurations of OCT. Typical scanning patterns with associated synchronisation requirements are discussed.

7904-16, Session 4

Computational model of optical scattering by elastin in lung

T. B. Swedish, J. P. Robinson, D. R. Kaeli, C. A. DiMarzio, Northeastern Univ. (United States)

Little is understood about the detailed microstructure of lung in vivo. Attempts to improve imaging are hampered by heterogeneity of the tissue. One common ex vivo technique is Optical Coherence Tomography (OCT). Simulated OCT with a Finite-Difference Time-Domain (FDTD) computer model elucidates the relationship between captured images and the physical geometry of the lung. Parallel computation and improved processing power make accurate coherent imaging models feasible. A previous FDTD model of pulsed laser wave propagation in the lung produced images which displayed many of the properties of experimental images. The model was improved with the addition of elastin and increased computational volume. Elastin plays an important role in the simulation because the combination of its fibrous structure and high index of refraction acts as an excellent scatterer of light. This strong scattering increases the signal reported by the simulated OCT scan in areas where elastin is most abundant, improving visualization of the structure as more light is reflected back from the heterogeneous elastin network. However, scattering by elastin decreases the depth of penetration and leads to images that are more difficult to interpret. Gaining a better understanding of how lung structures affect light propagation will lead to improved signal processing, instrumentation, and the development of new probing techniques. This image modeling technique can also be applied to other imaging modalities such as confocal and other laser scanning methods.

7904-17, Session 4

Mirror-assisted dark-field optical coherence microscopy

M. L. Villiger, A. Bouwens, C. Pache, T. Lasser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Interferometric microscopy techniques are common to image near transparent cell samples. Many of these methods work in the transmission mode treating the cell as a pure phase object and measure the optical path difference induced by the sample. The microscope acts as a low-pass filter along the axial spatial frequencies in this case. In an epi-configuration on the other hand it acts as high pass-filter and captures the signal backscattered by more abrupt variations of the refractive index within the cell. We have recently introduced dark-field Optical Coherence Microscope, which uses decoupled detection and illumination paths to suppress specular reflection and achieve improved backscattering contrast. Together with the high sensitivity of the interferometric detection in the Fourier domain, this allows detecting the little light backscattered by the cell sample. Here, we extend this modality by placing a mirror behind the sample to create a well-defined specular reflection, which contains transmission information. Instead of blocking this reflection signal in the detection path, it is delayed by a glass mask allowing separation of the simultaneously recorded backscatter
and reflection signals. With a careful analysis of the coherency transfer function of each wavenumber channel for both the transmission and the reflection paths, the cell tomogram can be reconstructed by combining this complementary information. This architecture gives access to an increased set of spatial frequencies and helps to resolve the ambiguity between index fluctuations and physical displacements inside the cell.

7904-18, Session 4

Real-time dual-mode standard/complex Fourier-domain OCT system using graphics processing unit accelerated 4D signal processing and visualization

K. Zhang, J. U. Kang, The Johns Hopkins Univ. (United States)

The acquisition line (A-scan) speed of Fourier-domain optical coherence tomography (FD-OCT) has been advancing rapidly to >100,000 line/s level in the last few years. However, parallel efforts have not been embarked on data processing and visualization at the matching speed of the acquisition. In this work, we realized a real-time dual-mode standard/complex FD-OCT system using graphics processing unit (GPU) accelerated real-time 4D (3D+time) signal processing and visualization.

For both standard and complex FD-OCT modes, the signal processings were implemented on a GPU (NVIDIA GTX 480), including -to-k spectral re-sampling, fast Fourier transform (FFT), modified Hilbert transform, and logametric-scaling. The maximum A-scan processing speed achieved is 1,000,000 line/s for the standard 1024-pixel-FD-OCT (1024-OCT), and 300,000 line/s for the complex 1024-OCCT.

A CMOS camera with actual imaging speed of 256,000 line/s for 1024 pixels is used as an acquisition device for the both OCT modes. A phase modulation is applied to each B-scan’s 2D interferogram frame by adding a third galvanometer to the reference arm to realize complex OCT mode. For the both OCT modes, 3D Data sets are continuously acquired in real time, online processed and visualized as high as 10 volumes/second (25,000 A-scans/volume) by either end face slice extraction or ray-casting based volume rendering from 3D texture mapped in graphics memory.

The GPU-acceleration technique is highly cost-effective and can be easily integrated into most ultrahigh speed FD-OCT systems to overcome the 3D data processing and visualization bottlenecks.

7904-19, Session 4

Fluorescence lifetime optical projection tomography

J. A. McGinty, Imperial College London (United Kingdom); D. W. Stuckey, Imperial College Healthcare NHS Trust (United Kingdom); G. Sun, H. B. Taylor, G. A. Rutter, Imperial College London (United Kingdom); A. Sardinia, Imperial College Healthcare NHS Trust (United Kingdom); J. R. Lamb, Imperial College London (United Kingdom); G. W. Stamp, The Royal Marsden NHS Foundation Trust (United Kingdom); M. J. Dallman, P. M. W. French, Imperial College London (United Kingdom)

Optical Projection Tomography (OPT) is a relatively rapid and robust technique for measuring the three-dimensional (3-D) distribution of fluorescence and/or absorption in transparent (i.e. non-scattering) samples that utilise wide-field illumination and detection. We have previously extended the intensity-based OPT technique to 3-D fluorescence lifetime tomography (tomoFLIM), noting that fluorescence lifetime measurements are more robust to intensity-based artefacts (e.g. concentration, uneven illumination/detection efficiency, etc). In OPT a series of wide-field images of a “transparent” sample is acquired as the sample rotates. Each row of pixels can be treated as a projection through a slice of the sample and therefore back-projected to reconstruct the 2-D intensity distribution in an analogous way to X-ray computed tomography. In tomoFLIM the acquisition is extended to acquire a series of time-gated fluorescence intensity images at each angular position, resulting in time-gated 3-D reconstructions at different relative time delays. An appropriate function describing the fluorescence decay profile can then be attributed to each voxel producing a 3-D fluorescence lifetime reconstruction. Here we present a detailed characterisation of an intensity-based and time-resolved OPT system that permitted optimisation of the acquisition procedure in terms of spatial resolution, signal-to-noise and total acquisition time. This system has been applied to cm scale volumetric imaging of chick embryos, mouse pancreas (to measure the islet volume in excised murine pancreata) and human tissue resections (for 3-D histology without the need for serial sectioning). We have also developed a higher magnification system implementing modulation transfer function-dependent filters for imaging zebrafish expressing fluorescent protein labels.

7904-20, Session 4

High-resolution optical projection tomographic microscopy for 3D tissue imaging

Q. Miao, Univ. of Washington (United States); J. W. Hayenga, M. G. Meyer, T. Neumann, F. W. Patten, A. C. Nelson, VisionGate, Inc. (United States); E. J. Seibel, Univ. of Washington (United States)

Optical projection tomography (OPT) requires a large depth of field (DOF) of a low numerical aperture (NA) lens resulting in low resolution of the image. However, DOF of a high NA objective can be extended by scanning the focal plane through the sample. This extended DOF image is called pseudoprojection, which is used by optical projection tomographic microscope (OPTM) for tomographic reconstruction. The advantage of OPTM is the acquisition of relatively high resolution and large depth of field concurrently. In OPTM, pseudoprojections from different perspectives are taken by rotating the sample in a microcapillary rotation stage. Multicellular samples are fixed, stained, and mixed with optical gel. By applying pressure, the multicellular sample is flowed within the capillary tube for imaging. The optical gel, capillary, and surrounding medium have the same refractive index, thus minimizing the optical distortion caused by the curvature of the capillary. In this paper, we image clusters of multicellular spheroids stained with hematoxylin inside a capillary tube having an inner diameter of 320um. We use 10X objective lens (NA 0.3) to obtain isometric resolution of 1.1um. In contrast, for conventional OPT lens of NA about 0.04 is needed for DOF to cover the 320um sample, so the resolution would be 8 um. OPTM can be used for 3D histological analysis of H&E stained biopsy specimen with sub-cellular resolution. Tissue can be cleared by clearing agents, so distortion will be minimized when focus goes deep into tissue.

7904-94, Session 4

High-resolution optical projection tomographic microscopy for 3D tissue imaging

S. Yan, C. Preza, The Univ. of Memphis (United States)

In 3D wide-field computational microscopy, image estimation methods have played an important role. The accuracy of the forward model has a significant impact on the complexity of the estimation method and consequently on the accuracy of the estimated intensity. Previous studies have shown that a forward model based on a depth-varying point-spread function (DV-PSF) leads to a substantial improvement in the resulting images [1, 2]. In this depth-varying model, the depth-dependent imaging effects are handled using a strata-based interpolation method defined on discrete, non-overlapping layers or strata along the Z axis. Recently, a new approximation method based on principle component analysis...
(PCA) was developed to predict depth-varying PSFs [3]. It has been reported that DV-PSFs developed by the new method have improved accuracy over the DV-PSFs predicted by the strata interpolation method of [1] and that this method can eliminate noise in measured PSFs. In this study, we developed an image estimation method that uses DV-PSFs predicted by the PCA model. The performance of the method was evaluated and compared to the depth-variant expectation maximization (DV-EM) algorithm [1] that uses the strata interpolation method. DV-PSFs were computed using both the strata-based approximation scheme and the new PCA-based approximation scheme and were used in 3D image estimation. Results obtained from both simulated objects and fluorescence bead experiments are presented and compared.

References:

7904-21, Session 5

Mueller matrix microscopy

M. Mujat, N. V. Ittimia, R. D. Ferguson, D. X. Hammer, Physical Sciences Inc. (United States)

Polarization is a fundamental property of light and its measurement is one of the effective means of investigating the light-matter interaction. Polarization imaging provides additional contrast mechanisms as compared to traditional intensity imaging, without having to stain or label the sample. The most complete spatial polarimetric characterization of biological tissue is provided by Mueller matrix imaging. In addition to the classical intensity image, the Mueller matrix contains information on the refractive index in biological samples. We describe here a new imaging technique, Mueller matrix microscopy, for investigating the anisotropic properties of the refractive index in biological samples. The system's capabilities are demonstrated first on mica, quartz and biological samples. Current polarization microscopes are working in transmission through transparent samples and in general measuring only in orthogonal polarization channel or doing partial polarization measurements. We have developed a Mueller matrix microscope capable of performing complete Mueller matrix imaging in both transmission and reflection configuration and at different wavelengths. Polarimetric microscopy can provide unprecedented details in biophysical measurement of cell functions, in analyzing the effects of electric or magnetic fields, photoactivation, testing of drugs or biocompatible polymers on live tissue, or in longitudinal studies on interacting cellular structures during cell division, motility and apoptosis.

7904-22, Session 5

Dynamic phase imaging utilizing a 4-dimensional microscope system

K. Creath, 4D Technology Corp. (United States) and Optineering (United States) and The Univ. of Arizona (United States)

This paper describes a new, novel interference Linnik microscope system and presents images and data of live biological samples. The specially designed optical system enables instantaneous 4-dimensional video measurements of dynamic motions within and among live cells without the need for contrast agents. This “label-free”, vibration insensitive imaging system enables measurement of biological objects in reflection using harmless light levels with a variety of magnifications and wavelengths with fields of view from several hundred microns up to a millimeter. At the core of the instrument is a phase measurement camera (PMC) enabling simultaneous measurement of multiple interference patterns utilizing a pixeled phase mask taking advantage of the polarization properties of light. Utilizing this technology enables the creation of phase image movies in real time at video rates so that dynamic motions and volumetric changes can be tracked. Objects are placed on a reflective surface in liquid under a coverslip. Phase values are converted to optical thickness data enabling volumetric, motion and morphological studies. Data from a number of different organisms such as paramecium, flagellates and rotifers will be presented, as well measurements of human breast cancer cells with the addition of various agents that break down the cells. These data highlight examples of monitoring different biological processes and motions. The live presentation features 4D phase movies of these examples.

7904-23, Session 5

Quadriwave lateral shearing interferometry for quantitative phase microscopy: correlation with fluorescence measurements

P. Bon, PHASICS S.A. (France); J. Savatier, Institut Fresnel (France); B. F. Wattellier, PHASICS S.A. (France); D. D. Marguet, Ctr. National de la Recherche Scientifique (France); S. Monneret, Institut Fresnel (France)

Phase visualization of biological samples requires a specific setup as Zernike phase contrast or Nomarski-DIC equipments. Those techniques implicitly use the fact that light passing through a sample accumulates phase shift. Accessing to the actual value of this phase shift is very difficult, explaining that such techniques are commonly used as contrast enhancer only. We describe here the use of quadri-wave lateral shearing interferometry (QWLSI) [1] for wavefront sensing, in order to measure quantitatively the local phase shift within a sample. We use a SiD4Bio wavefront sensor (Phasics) and we get a 300x400 sampling points on the sample, with both phase and intensity information. The method can be considered as “plug and play” when used with a conventional microscope: its native bright-field illumination system is used as the light source, and the wavefront sensor has only to be mounted on one of its video port. Correlation between images obtained either with phase or fluorescence is considered. The phase visualization of intracellular elements is clearly possible with a high contrast, and targeted fluorescence allows a precise identification of each component. A multidimensional image is then obtained by combining those two images.


7904-24, Session 5

Quantitative measurement of phase using an unmodified differential interference contrast microscope

D. D. Duncan, Portland State Univ. (United States); D. G. Fischer, NASA Glenn Research Ctr. (United States); A. L. Dayton, S. A. Prah, Providence St. Vincent Medical Ctr. (United States)

Differential contrast microscopy is a commonly used method of acquiring qualitative information. Here we demonstrate a simple procedure for
deriving quantitative information from an unmodified DIC microscope. The method is based on a calibration step that establishes the amount of image shear and the phase increment per revolution of the bias screw on the second Wollaston prism. Subsequent to the calibration step, we demonstrate a phase stepping procedure based on the Carrè four-step algorithm that yields quantitative information on the phase gradient. Together with the estimate of shear derived from the calibration, this phase gradient can be interpreted directly in terms of a ray deviation. By this procedure a characterization of the object in terms of first and second order statistics of the scatter direction (polar and azimuthal) is possible.

We discuss the calibration process using an optical wedge commonly used to produce a known ray deviation, the phase stepping procedure, the interpretation of the quantitative phase gradient information, and statistical characterizations in terms of the first and second order statistics. Typical results are shown for a variety of tissue samples.

7904-25, Session 5

Refractive index reconstruction of biological samples from multimodal phase microscopy

H. Sierra, D. H. Brooks, C. A. DiMarzio, Northeastern Univ. (United States)

Extraction of quantitative information is important to better understand cellular activity in biological preparations. In particular the optical refractive index can be used to analyze the results of cellular processes such as the dry mass of biological samples. Phase microscopy modalities are widely used to image unstained biological samples because of their ability to obtain high-contrast images without introducing exogenous agents. The most common phase modalities are predominantly qualitative. However quantitative phase microscopy can provide more specific information about optical thickness and refractive index. In biological samples with several internal inhomogeneities and thickness variations, refractive index calculation becomes challenging to achieve by direct analysis of the images. Here we present a multimodal iterative method to reconstruct the spatial distribution of refractive index, combining information from two phase microscopy techniques. We use a constrained boundary iterative method under the assumption that the index of refraction inside the object can be approximated as piecewise constant. The boundary locations of all inhomogeneities are obtained by leveraging measurements from two phase imaging modalities, and then the index of refraction is estimated based on those boundaries and a quantitative forward model for one modality. Simulations have confirmed the reliability of the proposed method. Experiments with measurement from mouse embryos at several development stages show that the proposed approach can reconstruct the distribution of the refractive index of these samples.

7904-26, Session 6

Beyond the lateral resolution limit by phase imaging

Y. Cotte, C. D. Depeursinge, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

We present a theory stating how to overcome the classical Rayleigh-resolution limit. It is based upon a new resolution criterion in phase of coherent imaging process and its spatial resolution is thought to be only SNR limited. Recently, the experimental observation of systematically occurring phase singularities in coherent imaging of sub-Rayleigh distanced objects has been reported [1]. The phase resolution criterion relies on the unique occurrence of phase singularities. Thus, an in-phase object yields a lateral resolution of 1.64 superior to the Rayleigh-limit. However, by introducing an arbitrary phase difference, the lateral as well as the longitudinal resolution can be tremendously enlarged.

Proposals for experimental realisation are debated and first experimental results are shown. The experimental setup is based on Digital Holographic Microscopy (DHM), an interferometric method providing access to the complex wave front. In off-axis transmission configuration, a sub-wavelength nano-metric hole on metallic films acts as the customized high-resolution test target. The nano-metric apertures are drilled with focused ion beam (FIB) and controlled by scanning electron microscopy (SEM). In this manner, Rayleigh’s classical two-point resolution condition can be rebuilt by interfering complex fields emanated from multiple single circular apertures on an opaque metallic film. By introducing different offset phases, enhanced resolution is demonstrated. Furthermore, the measurements can be exploited analytically or within the post processing of sampling a synthetic complex transfer function (CTF).


7904-27, Session 6

Wide-field reflection phase microscope

Z. Yaqoob, Massachusetts Institute of Technology (United States); T. Yamauchi, Hamamatsu Photonics K.K. (Japan); D. Fu, Massachusetts Institute of Technology (United States); W. Choi, Korea Univ. (Korea, Republic of); R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

Bio-micro rheology studies mechanical properties of live cells. Most of the cells in our body are eukaryotic and have complicated 3-D cytoskeleton. Existing techniques such as transmission phase microscopy probe a combination of membrane as well as bulk properties of cell that are difficult to decouple. In this context, properly designed reflection-based phase microscopy can play vital role to exclusively access the membrane dynamics of eukaryotic cells. Moreover, reflection-based optical methods also promise measurement sensitivity advantage over transmission-based optical techniques. In recent past, we designed and developed a quantitative phase microscope based on spectral domain optical coherence tomography and line-field illumination, which offered major improvements over point illumination techniques such as multi-point phase measurement along the line of illumination and high measurement sensitivity.

Here, we report the design and development of a single-shot wide-field reflection phase microscope based on off-axis interferometry and time-domain optical coherence tomography. Its unique design provides the desired wavefront tilt in the reference beam for off-axis interferometry such that it interferes with the sample beam across the whole field. The single-shot interferograms are processed to determine the optical phase of the returning sample beam and hence sub-nanometer motions associated with the sample under study. We have successfully applied this new instrument to measure membrane fluctuations in HeLa cells. We also note that an appropriate model can relate the measured membrane fluctuations to physical parameters such as bending modulus and tension coefficient of the cell membranes.

7904-28, Session 6

Three-dimensional refractive index measurement and its biological applications

Y. Sung, Massachusetts Institute of Technology (United States); W. Choi, Korea Univ. (Korea, Republic of); R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

Refractive index is an intrinsic property which is proportional to the concentration of biomolecules inside a biological sample. High resolution imaging of refractive index can provide an alternative way to quantitatively monitor a live biological sample.

To measure the refractive index of a biological sample, we have developed a three dimensional diffraction tomography based on off-axis holography and scanning beam geometry. To overcome the missing cone effect, we tried a regularization technique together with positivity...
constraint as a priori knowledge. In this presentation, we will show how additional information such as a boundary constraint can improve the image quality more. Also, we will introduce our recent work to completely remove the missing cone problem, which we call 4-Pi tomography.

By handling the diffraction effect, we show that accurate estimation of dry mass is possible. The accurate measurement of dry mass enables to investigate the growth and regulation of cell cycle without adding any extra agent. By combining our imaging technique with an on-stage cell culture system, we have successfully measured the growth of adherent cells for the entire cell cycle without adding any extra agent. We will show how the cell divide and grow based on the refractive index measurement. We will also present our recent effort to monitor the drug effect on myeloma cells.

7904-29, Session 6

Optical slicing of wide-field microscopy images by integral imaging
M. Martinez-Corral, H. Navarro, Univ. de València (Spain); R. Martinez-Cuenca, Univ. Jaume I (Spain); B. Javidi, Univ. of Connecticut (United States); G. Saavedra, Univ. de València (Spain)

Integral Imaging (InI) is a very promising technique for the acquisition and display of images of 3D scenes. Based on an old concept published by Lippmann more than one century ago, InI produces auto-stereoscopic 3D images which can be observed directly with no need of additional viewing devices such as special goggles.

The Lippmann idea was that one can record in a 2D matrix sensor many elemental images of a 3D scene, so that any elemental image stores the information of different perspectives of the object. When this info is projected onto a display panel placed in front of an array of pinholes, any pixel of the display generates a conical ray-bundle. And it is, precisely, the intersection of ray-bundles which produces the local concentration of light-density that per-mits the reconstruction of the scene. This reconstructed scene is perceived as 3D by the observer, whatever his/her position.

Although InI concept was initially intended for the capture and display of 3D pictures or movies, along the last decade it has been used for other interesting applications. One is the digital reconstruction, by means of back-projection algorithms, of spatially incoherent 3D scenes. The main advantage of this reconstruction procedure is its capacity of producing an impressive blurring of out-of-focus parts of the 3D scene. This capacity can be used in fluorescence microscopy, to obtain high-contrast optical slices of 3D samples by combination of InI concept.

7904-30, Session 6

Autostereoscopic visualization of 3D time-varying complex objects in volumetric image sequences
A. Benassarou, G. Valette, Univ. de Reims Champagne-Ardenne (France); D. G. Debons, 3DTV Solutions (France); Y. Remion, L. Lucas, Univ. de Reims Champagne-Ardenne (France) and 3DTV Solutions (France)

Methods and tools for visualizing biological data have improved considerably over the last decades, but they are still inadequate for some highly dense data sets. For most users, a key challenge is to maximum benefit of data without being overwhelmed by them. To achieve this, we propose to take advantage of true 3D depth perception offered by autostereoscopic displays, and to give more informative visual presentations of biological data, thanks to our method of interactive analysis of 3D time-varying complex hierarchical objects.

In this paper, we describe 4DvizMED, a complete and coherent framework combining a deformable surface model, which automatically tracks volumetric features by adapting its topology, real-time multi-view stereo volume rendering, and some interactive tools for manipulation and quantization. Our method is based on a topological feature tracking process, using a flow-based paradigm and a deformable surface model. It tracks through time the evolution of the components of an isosurface and their interaction with other components. In this paper, we focus on the difficulties of visualizing 4D volume data, and we report the results of preliminary experiments designed to evaluate the utility of autostereoscopic displays for this purpose.

The remainder of the paper is organised as follows. In the first section we outline the specific problem we are trying to solve: the interactive visualization and manipulation of 3D time-varying objects in volumetric image sequences. We briefly introduce our surface tracking and direct volume rendering methods. Next, we address some of the arising problems, and explain how autostereoscopic displays may help to alleviate these. We then describe our results, concluding with a discussion of their significance.

7904-32, Session 7

Fourier-excitation hyperspectral fluorescence lifetime imaging
L. L. Peng, M. Zhao, The Univ. of Arizona (United States)

We report a novel confocal fluorescence lifetime imaging microscope (FLIM) that images lifetimes as a 2D function of excitation wavelengths and emission colors at 22,000 pixels per second. The instrument measures with multiple excitation wavelengths in parallel, which was the biggest bottleneck in hyperspectral fluorescence imaging.

Our instrument uses a Michelson interferometer with a scanning delay line to generate intensity modulations on multi-wavelength excitation light, and measures fluorescence lifetimes with the frequency domain lifetime method. Because the interference frequency is wavelength-dependent, excitation wavelengths are separated in the frequency
domain by differences in modulation frequencies. Therefore, fluorescence emission associated with each excitation wavelength can be separated by Fourier signal analysis. In the Michelson interferometer, the scanning delay line is based on a 24-facet polygon mirror spinning at 55,000 RPM. It generates interference intensity frequencies sweeping continuously from about 150 MHz to 0 MHz then back to 150 MHz within a single facet scan (45.5 microseconds), which allows lifetime measurements at 22,000 points/s. Modulated emission is further separated into multiple emission color channel. As the result, lifetimes are measured as 2D functions of multiple excitation lines and emission channels at 22,000 pixels per second, or 3 second per frame (256×256 pixels) with a confocal scanner. The instrument provides 7-dimensional information (intensity, lifetime, excitation wavelength, emission wavelength, X, Y and Z) at a frame rate suitable for live cell imaging. It opens the door to study complex fluorescent phenomenon such as Förster resonant energy transfer between four or more fluorescent labels.

7904-33, Session 7
Fluorescence optofluidic microscope (OFM) based on zone plate array
S. Pang, L. M. Lee, C. Yang, California Institute of Technology (United States)
The lensless and fully on-chip microscope systems, termed optofluidic microscope (OFM) reported recently, can potentially improve the modern clinical diagnostics and biology research. Due to its sensitivity and specificity, fluorescence is probably the most important readout mode in biological microscopy. Here we present an OFM system design based on zone plate array that is capable to capture high resolution fluorescence image. Similar to the precedent modalities, the fluorescent OFM system is compact, low-cost and suitable for mass production. Each Fresnel zone plate creates a high-resolution focus inside a microfluidic channel, and each focus generates an excitation trace on fluorescence-tagged sample while it flowing through the channel. The array is orientated to a specific angle to the fluidic channel so that the distance between adjacent line traces is less than the dimension of the focus. Thus, neither the zone plate dimension nor the sensor pixel size has impact on the system’s resolution. A filter layer rejects the excitation light. Taking advantage of the dimension, the CMOS sensor integrated with the device effectively collects the emission without using a high numerical aperture objective. The device is capable of high throughput fluorescence imaging applications.

7904-34, Session 7
Time-resolved cuvette system study of calcium sensors: toward obtaining biophysical properties of the construct
R. Laine, H. B. Manning, Imperial College London (United Kingdom); D. W. Stuckey, Imperial College Healthcare NHS Trust (United Kingdom); G. T. Kennedy, M. A. Neil, D. Carling, C. W. Dunsby, P. M. W. French, Imperial College London (United Kingdom); A. Sardini, Imperial College Healthcare NHS Trust (United Kingdom)
We present the study of two FRET ( Förster resonance energy transfer) calcium sensors based on the non-ubiquitous Troponin C protein [1]. We applied an analysis, developed by Visser et al. [2], for evaluating the distance and angular separation between two fluorophores in a eCFP/Citrine-based calcium FRET sensor and a new mTFP/Citrine-based calcium FRET sensor. Our approach directly estimates the fluorophore orientation factor. We applied the TCSPC method to calculate the average lifetime of each fluorophore in a home-built multidimensional fluorometer resolving fluorescence lifetime, spectrum, and polarization. The detection system combines time-correlated single photon counting (TCSPC, Becker & Hickl, SPC 730) for precision lifetime measurements with a scanning monochromator and automated polarizers for spectrally- and polarization-resolved fluorescence emission analysis. The excitation source is a picosecond fiber laser-pumped supercontinuum source that is spectrally filtered to provide the optimum excitation wavelength. Data analysis was performed with discrete exponential models (single, double or triple exponential models) or the full polarized decay model using the TRFA (STTC) software package. Replacing CFP with teal fluorescent protein (mTFP) that exhibits a mono-exponential fluorescence decay, simplifies the data analysis by reducing the complexity of the models and therefore increasing the confidence in the outcome. In order to make samples for such cuvette measurements, we developed a novel protocol for cytosol extraction from mammalian cells. This provides a biologically relevant system in solution phase that can be readily and rapidly made compared to protein purification.

[2] Visser et al. (2008), Biophysical Journal

7904-35, Session 7
High-speed multiple-process imaging using FLIM-FRET for complex and dynamic live-cell studies
R. Laine, A. Margineanu, S. Kumar, G. T. Kennedy, D. M. Grant, J. A. McGinty, C. B. Talbot, D. Carling, C. W. Dunsby, M. A. Neil, Imperial College London (United Kingdom); M. Katan-Muller, The Institute of Cancer Research (United Kingdom); A. Sardini, Imperial College Healthcare NHS Trust (United Kingdom); P. M. W. French, Imperial College London (United Kingdom)
We report the first high-speed optically sectioned fluorescence lifetime imaging (FLIM) microscope capable of multiplexing several FRET (Förster resonance energy transfer) readouts for fixed or live cell studies. Optical sectioning is implemented using a Nipkow confocal spinning disk (Yokogawa, CSU-X) and rapid wide-field time-gated FLIM is achieved using a time-gated optical intensifier system (Kentech Instruments, HR1) with an ultrafast tunable excitation source based on a supercontinuum laser source (Fianium, SC-450-6) or a frequency-doubled mode-locked Ti:Sapphire laser (Spectra-Physics MaiTai). The spectral channel multiplexing, z-stack imaging and acquisition of time-lapse images are fully automated, permitting the spatial and temporal behaviour of several cellular signalling events to be followed in real time. A typical FLIM acquisition reading out FRET in live cells transfected with GFP takes ~1 to 5 s. An initial demonstration of time-lapse imaging of living cells transfected with a FRET calcium sensor (TNL15 [1]) and loaded with a calcium dye (FluoForteTM © 2009 Enzo Life Sciences, Inc.) showed a concomitant increase in calcium level in both channels after stimulation with ionomycin, as evinced by a decrease in lifetime of the TNL15 FRET sensor and an increase in lifetime of the calcium dye (resulting from an increased quantum efficiency). This novel instrument is being applied to readout multiple components of cell signalling pathways in live cells, including kinase cascades and energy level-related studies, for which we aim to combine FRET sensors with other cell signalling and metabolic readouts.


7904-36, Session 8
Myopic deconvolution of adaptive optics retina images
L. Blanco, Observatorio de Paris à Meudon (France); L. Mugnier, ONERA (France); M. Gianc, Observatoire de Paris à Meudon (France)
Adaptive Optics corrected flood imaging of the retina is a well-developed technique. The raw images are usually low contrasted because they are
dominated by an important background, and because AO correction is only partial. Interpretation of such images is difficult without an appropriate post-processing, typically background subtraction and image deconvolution. Deconvolution is difficult because the PSF is not well-known (myopic/blind deconvolution) and because the image contains in-focus and out-of-focus information from the object.

In this communication, we tackle the background removal and the deconvolution. Background is estimated on a single raw image using a parametric model of the background shape. Subtraction has been validated on experimental data from the LESIA setup at 15-20 hospital.

We model the 3D imaging by assuming that the object is approximately the same in all planes within the depth of focus. The 3D model becomes a 2D model with the global PSF being an unknown linear combination of the PSF for each plane. The problem is to estimate the coefficients of this combination and the object. We show that the traditional method of joint estimation fails even for a small number of coefficients. We derive a Marginal estimation of unknown hyperparameters (coefficients, object Power Spectral Density, noise level) followed by a MAP estimation of the object. Such a marginal estimation has better statistical convergence properties, and allows us to obtain an "unsupervised" estimate of the object. Results on simulated data and experimental data from both the LESIA and Imagine Eyes retinal imagers are shown.

7904-37, Session 8

Three-dimensional data acquisition with aberrations correction capability for video-rate microscopy

M. Samim, Univ. of Toronto (Canada); R. Cisek, D. Sandkujl, S. Musikhin, V. Barzda, Univ. of Toronto Mississauga (Canada)

Major developments in microscopy have recently pushed imaging speeds particularly for biological applications into new frontiers. Hardware and software used for diverse detection schemes are rearranged to perform video-rate imaging more optimally and in the cost-effective manners. Multi-contrast, multi-foci nonlinear optical microscopes are the most recent examples in this field. These microscopes are, for example, capable of capturing simultaneous images of orthogonal polarization from two distinct optical sections, in both forward and backward directions. The converging, diverging and/or reference beams, that are reflected off of the surface of the deformable mirrors, and are conjugated with the back-aperture of a microscope objective, can be focused at different axial depth. One such microscope is currently being developed in our lab. The optical wavefronts of a novel Yb:KGW femto-second (1028nm) laser-beams are monitored by the microdens-array Shack-Hartmann wavefront sensor. We have already demonstrated the ability of our 39-actuator deformable mirrors in reshaping the phasefront and correcting for the optical aberrations to achieve the diffraction limited focal volume for deep excitation inside biological tissues. Now, we present data illustrating the ability to focus at distinct axial depths in our newly developed microscope. The three-dimensional video rate scanning capability will enable us to study the rapid dynamics of bio-organisms, such as their blood flow, cardiac activities, and microorganisms’ motility in a three-dimensional volume.

7904-38, Session 8

Wide-field adaptive optics for microscopy

C. Bourgenet, C. D. Saunter, J. M. Girkin, G. D. Love, Durham Univ. (United Kingdom)

Adaptive optics is used to correct for sample-induced aberrations in a brightfield microscope. As in astronomy, the adaptive optics system will have a rather narrow field of view due to the fact that light forming different points in the image will have passed through different parts of the sample and therefore have different aberrations. Here we report on work aimed towards wide field imaging by “foveated” imaging - i.e. a steerable corrected field.

We will report on experiments using both image optimization techniques, wavefront sensing techniques, and methods for measure aberrations volumetrically.

7904-39, Session 8

Comparative assessment of three algorithms to control a deformable mirror for an adaptive optics system with no wavefront sensor

M. R. Nasiri Avanaki, S. A. Hojjatoleslami, Univ. of Kent (United Kingdom); H. Sarmadi, Univ. of Tehran (United States); A. Meadoway, A. G. Podoleanu, Univ. of Kent (United Kingdom)

In confocal imaging systems, the images present less resolution than the theoretical limit due to imperfection of the optical devices used; lens, mirror, dispersion compensation glass, coupler, and beam splitter. This deteriorates the wavefront and introduces aberrations to the optical system [1]. The aberration is in fact a wavefront deformation which lessens the optical and electrical signal to noise ratio and as a consequence lessens the quality of the image [2]. Owing to this, there is a great interest in aberration reduction in such imaging systems. Adaptive optics (AO) systems composed of a wavefront sensor (WFS) and a deformable mirror represent the most used solution to this problem [3]. Such adaptive optics systems are expensive. In microscopy however, WFSs cannot be used due to stray reflections in the system and high aberrations introduced by the specimen [4]. In sensor-less AO, a deformable mirror (DM) is used along an optimization algorithm (blind optimization algorithm) [5]. The optimization algorithm works in a closed-loop in an iterative manner. In each iteration, the algorithm changes the shape of the mirror so as to optimize the photodetector current of the imaging system. In this paper three optimization techniques are implemented with an electromagnetic deformable mirror to control the aberration. The deformable mirror has 52 actuators which are pulled and pushed by the voltage produced from the optimization algorithms. Three well established optimization techniques are explained and compared [6-7]; simulated annealing, genetic algorithm and particle swarm optimization.

REFERENCES


7904-40, Session 8

Pupil engineering for a confocal reflectance line-scanning microscope for imaging human skin

Y. G. Patel, Northeastern Univ. (United States); M. Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States); C. A. DiMarzio, Northeastern Univ. (United States)

Confocal reflectance microscopy may enable screening and diagnosis of skin cancers noninvasively and in real-time, as an adjunct to biopsy and pathology. Current confocal point-scanning systems are large, complex, and expensive. A confocal line-scanning microscope, utilizing a line array detector will be simpler, smaller, less expensive, and may accelerate the translation of confocal microscopy in clinical and surgical dermatology. A line array detector may be implemented with a divided-pupil, half used for transmission and half for detection, or with a full-pupil using a beamsplitter. The premise is that a confocal line-scanner with either a divided-pupil or a full-pupil will provide high resolution and optical sectioning that would be competitive to that of the standard confocal point-scanner.

We have developed a confocal line-scanner that combines both divided-pupil and full-pupil configurations. This combined-pupil prototype is being evaluated to determine the advantages and limitations of each configuration for imaging skin, and comparison of performance to that of commercially available standard confocal point-scanning microscopes. With the combined configuration, experimental evaluation of line spread functions (LSFs), contrast, signal-to-noise ratio, and imaging performance is in progress under identical optical and skin conditions. Experimental comparisons between divided-pupil and full-pupil LSFs will be used to determine imaging performance. Both results will be compared to theoretical calculations using our previously reported Fourier analysis model and to the confocal point spread function (PSF). These results may lead to a simpler class of confocal reflectance scanning microscopy for clinical and surgical dermatology.

7904-41, Session 8

Sagnac-interferometry based digital phase conjugation system for turbidity suppression

T. R. Hillman, Y. Park, D. Fu, Z. Yaoqob, Massachusetts Institute of Technology (United States); W. Choi, Korea Univ. (Korea, Republic of); T. Yamauchi, R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

Multiple scattering has been a significant obstacle in the optical imaging of biological samples. It has been generally been treated as stochastic noise; its correlation with sample structure has seldom been exploited. Recently, significant breakthroughs have been achieved by treating light scattering as a deterministic phenomenon. It has been shown that optical phase conjugation of the scattered field can reverse the effects of multiple scattering. Here, we present a simple and robust experimental setup for digital optical phase conjugation (DOPC). In DOPC, the scattered wavefront is detected by digital holography and the conjugated field is shaped and applied by a spatial light modulator (SLM). The detector and SLM are set up in the geometry of a Sagnac interferometer, which enables the simple design and robust instrumentation for DOPC. We show that optical information can be recovered even after entanglement by a highly scattering medium (of length 1 to 20 scattering mean-free paths).

The SLM provides greater beam-shaping control than merely reproducing the conjugated, detected wavefront. To exploit this fact, we relied on some insights from random matrix theory: that propagation through a turbid medium is dominated by the contributions of a few, "open" transmission channels, corresponding to input wavefield distributions. We show that our experimental geometry can be used to isolate the open channels. When the SLM-generated wavefield applied to the medium is restricted to the open-channel subspace, the total power in the object reconstruction is increased, yet the essential image information is preserved.

References

7904-42, Session 9

High-resolution lensfree on-chip imaging over a wide field-of-view using source-shifted pixel superresolution

W. Bishara, T. Su, A. F. Coskun, A. Ozcan, Univ. of California, Los Angeles (United States)

We demonstrate high-resolution lensfree holographic microscopy on a chip by overcoming the physical detector pixel-size limitations through mechanical shifting of the illumination source and a Pixel Super-Resolution (SR) algorithm. This lensfree on-chip microscope achieves ~0.6um spatial resolution over a 24mm2 field-of-view (FOV), which is >100 fold larger than the FOV of a typical 40X objective-lens. This on-chip imaging modality utilizes a partially-coherent illumination source filtered by a large aperture (>50um) in an in-line holography configuration, and a digital sensor-array with a pixel-size of 2.2um and an imaging area of 24mm2. With this configuration, a single raw hologram yields, after digital processing and twin-image elimination, a microscopic image with resolution of ~1.5-2.0um corresponding to a numerical-aperture (NA) of ~0.15-0.20. To effectively increase the NA of this platform, multiple holograms with sub-pixel shifts can be recorded by mechanical shifting of the illumination source by relatively large distances due to the demagnification (e.g., >100X) inherent in this set-up. Such multiple sub-pixel shifted holograms are then digitally combined using a Pixel SR algorithm to synthesize a high-resolution hologram and a reconstructed microscopic image with ~0.6um resolution, corresponding to an NA of ~0.5, over an FOV of ~24mm2. The performance of this lensfree on-chip microscope is validated by imaging patterned transparent substrates and blood smear samples. This lensfree holographic imaging modality could enable the design of a light-weight, compact and cost-effective microscope with sub-micron resolution over a large field-of-view, which would especially be valuable for point-of-care applications as well as for field-use in resource-poor settings.

7904-43, Session 9

Measuring spatial distribution of mitochondria to assess embryo viability

J. L. Hollmann, Pathfinder Energy Service (United States); C. A. DiMarzio, Northeastern Univ. (United States)

This paper describes a novel approach to classifying mitochondrial patterns within an oocyte utilizing the distribution of amplitudes at different spatial frequencies from a slice of a three-dimensional image of the oocyte. The amplitudes associated with low spatial frequencies provide quantification of the qualitative terms “diffuse” and “aggregated” - patterns thought to be key indicators for an oocyte’s health and its potential for survival post-implantation. Mitochondrial patterns are imaged utilizing a confocal microscope. The oocyte under analysis is isolated within the image. A coarse grid is superimposed upon the oocyte and the spatial spectrum for each grid...
For lensfree-imaging of incoherent objects, such as fluorescent-samples, sampling (CS) is one such theoretical framework which provides perfect simpler, compact, light-weight and cost-effective ones. In doing so, the major direction in lensfree-imaging is to utilize digital algorithms on a chip, the emitted-light from the object-plane diffracts to be sampled by a sensor-array. This sampling-process is linear in intensity, and any arbitrary distribution of incoherent sources can be decoded (using CS algorithms) from the measured 2D light-pattern. Crucial for this decoding is the incoherent-point-spread-function, which can easily be measured for a given lensfree-architecture. Since most objects-of-interest (such as rare-cells within a microfluidic-device) are by-definition sparse, enforcing sparsity as a constraint in compressive decoding can recover the incoherent object-distribution with a resolution that is close to the sensor pixel-size. We experimentally validated this by achieving ~10um resolution over >8cm2 field-of-view (pixel-size: 9um) without the use of any lenses/mechanical-scanners. This resolution was further improved using CS to sub-pixel level (e.g., 2-3um) by nano-structuring of the chip-surface which purposely modulated the incoherent emission from the object-plane.

Semi-automated algorithm for localization of dermal/epidermal junction in reflectance confocal microscopy images of human skin

The examination of the dermis/epidermis junction (DEJ) is clinically important for skin cancer diagnosis. Reflectance confocal microscopy (RCM) is an emerging tool for detection of skin cancers in vivo. However, visual localization of the DEJ in RCM images, with high accuracy and repeatability, is challenging, especially in fair skin, due to optically subtle changes, low contrast, heterogeneous topography and high inter- and intra-subject variability. We propose a semi-automated algorithm to localize the DEJ in z-stacks of RCM images, based on feature segmentation and classification. The algorithm operates on small tiles (regions) within images and partitions z-stacks of tiles into homogenous segments by fitting a predictive model to a set of texture features. The segments are then classified as epidermis, dermis or transitional DEJ. The algorithm was trained on a small number of labeled images and tested on image stacks from fair skin. Preliminary results show that the epidermis/dermis misclassification rates are 10% and the average localization error relative to expert marked DEJs are 13um. Current work is on applying the algorithm to dark skin, in which strong backscatter from the pigment melanin causes the DEJ to appear with high contrast and is easier to detect. In these cases, the algorithm will find the appropriate peak of the smoothed average intensity depth profile of each tile. Current work is also on generalizing the algorithm to process all skin types. The algorithm will first decide the skin type and then apply the appropriate DEJ localization method.

Compressive decoding for incoherent lensfree on-chip imaging

Lensfree on-chip imaging aims to create new microscopes that better suit the needs of on-chip platforms, and would be quite useful for high-throughput cytometry and rare-cell analysis, among others. A major direction in lensfree-imaging is to utilize digital-algorithms along with novel theories to replace advance optical-hardware with simpler, compact, light-weight and cost-effective ones. In doing so, the throughput and the field-of-view can also be significantly increased, which would be exceedingly useful for micro-fluidics. Compressive Sampling (CS) is one such theoretical framework which provides perfect signal-recovery under certain conditions even at sub-Nyquist sampling-rates. Here we present a new application for CS and discuss its impact for incoherent on-chip imaging.

For lensfree-imaging of incoherent objects, such as fluorescent-samples with high fidelity. We believe this is the first time to our knowledge that CS is used to process experimentally obtained OCT signal.

Sparse OCT: the application of compressed sensing in spectral-domain optical coherence tomography

Spectral domain optical coherence tomography (SD-OCT) detects interferograms in Fourier domain (k-space) using a spectrometer. Conventional image reconstruction algorithms for SD-OCT require k-space sampling beyond Nyquist rate to achieve system’s maximum imaging depth. To achieve this, SD-OCT usually uses a linear array CCD camera with a large number of pixels to guarantee that at least two data points are sampled within one period of the spectral interferogram. Such array detector and associated electronics are usually slow and expensive. Moreover, it is challenging to transfer and process the vast amounts of data acquired from the large detector array. However, when images have a sparse representation, highly undersampled random measurements in k-space can yield accurate image recovery through the use of Compressed Sensing (CS). CS has been successfully applied to MRI and photoacoustic tomography. In this study, we explore the potential of using CS in SD-OCT. If OCT interferograms have a sparse representation, CS would allow using an array detector with fewer pixels to obtain high quality OCT images. We tested the method by randomly undersampled k-space SD-OCT signal of a biological sample. Images are reconstructed by solving an optimization problem that minimizes the l-1 norm of a transformed image to enforce sparsity, subject to data consistency constraints. Results show that randomly sampling as low as 20% of original spectral data allows the full reconstruction of the image with high fidelity. We believe this is the first time to our knowledge that SD-OCT imaging depth. To achieve this, SD-OCT usually uses a linear array CCD camera with a large number of pixels to guarantee that at least two data points are sampled within one period of the spectral interferogram. Such array detector and associated electronics are usually slow and expensive. Moreover, it is challenging to transfer and process the vast amounts of data acquired from the large detector array. However, when images have a sparse representation, highly undersampled random measurements in k-space can yield accurate image recovery through the use of Compressed Sensing (CS). CS has been successfully applied to MRI and photoacoustic tomography. In this study, we explore the potential of using CS in SD-OCT. If OCT interferograms have a sparse representation, CS would allow using an array detector with fewer pixels to obtain high quality OCT images. We tested the method by randomly undersampled k-space SD-OCT signal of a biological sample. Images are reconstructed by solving an optimization problem that minimizes the l-1 norm of a transformed image to enforce sparsity, subject to data consistency constraints. Results show that randomly sampling as low as 20% of original spectral data allows the full reconstruction of the image with high fidelity. We believe this is the first time to our knowledge that CS is used to process experimentally obtained OCT signal.

Effect of double-helix point-spread functions on 3D imaging in the presence of spherical aberrations

Double Helix point-spread functions (DH-PSFs), the result of PSF engineering have been used for super resolution microscopy [1]. The DH-PSF design features 2 dominant lobes in the image plane whose angular orientation rotates with the (z) position of the emitter. The center of the DH-PSF gives the precise location of the fluorophores, while the orientation of the lobes when matched to a given graph gives the location on the Z axis. In this paper we investigate the effect of spherical aberrations on the DH-PSF. Physical parameters such as the lens used, the size of the object and its refractive index contribute to the amount of spherical aberration present in the PSF. DH-PSFs were computed for different imaging conditions with spherical aberrations and images were...
simulated using these aberrant PSFs and computer-generated objects. A depth-variant approach was used to estimate the underlying object from these images as well as extract depth information from the rotation of the DH-PSF.

Reference

7904-48, Session 9
Reducing noise in microscope images by optical manipulation of point spread functions
R. N. Zahreddine, R. H. Cormack, C. J. Cogswell, Univ. of Colorado at Boulder (United States)

This work describes using computational optics principles to first encode the specimen signal information optically into specially-engineered PSFs (that are recorded at the image detector plane), then decoding the signal and separating it from the system noise using specially-tailored algorithms. This approach should greatly improve a microscope’s imaging capabilities in photon-starved applications such as live-cell fluorescence and object tracking.

7904-49, Session 10
Time-resolved confocal microscopy of cryogenic processes in biological tissues
M. Schellenberg, M. Kloster, E. Peev, J. Napier, W. Neu, Fachhochschule Oldenburg/Ostfriesland/Wilhelmshaven (Germany)

Cryogenic procedures for conservation of living biological cells and tissues as well as for the purification of e.g. virus particles are fundamental tools in modern biology. In complex tissues the transport processes of cryoprotective compounds are much slower compared the ones in single cell suspensions, and are much less investigated. For research on and optimization of the processes involved in the freezing and thawing of biological material, a method for fast 3D-/4D-microscopy combined with a way of applying defined temperature profiles on the sample would be of great benefit.

The technique of DMD-microscopy enables confocal imaging at high spatial and temporal resolution by utilizing a large amount of pinholes simultaneously at high scanning frequencies. Resolutions of 300x300x1000nm on volumes up to 300x300x80µm can be achieved at measurement speeds down to a few seconds for a complete scan, which is orders of magnitude faster than in conventional confocal microscopy. This elevated acquisition speed offers new insights into dynamic processes occurring on a (sub-)cellular level. For doing so well established methods like staining with fluorescent markers are used for precise differentiation of the interesting structures, while two-photon-excitation reduces the damage forced onto the sample by the excitation of the fluorophores.

Due to changes of the optical characteristics of the samples during the freezing and thawing procedure an automated adjustment of the measurement parameters has to be accomplished whilst the cryogenic process. For optimization purposes it is furthermore crucial to be able to apply exactly defined temperature profiles to the process.

7904-50, Session 10
In-vivo third-harmonic generation microscopy at 1550 nm: three-dimensional long-term time-lapse studies in living C. elegans embryos
R. A. Aviles-Espinosa, S. I. C. O. Santos, ICFO - Instituto de Ciencias Fotónicas (Spain); A. Brodschelm, W. G. Kaenders, TOPTICA Photonics AG (Germany); C. Alonso-Ortega, ICFO - Instituto de Ciencias Fotónicas (Spain); D. Artigas-García, ICFO - Instituto de Ciencias Fotónicas (Spain) and Univ. Politècnica de Catalunya (Spain); P. Loza-Alvarez, ICFO - Instituto de Ciencias Fotónicas (Spain)

In-vivo microscopic long term time-lapse studies require controlled imaging conditions to preserve sample viability. Therefore it is crucial to meet specific exposure conditions as these may limit the applicability of established techniques such as those based in linear and nonlinear fluorescence. In this work we demonstrate for the first time the use of third harmonic generation (THG) microscopy for long term time-lapse three-dimensional studies (4D) in living Caenorhabditis elegans embryos employing a 1550nm femtosecond fiber laser.

On one hand, we take advantage of the fact that THG only requires the existence of interfaces to generate signal (change in the refractive index or in the X^3 nonlinear coefficient) therefore no markers are required.

On the other hand, by using this wavelength the emitted THG signal is generated at visible wavelengths (516nm). Given this, standard collection optics and detectors operating near their maximum efficiency enable an optimal signal collection. As result, the incident light intensity at the sample plane can be considerably decreased enabling several hours of sample exposure.

We obtain THG signal in all embryo development stages, providing different tissue/structure information. By means of control samples, we demonstrate that the expected water absorption at this wavelength does not severely compromise sample viability.

Certainly, this technique reduces the complexity of sample preparation (i.e. genetic modification) required by established linear and nonlinear fluorescence based techniques. We demonstrate the non-invasiveness, reduced specimen interference, and strong potential of this particular wavelength to be used to perform long-term 4D studies (such as cell lineage).

7904-51, Session 10
High-depth discrimination property of saturated excitation (SAX) microscopy
M. Yamanaka, S. Kawano, N. I. Smith, Osaka Univ. (Japan); S. Kawata, Osaka Univ. (Japan) and RIKEN (Japan); K. Fujita, Osaka Univ. (Japan) and Japan Science and Technology Agency (Japan)

We report the depth-discrimination property of saturated excitation (SAX) microscopy. In SAX microscopy, the three-dimensional imaging capability is improved by the use of nonlinear fluorescence response resulting from saturation phenomenon in single-photon excitation of fluorescence molecules. Since the saturation effect is induced by high excitation intensity, the nonlinear fluorescence signal appears prominently at the center of the focus spot, which can be extracted by modulation of the excitation intensity at a single frequency and demodulation of the fluorescence intensity at the harmonic frequencies. The use of the nonlinear fluorescence response in confocal microscopy suppresses fluorescence signal from the out-of-focus plane significantly. Thus, the defocused image is eliminated more effectively in SAX microscopy than single-photon excitation confocal fluorescence microscopy. Using SAX microscopy, we observed actin filaments stained with ATTO Rho6G phalloidin in HeLa cells. The sample was excited with a continuous-wave (CW) laser whose wavelength was 532 nm and the excitation laser was focused with an NA 1.49 oil-immersion objective lens. The excitation
intensity was modulated at 10 kHz and the fluorescence intensity was demodulated at 20 kHz. We took fluorescence images of the actin filaments in the focal plane (x-y image) and along the optical axis (x-z image). From the observation, we confirmed the effective removal of fluorescence from the out-of-focus plane and improvement of the spatial resolution.

**7904-52, Session 10**

**Diffraction-unlimited three-dimensional optical microscopy with opposing lenses**

A. Egner, R. Schmidt, D. Aquino, C. Geisler, A. Schönlé, S. W. Hell, Max-Planck-Institut für biophysikalische Chemie (Germany)

Far field optical microscopy and especially confocal fluorescence microscopy are well established methods for the non-invasive 3D-investigation of cellular structures. However, the resolution of conventional light microscopy is limited by diffraction to ~200nm in the focal plane and ~600nm along the optic axis. In order to discern identical labels which are much closer than this, one has to overcome the diffraction barrier.

The utilization of optical switching events allows one to circumvent Abbe's diffraction limit: The switching of only markers within an area which is much smaller than the size of a diffraction limited spot to a visible “bright” state while all other markers are switched to a non-visible “dark” state defines a sub-diffraction area. By sequentially recording all areas within the diffraction spot, it is possible to assemble a sub-diffraction image.

The first radical concept for improving the resolution of a far field microscope was Stimulated Emission Depletion (STED) microscopy. In this concept the saturated depletion of the excited state of the fluorescent molecule is used to generate a fluorescent spot that is narrower than the diffraction limit. isoSTED microscopy proved a resolution of up to 21 nm in the lateral and 30 nm in the axial direction, meaning that the resolution is higher by more than an order of magnitude as compared to confocal microscopy. Consequently, it was possible to reveal the distribution of mitochondrial proteins. Further, the 3D structure of nanostructured block copolymers in the 50nm range could be resolved. Two color imaging can be performed using a single STED beam which avoids spatial distortion of the channels with respect to each other due to chromatic effects.

An other method utilizing molecular switching events for achieving nanoscale resolution in microscopy uses a more pointillist approach. For example photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM) and PALM with independently running acquisition (PALMIRA) all bear different names but are based on the same principle: Single molecules which are initially in a dark state are sequentially activated, located and deactivated.

The localization accuracy of each molecule depends, of course, on the number of detected photons per molecule and can be has high as 2 nm. Over the whole field of view, these methods provide an average resolution in the order of several tens of nanometers.
Conference 7905: Single Molecule Spectroscopy and Imaging IV

7905-01, Session 1

Ultra-high-throughput single-molecule spectroscopy with a 1024-pixel SPAD

R. A. Colyer, G. Scalia, Univ. of California, Los Angeles (United States); F. A. Villa, F. Guerrieri, S. Tisa, F. Zappa, S. D. Cova, Politecnico di Milano (Italy); S. Weiss, X. Michalet, Univ. of California, Los Angeles (United States)

Single-molecule spectroscopy is a powerful approach to measuring molecular properties such as size, brightness, conformation, and binding constants. Due to the low concentrations in the single-molecule regime, measurements with good statistical accuracy require long acquisition times. Previously we showed an order of magnitude improvement in acquisition speed using a custom-CMOS 8x1 SPAD array. Here we present preliminary results of up to three orders of magnitude improvement obtained using a liquid crystal on silicon spatial light modulator (LCOS-SLM) and a novel standard CMOS 1024 pixel SPAD array, opening the way to truly high-throughput single-molecule spectroscopy.

7905-02, Session 1

Parallel fluorescence photon timing module with monolithic SPAD array detector

I. Rech, A. Gulinatti, C. Cammi, F. Panzeri, M. Ghioni, Politecnico di Milano (Italy)

Time Correlated Single Photon Counting (TCSPC) has reached a considerable importance and a great deal of techniques have been developed based on it, such as Fluorescence Lifetime Imaging (FLIM), optical tomography and time-resolved laser scanning microscopy. Recently, new improvements in the TCSPC technique allowed its application to multidimensional measurements; this consequently required the development of new arrays of detectors with high photon detection efficiency, low dark counting rate and afterpulsing, and high time resolution. In recent years, Single Photon Avalanche Diodes (SPADs) have emerged as a solid state alternative, in particular for the production of arrays, since they present remarkable advantages such as: low cost, high integrability, low power dissipation and higher performance. We have already shown that it is possible to fabricate good detectors with diameters of 50, 100 and 200 μm; we also proved that it is possible to obtain time resolutions down to 30-35 ps FWHM not only using the smaller detectors, but even with diameters as large as 200 μm.

We present here a multichannel photon timing module that exploits a monolithic array of single-photon avalanche diodes (SPADs). The detector array consists of eight 50μm diameter SPADs featuring low dark counting rate, high photon detection efficiency (50% at 550nm) and high time resolution (less than 100ps FWHM). The use of highly integrated active quenching circuits and an integrated front-end electronics close to the device makes it possible to design a very compact read-out circuit, yet providing eight fully independent timing channels and gating capability.

7905-03, Session 1

Confocal microscopy: well established and no more cool?

F. Koberling, PicoQuant GmbH (Germany); M. König, PicoQuant GmbH (United States); S. Fore, PicoQuant Photonics North America, Inc. (United States); S. Rüttinger, M. Wahl, PicoQuant GmbH (United States); O. Schulz, R. Ros, Arizona State Univ. (United States); T. Haisen, D. Herten, Ruprecht-Karls-Univ. Heidelberg (Germany); R. Erdmann, PicoQuant GmbH (United States)

Single molecule based techniques made their way from the early idea to overcome ensemble averaging via studies of biological dynamics and conformations, towards DNA sequencing and ultra high resolution imaging. From the very beginning, confocal microscopy was the workhorse due to its versatility and straight forward multiparameter detection capability. We will show that being fairly standard in its core components, carefully chosen extra equipment (novel combinations with different techniques such as AFMs or UV detection) can significantly expand its capabilities, making sub-diffraction imaging features or sub ensemble single fluorophore counting possible.

Adding an AFM tip to the confocal observation volume allows for the manipulation of single emitters while at the same time their response can be monitored. Via tip induced quenching of a single molecule’s fluorescence the diffraction limit is overcome, paving the way for tailoring photophysical properties like the fluorescence lifetime. Photon coincidence measurements (Antibunching) started as the ultimate proof for the existence of a single emitter but nowadays also decipher the true number of immobilised emitters in the sub-micron observation volume. We show that an increase in independent detection channels in combination with a truly parallel data acquisition is ready to find its way into quantitative biology, e.g. to determine the stoichiometry of protein aggregates or receptor clusters on cell membranes.

Recent advances in UV optics and detection enable also to expand the spectral observation window into the deep UV. We present first steps towards single molecule studies of intrinsic fluorescence overcoming the need of elaborated labeling strategies.

7905-05, Session 1

Second-harmonic generation of single gold metallic nanoparticles


We report the Second Harmonic Generation from a single gold metallic nanoparticle, the diameter of which is 150 nm, dispersed in a homogeneous gelatin matrix. The homogeneous embedding media prevent symmetry breaking as opposed to the case of nanoparticles deposited on substrates. These experiments allowed us to investigate the intrinsic nonlinear optical properties of the nanoparticles. The results were compared to ensemble measurements performed in aqueous solutions and Finite Elements Method simulations. In particular, a polarization analysis was performed to ensure that indeed the SHG response is collected from a single gold metallic nanoparticle. This work paves the way for a deeper understanding of SHG from non spherical nanoparticles preventing averaging procedures over the orientation distribution.

Besides, the sensitivity of our experimental set-up for nonlinear scattering, also known as Hyper Rayleigh Scattering, is presented with a statistical analysis of the SH response as the volume density of 80 nm diameter the gold metallic nanoparticles is reduced down to one particle in the volume sampled.
New microscope objective for parallel near- and far-field microscopy

T. Ruckstuhl, C. M. Winterflood, D. Verdes, S. Seeger, Univ. of Zürich (Switzerland)

A new microscope objective is introduced for straightforward implementation of parallel near- and far-field microscopy on common microscope platforms opening up new perspectives for single molecule detection at interfaces. Fluorophores in direct vicinity of a microscope coverslip emit a significant proportion as supercritical angle fluorescence (SAF) above the critical angle into the glass. As the coupling of the near-field decays exponentially within a wavelength distance to the surface the SAF intensity is extremely sensitive to the surface distance of its source. On the other hand, the emission below c (far-field) is barely affected by the axial position and can serve to determine the emitter’s brightness. The parallel measurement of the intensities emitted below and above the critical angle allows the z-localization of emitters with a resolution far beyond the diffraction limit. This is demonstrated by localizing fluorescent point sources along the optical axis with up to 1 nm accuracy. The two detection volumes of different axial extent open up new perspectives for single molecule detection applications based on tight laser focusing, such as FLIM and FCS. The scope of parallel near- and far field FCS will be discussed.

Polarized fluorescence nanospheres

Z. K. Gryczynski, R. Luchowski, P. Sarkar, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); Z. Földes-Papp, ISS, Inc. (United States); A. Chang, S. Raut, J. Borejdo, I. Gryczynski, Univ. of North Texas Health Science Ctr. at Fort Worth (United States)

Fluorescent beads (nanoparticles, nanospheres) are commonly used in fluorescence spectroscopy and microscopy. Due to the random distribution of dyes and high dyes to nanoparticles ratio (high dye concentration), the fluorescence polarization observed from the beads is low. Therefore beads are not useful for polarization based studies. We demonstrate that photoselective bleaching can create beads with highly polarized fluorescence. A simple technology for creating beads with highly aligned dyes molecules will be presented. We will demonstrate experiments exploiting anisotropy decays, Fluorescence Correlation Spectroscopy (FCS) and steady state fluorescence to compare photophysical properties of photobleached and not bleached nanospheres in water. Highly polarized beads are very useful for rotational diffusion studies of large molecular complexes using FCS approach.

Imaging properties of supercritical angle fluorescence optics

J. Enderlein, Georg-August-Univ. Göttingen (Germany); T. Ruckstuhl, Univ. of Zürich (Switzerland)

In recent years, new optical systems have been developed with the ability to collect light at very high angles of emission, thus permitting also the detection of evanescent modes of fluorescent emitters at an interface (supercritical angle detection). Prominent examples are solid-immersion lenses or the paraboloid supercritical angle fluorescence objective. To date, a rather neglected issue are the imaging properties of such systems. For conventional optical systems with high numerical aperture, there exists a precise wave-optical theory of imaging which was developed by Richards and Wolf in the fifties of the last century. However, their theory is not easily applicable to systems that collect a significant part of light above the critical angle of total internal reflection. Here, we extend the theory to describe the imaging properties of such optical systems, and we find some fascinating caustic properties of the propagating wave front within these systems.
established single molecule spectroscopy methods. The approach will not only aid in the analysis of isolated dyes or nanoparticles but also prove valuable in analyzing complex emitting systems like FRET pairs, fluorescent proteins and upconversion particles.

7905-10, Session 2

**Determination of position and 3D orientation of single quantum emitters in a tunable microcavity**

R. Gutbrod, A. I. Chizhik, F. Schleifenbaum, A. M. Chizhik, S. Bär, A. J. Meixner, Eberhard Karls Univ. Tübingen (Germany)

Optical microresonators are structures which confine light to a small region in the range of one wavelength. The radiation of a quantum emitter is coupled to cavity resonances which lead to an optical confinement of the broadband fluorescence. A practical design for this single-mode microresonator is formed by two silver mirrors enclosing a transparent dielectric medium with single quantum emitters. The fluorescence spectra and decay lifetimes of single molecules in this Fabry-Perot type microresonator are strongly dependent on the resonator length.

In the novel tunable microresonator we present here, the resonator length can be changed reversibly with piezoelectric elements to a distinct position corresponding to a specific emission wavelength. The local mode structure of the electromagnetic field is changed at this position which results in a redistribution of the fluorescence and a modification of the lifetime for the same single molecule.

The radiative coupling of the emitter to the electromagnetic field is also determined by the orientation of its transition dipole moment with respect to the cavity normal. The higher order laser modes used for illumination of the single molecule are changed by the resonator with respect to free space excitation, which allows us by analyzing the emission patterns to determine its three-dimensional orientation in the microresonator. In addition, this beam provides an excitation pattern which can be used to detect the longitudinal position of a fluorescent bead in the microresonator with an accuracy of a few nanometers.

7905-11, Session 2

**Time-resolved 3D orientation spectroscopy: experimental realization and simulation**

R. Börner, Univ. zu Lübeck (Germany); D. Kowerko, S. Krause, C. von Borczyskowski, Technische Univ. Chemnitz (Germany); C. G. Hübler, Univ. zu Lübeck (Germany)

Confocal microscopy is a powerful tool for single molecule investigation of fluorescent macromolecules. Besides the commonly studied features in single molecule detection, the 3D orientation determination of the emission dipole via a sophisticated splitting of the collected fluorescence intensity in a high and low aperture angle region [1,2] enables analyses of different conformational states. These conformational states can be represented as state depending dipole orientations intrinsic to the fluorescent molecule and/or in relation to the molecular frame.

On the other hand, conformational states can be subject to intramolecular dynamics, which may lead to spectral diffusion, fluorescence intensity and/or lifetime fluctuations and changes in the orientation of the emission dipole [3]. Therefore, a simultaneous measurement of all parameters of fluorescence is mandatory. To this end, we implement a detection scheme that allows for simultaneous determination of the full 3D emission dipole orientation, the fluorescence intensity, the fluorescence lifetime and the emission spectra of single fluorescent molecules.

We demonstrate the feasibility of our approach using pyridyl functionalized perylene bisimide (PBI) bound to silicon dioxide surface. Especially we observe the group-specific adsorption of PBI to the surface and how this restricts conformation and orientation dynamics.

7905-12, Session 3

**Multiparameter fluorescence image spectroscopy to characterize autofluorescence in biological samples**

S. Weidtkamp-Peters, S. Felekyan, H. Hornen, R. Kühnemuth, C. A. Seidel, Heinrich-Heine-Univ. Düsseldorf (Germany)

Multiparameter Fluorescence Image Spectroscopy (MFIS) is used to monitor simultaneously a variety of fluorescence parameters in confocal fluorescence microscopy. As the photons are registered one by one, MFIS allows for fully parallel recording of Fluorescence Correlation / Cross Correlation Spectroscopy (FCS / FCCS), fluorescence lifetime and pixel / image information over time periods of hours with picoseconds accuracy.

The subsequent analysis of the pixel fluorescence information in more dimensional histograms maximizes the selectivity of fluorescence microscopic methods. Moreover it facilitates a statistical relevant data analysis of the pixel information which makes an efficient detection of heterogeneities possible.

The expression of fluorescent proteins fused to a protein of interest in cultured cells or whole organisms like drosophila embryos has become a well established tool to investigate e.g. the dynamic behavior, complex formation and interaction of these proteins under physiological conditions. However the detected fluorescence signal emerging from most biological samples is affected by auto-fluorescence. Auto-fluorescence is caused by diverse biological molecules and structures with a high variability between different samples. Here we use MFIS to characterize auto-fluorescence by looking at different fluorescence parameters like fluorescence lifetime and spectral properties of auto-fluorescence, and subsequently we can filter pixel-wise for the contribution of auto-fluorescence to the detected fluorescence signal. This helps to increase the robustness of the analysis significantly. It also supports the economic use of photon information and thus allows one to keep the expression levels of fluorescent protein-fusion proteins as low as possible to preserve physiological conditions as much as possible.

7905-13, Session 3

**Fluorescence excitation-emission lifetime matrix measurements by Fourier transform spectroscopy**

M. Zhao, L. L. Peng, College of Optical Sciences, The Univ. of Arizona (United States)

Multi-parameter fluorescence lifetime measurements can greatly enhance multi-label single molecule study. Whereas lifetimes of multi-color emissions can be measured in parallel by spectral emission detector, lifetime measurements of multiple excitation wavelengths are mostly achieved by time-sharing, in which a switching mechanism is used to limit the excitation to a single wavelength at a time. We developed a multi-excitation fluorescence lifetime spectrometer that simultaneously measures fluorescence lifetimes at each individual excitation lines. With a spectral TSCPS detector array, the instrument can measure excitation-emission lifetime matrix with single molecule sensitivity.

The instrument uses a Fourier transform interferometer to generate Monte Carlo simulations of rotational dynamics taking into account the emission characteristics of dipoles close to optical interfaces in conjunction with the 3D orientation determination complement our experiment.

intensity modulations on the excitation light, and measures fluorescence lifetimes with the frequency domain lifetime method. A Michelson interferometer with a double passing high-speed optical delay-line is used to interferometrically modulate the excitation light intensities. A 24-facet polygon mirror spinning at 55,000 RPM provides the optical delay scan, which generates interference signal whose frequency sweeps continuously from about 150 MHz to zero then back to 150 MHz within a single facet scan (45.5 µs). Multiple excitation wavelengths are separated in the frequency domain by their different modulation frequencies, and fluorescence emissions associated with each wavelength can be separated by Fourier analysis. The instrument further uses a multi-color emission detector array to separate emission wavelengths. As the result, nanosecond lifetimes in the form of excitation-emission matrix can be measured at 22,000 matrices per second. We calibrated excitation-emission lifetime matrix measurements with a mixture solution of fluorescein and rhodamine B. The resulting fluorescence lifetimes are 3.6±0.4 ns for fluorescein and 1.7±0.2 ns for rhodamine B, both matching results from literature. Multi-color single molecule study via excitation-emission lifetime matrix is underway.

7905-14, Session 3

Performance of in-solution single molecule measurements for bioanalytical and in-vivo studies

A. Mazouchi, A. Bahram, B. Liu, Univ. of Toronto Mississauga (Canada); W. Houry, Univ. of Toronto (United States); C. C. Gradinaru, Univ. of Toronto Mississauga (Canada)

A home-built, multiparameter photon-counting microscope is used to implement in-solution single molecule techniques like fluorescence correlation spectroscopy (FCS) and burst analysis. The performance of standard FCS spectroscopy was studied over a wide range of sample concentrations, excitation power and background signal. Our analysis gave robust results, i.e. local concentrations and diffusion constants, up to cellular relevant concentrations. A combination of FCS and brightness analysis was employed to measure the anomalous diffusion of EGFP in the Drosophila cell nucleus. The binding affinity of a novel peptide-based drug to the cancer-regulating STAT3 protein is quantified simultaneously by FCS and polarization analysis on the same photon data set. In the pM concentration limit, lifetime and anisotropy analysis of photon bursts reveal the substrate and chaperon induced dynamics in the handle domains of the caseinolytic protease (ClpP) from E. coli. The results show an increased flexibility in the handle domain in the presence of both substrate and the molecular chaperone ClpX. This is a direct evidence for the mechanism of release of products from ClpP proteolytic chamber, hypothesized to involve transient pores in the handle domain.

7905-15, Session 4

Fluorescence correlation spectroscopy as tool for high-content-screening in yeast (HCS-FCS)

C. J. Wood, Stowers Institute for Medical Research (United States); J. Huff, Carl Zeiss MicroImaging, Inc. (United States); W. A. Marshall, J. R. Unruh, W. Wiegraebe, Stowers Institute for Medical Research (United States)

To measure protein interactions, diffusion properties, and local concentrations in single cells, Fluorescence Correlation Spectroscopy (FCS) is a well established and widely accepted method. However, measurements can take a long time and are laborious. Therefore investigations are typically limited to tens or a few hundred cells. We developed an automated system to overcome these limitations and make FCS available for high-content-screening (HCS). We will present data from an auto-correlation screen of more than 4000 of the 6000 proteins of the yeast Saccharomyces cerevisiae, tagged with eGFP. We expanded the HCS to use cross-correlation between eGFP and mCherry tagged proteins to screen for molecular interactions. All high-content FCS screens (HCS-FCS) were performed in a 96 well plate format. The system is based on an extended Carl Zeiss fluorescence correlation spectrometer ConfoCor 3 attached to a confocal microscope LSM510. We developed image-processing software to control these hardware components. The confocal microscope obtained overview images and the algorithm searched for and detected single cells. At each cell we positioned a laser beam at a well-defined area and recorded the fluctuation signal. We used automatic scoring of the signal for quality control. All data was stored and organized in a database based on the open source Open Microscopy platform. Data analysis was performed in the image processing language IDL and the open source statistical software package R.

7905-16, Session 4

Plasma membrane micro-organization of LR73 multidrug-resistant cells revealed by FCS

P. Winckler, R. Jaffiol, Lab. de Nanotechnologie et d’Instrumentation Optique (France); A. Cailler, H. Morjani, P. Jeannesson, UMR CNRS 6237 Matrice Extracellulaire et Dynamique Cellulaire (France)

Tumoral cells could present a multidrug resistance (MDR) to chemotherapy treatments. This drug resistance would be associated to biomechanisms occurring at the plasma membrane level, involving modification of membrane fluidity, drug permeability, presence of microdomains (rafts, caveolae...), and membrane proteins expression such as P-glycoprotein. Fluorescence correlation spectroscopy (FCS) is a powerful method to investigate locally the fluidity of biological membranes through the lateral diffusion of a fluorescent membrane probe. Thus, we use FCS to monitor the membrane organization of LR73 carcinoma cells and three multidrug-resistant cancer cell lines derived from this sensitive line. Measurements were conducted at the single cell level, which enabled us to get a detailed overview of the typical plasma membrane microviscosity distribution of each cell line studied. Moreover, we perform a Monte Carlo simulation based on a 2D diffusion model in a matrix including microdomains. This simulation allows us to link the fluidity distributions with the plasma membrane organization of MDR cells.

7905-17, Session 4

Analyzing activity of Neurokinin 1 receptor inserted in nanolipoproteins by fluorescence correlation spectroscopy

T. Gao, UC Davis Medical Ctr. (United States); J. Petriova, Univ. of California, Davis (United States); W. He, UC Davis Medical Ctr. (United States); F. Katzen, W. A. Kudlick, Life Technologies Corp. (United States); T. R. Huser, UC Davis Medical Ctr. (United States); J. Voss, Univ. of California, Davis (United States); M. A. Coleman, Lawrence Livermore National Lab. (United States)

We used cell-free expression to simultaneously produce apoA1 NLPs with an inserted membrane protein neurokinin receptor (NK1), a GPCR involved in pain perception). By labeling individual proteins and lipid species before NLP synthesis, fluorescence correlation spectroscopy (FCS) measured the specific diffusion times in the mixture using the cross correlation of fluorescence signals from each constituent after NLP formation. The functionality of NK1 was tested using FCS and Electron Paramagnetic Resonance (EPR). Both techniques confirmed NK1 inserted in NLPs was not only soluble, but more importantly functional when properly folded within NLPs. The binding affinity between NK1-
loaded NLPs and substance P (a NK1-specific ligand) was detected through a competitive binding assay designed for FCS, which allowed for direct comparison with EPR studies.

7905-18, Session 4

Subunit rotation in a single FoF1-ATP synthase in a living bacterium monitored by FRET

K. Seyfert, Univ. Stuttgart (Germany); H. Yagimuna, T. Oosaka, Osaka Univ. (Japan); S. Ernst, Univ. Stuttgart (Germany); R. lino, H. Noji, Osaka Univ. (Japan); M. Börsch, Univ. Stuttgart (Germany)

FoF1-ATP synthase is the ubiquitous membrane-bound enzyme in mitochondria, chloroplasts and bacteria which provides the 'chemical energy currency' adenosine triphosphate (ATP) for cellular processes. In Escherichia coli ATP synthesis is driven by a proton motive force (PMF) comprising a proton concentration difference 'delta'pH plus an electric potential 'deltaPsi' across the lipid membrane. Single-molecule in vitro experiments have confirmed that proton-driven subunit rotation within FoF1-ATP synthase is associated with ATP synthesis. Based on intramolecular distance measurements by single-molecule fluorescence resonance energy transfer (FRET) the kinetics of subunit rotation and the step sizes of the different rotor parts have been unraveled. However, these experiments were accomplished in the presence of a PMF consisting of a maximum 'delta'pH ~ 4 and an unknown 'deltaPsi'. In contrast, in living bacteria the maximum 'delta'pH across the plasma membrane is likely 0.75, and 'deltaPsi' has been measured between -80 and -140 mV. Thus the problem of in vivo catalytic turnover rates, or the in vivo rotational speed in single FoF1-ATP synthases, respectively, has to be solved. In addition, the absolute number of enzymes in a single bacterium required to maintain the high ATP levels has to be determined. We report our progress of measuring subunit rotation in single FoF1-ATP synthases in vitro and in vivo, which was enabled by a new labeling approach for single-molecule FRET measurements.

7905-19, Session 5

Probing the hydrodynamic properties of GFP-tagged membrane proteins by rotational fluorescence correlation spectroscopy

S. Pallikkuth, A. Volkmer, Univ. Stuttgart (Germany)

By monitoring the Brownian rotational diffusion of a fluorescent biomolecule in solution, information regarding its hydrodynamic size and shape can be obtained. While the free rotational dynamics of a small biomolecule, typically occurring on the pico- and few-nanosecond time scale, is generally obtained from the measurement of its time-resolved fluorescence anisotropy upon pulsed excitation, the accurate measurement of the rotational diffusion of a large biomolecules, occurring within tens of nanoseconds to milliseconds, is prevented by the inherently short fluorescence lifetime of the fluorescent label. Based on recent advances in single-photon counting technology in combination with confocal fluorescence microscopy, we demonstrate polarization-sensitive detection of distinct photon arrival times with picosecond time resolution followed by an exact analysis in terms of their second-order correlation function. We will present the measurement of the rotational and translational Brownian diffusion properties of GFP-tagged membrane proteins in solution without the need for pulsed excitation. We believe this technique could provide a unique tool for studying simultaneously the hydrodynamic and photophysical properties of membrane proteins occurring on time scales covering more than twelve orders of magnitude.

7905-20, Session 5

Detection of pathogenic DNA at the single-molecule level

I. Yahiatène, T. Klamp, M. Sauer, Univ. Bielefeld (Germany)

Having a secure way for a fast and sensitive detection of viral or bacterial DNA in times of a rapidly growing population gets increasingly important. The key to health is an early medication which has to be started as soon as possible to maximize the healing probability. In this manner the greatest problem is that nowadays physicians try to culture the pathogens on growth medium. This takes a long time (days to weeks). During this time the potential disease progresses. The next step might be a PCR analysis to get a high amount of the pathogenic substance to be able to detect and count the specimen.

In the present work we portray the simplicity of a method for fast and sensitive detection of synthetic single stranded DNA target molecules with 100 nucleotides taken from Streptococcus Pneumoniae, which causes lower respiratory tract infections. We show the ability to detect single molecules at very low concentrations (< pM) bound to two fluorescent hairpins in homogeneous solution within minutes. This DNA-hairpin complex then diffuses through the excitation volume and gets excited, followed by a coincident burst of fluorescence photons. Detection is performed by a two color-confocal coincidence setup. Two lasers of different wavelength are overlayed by a dicroic mirror and directed into a high numerical aperture objective (60 X, 1.35NA), which generates an overlapping focus. Fluorescence emission is collected by the same objective and focused onto two spectrally separated avalanche photodiodes.

7905-21, Session 5

Ultra-sensitive bead-based pathogen detection

T. Klamp, I. Yahiatène, A. Lampe, Univ. Bielefeld (Germany); M. Sauer, Julius-Maximilians-Univ. Würzburg (Germany)

We introduce a new method for the ultra sensitive specific detection and quantification of pathogen single stranded DNA sequences in a bead-based fluorescence immunoassay. The target sequence of interest is captured by a specific/complementary DNA sequence (a short single-stranded oligonucleotide with a length of 25 bases) and coupled onto a magnetic microparticle via biotin/streptavidin binding. Iron oxide based particles embedded in polymeric matrices can be obtained in different sizes with various coatings and give the opportunity of target enrichment onto their surface in combination with an easy handling due to their magnetic properties. A second specific DNA sequence (again a short single-stranded oligonucleotide with a length of 25 bases), labeled with an appropriate fluorophore, is incorporated as efficient detector for standard fluorescence microscope systems. If the sequences are chosen carefully unspecific binding of detector DNA can be substantially reduced and the affinity of the capturing process can be increased. The introduced method provides single molecule sensitivity as well as the ability of using a simple and robust wide-field fluorescence microscope. Concerning most point of care system requirements, where incomplex applications and high detection sensitivities are needed, this is a great advantage. A further benefit of the concept is its huge modularity which enables the specific detection of various other pathogens.

We demonstrate the potential of the method detecting and quantifying synthetic gene-sequences of the two organisms S.pneumoniae and M.luteus at picomolar concentrations (1 nM to 1 pM) in a volume of only 20 µl. In comparison to other heterogeneous assays the bead-based assay is robust and can be performed within only 2 hours.
7905-22, Session 5

A photophysical study of two fluorogen-activating proteins bound to their cognate fluorogens

T. Gaiotto, H. B. Nguyen, Los Alamos National Lab. (United States); J. Jung, Stanford Univ. (United States); A. M. Bradbury, G. S. Gnanakaran, J. G. Schmidt, G. S. Waldo, R. M. Goodwin, Los Alamos National Lab. (United States)

We are exploring the use of fluorogen-activating proteins (FAPs) as reporters for single-molecule imaging. FAPs are single-chain antibodies selected to specifically bind small chromophoric molecules termed fluorogens. Upon binding to its cognate FAP, the fluorescence quantum yield of the fluorogen increases giving rise to a fluorescent complex. Based on the seminal work of Szent-Gyorgyi et al. (Nature Biotechnology, Volume 26, Number 2, pp 235-240, 2008) we have chosen to study two fluorogen-activating single-chain antibodies, HL1.0.1-T01 and H6-MG, bound to their cognate fluorogens, thiazole orange and malachite green derivatives, respectively. We use time-correlated single-photon counting, fluorescence correlation spectroscopy, and single-molecule imaging to study the photophysics of these fluorescent complexes.

7905-23, Session 6

Live-cell single-molecule and superresolution imaging of proteins in bacteria

J. S. Biteen, B. Coupland, B. Haas, N. Koropatkin, E. Martens, J. Matson, V. DiRita, Univ. of Michigan (United States); M. A. Thompson, L. Shapiro, W. E. Moerner, Stanford Univ. (United States)

Single-molecule imaging has extended the resolution of fluorescence microscopy down to the nanometer scale. Single-molecule-based super-resolution techniques (e.g., PALM) are non-invasive, tolerate simple sample preparation, and take advantage of well-developed labeling schemes. As a result, single-molecule imaging is especially powerful for studying structure and dynamics in live cells. Here, we focus on imaging live bacteria cells, with attention to the particular challenges that they present: these organisms are small, have short cell cycles, live in particular environments, and their organization is relatively poorly understood.

We resolve the superstructure of the bacterial actin homolog, MreB, in live Caulobacter crescentus cells to 40 nm based on the photoswitching of the common fluorescent protein EYFP. We then extend our technique to three dimensions to resolve the midplane ring formed by the prokaryotic cell-division protein, FtsZ, in Caulobacter. We utilize the natural dynamics of directed motion and polymerization to our advantage to increase our resolution and to extend the labeling possibilities. We also present preliminary results from applying live-cell single-molecule imaging to two novel problems of great biomedical significance: investigations of the regulatory pathway controlling pathogenicity in Vibrio cholerae, the agent of human cholera and studies of the mechanism of carbohydrate catabolism in the gut symbiont Bacteroides thetaiotaomicron.

7905-26, Session 5

DOPI and PALM imaging of single carbohydrate binding modules bound to cellulose substrates

D. Dagel, South Dakota School of Mines and Technology (United States); Y. Liu, National Renewable Energy Lab. (United States); L. Zhong, South Dakota School of Mines and Technology (United States); Y. Luo, Y. Zeng, M. E. Himmel, S. Ding, National Renewable Energy Lab. (United States); S. J. Smith, South Dakota School of Mines and Technology (United States)

We use single molecule fluorescence methods to study the binding of polysaccharide-specific Carbohydrate Binding Modules (CBMs) incorporating fluorescent proteins (FPs) to their target cellulose substrates. The CBMs are carbohydrate specific binding proteins, and a functional component of all cellulase enzymes, which in turn hydrolyze cellulose, releasing simple sugars suitable for fermentation to alcohol. The CBM plays the important role of locating the crystalline face of cellulose, a critical step in cellulase action. A biophysical understanding of the CBM action aids in developing a mechanistic picture of the cellulase enzyme, important for selection and potential modification. Towards this end, we observe a preferred orientation of the CBM-GFP complex relative to the crystalline basis of cellulose substrates derived from Valonia using the single molecule method known as Defocused Orientation and Position Imaging (DOPI), and used Photo Activated Localization Microscopy (PALM) to characterize the binding along and perpendicular to the microfibril axis with up to 10 nm localization accuracy. Carbohydrate Binding Modules, derived from fungal, bacterial, and cellulosomal sources, were genetically modified to incorporate green fluorescent proteins (GFP). Microfibrils composed of semi-crystalline cellulose, derived from Valonia and held in aqueous buffer, were exposed to the CBM-GFP and imaged in our home-built total internal reflection (TIRF) system. Subsequent analysis showed the CBMs bind to the opposite hydrophobic -1,1,0- faces of the cellulose microfibrils with a well-defined cross-orientation of about 70 Deg. Using PALM, we localized CBMs incorporating the photo-activated FP PAMCherry to visualize the binding along the microfibril and analyzed the nearest neighbor distributions.

7905-24, Session 6

Optical switching and time-sequential coherent detection of markers through opposing lenses enables multicolor 3D-nanoscopy with 10-nm resolution of large intracellular volumes

D. Aquino, A. Schöngle, C. Geisler, C. A. Wurm, S. W. Hell, A. Egner, Max-Planck-Institut für biophysikalische Chemie (Germany)

The resolution barrier in far-field optical microscopy has recently been broken by several methods all based on the concept of optically switching markers between states to allow their time-sequential recording. Here we present a novel implementation that allows three-dimensional (3D) image stacks to be acquired within thick specimens at a resolution of well below 10 nm in all spatial directions. By using two opposing lenses and taking into account their high focusing angles during data analysis, the positions and the type of isolated switched-on markers can be unambiguously determined within a 1 μm thick slice around the microscope’s focal plane. Allowing for non-invasive determination and colocalization of 3D structures on the nanoscale the device is ideally suited for studying the intracellular organization on the sub organelle level in the life-sciences and will enable local in situ analysis of three dimensional (3D) nanostructured materials.
Superresolution autofluorescence imaging of Nostoc punctiforme

D. L. Thompson, T. Gao, NSF Ctr. for Biophotonics Science and Technology (United States) and Univ. of California, Davis (United States); D. Ferreira, Univ. of California, Davis (United States); G. P. McNerney, NSF Ctr. for Biophotonics Science and Technology (United States) and Univ. of California, Davis (United States); J. C. Meeks, Univ. of California, Davis (United States); T. R. Huser, NSF Ctr. for Biophotonics Science and Technology (United States) and Univ. of California, Davis (United States)

Nostoc punctiforme (N.p.), a photosensory cyanobacterium, has been gaining interest as a source of renewable energy through nitrogen fixation due to its low resource working requirements, strong photosresponse, and overall versatility. Its broad range of biliprotein chromophores also make it a target for developing new biologically-based fluorescent probes as alternatives to green fluorescent protein. Our work aims to study intact chains of N.p. and characterize the local distributions and behavior of several fluorophores. Individual cells within chains of N.p. typically measure less than 3 µm in length and 2 µm in diameter. Diffraction-limited microscopy techniques, with resolution capabilities of ~250 nm, are only capable of revealing the general structure of the cells, while keeping many structural details hidden. Using deconvolution microscopy (DV), we have observed Förster resonance energy transfer (FRET), thus revealing colocalization of multiple chromophores near both cellular membranes and organelle structures. Super-resolution structured illumination microscopy (SIM) has enabled us to resolve even finer structures to better understand chromophore distribution within N.p. Here we will present sample results from our DV FRET and SIM autofluorescence imaging experiments, and discuss how chromophore distribution may impact the use of N.p. for renewable energy production.

Liver sinusoidal endothelial cell fenestrations revealed using structured illumination microscopy

G. P. McNerney, NSF Ctr. for Biophotonics Science and Technology (United States); V. Coggar, The Univ. of Sydney (Australia); M. T. Nyunt, NSF Ctr. for Biophotonics Science and Technology (United States); L. DeLeve, The Univ. of Southern California (United States); P. McCourt, B. Smetsroed, Univ. of Tromsø (Norway); D. Le Couteur, The Univ. of Sydney (Australia); T. R. Huser, NSF Ctr. for Biophotonics Science and Technology (United States) and Univ. of California, Davis (United States)

Blood plasma has to be separated from blood cells and debris before being delivered to the hepatocytes. There, plasma is partially cleaned of contaminants, such as toxins, while adding other components like glycoproteins. Lining the blood vessels that supply the hepatocytes is a single layer of liver sinusoidal endothelial cells (LSECs) which are thin (~200 nm) and have a distinct feature not found in other endothelial cells: clusters of tiny pores called fenestrations completely span through their cytoplasm. With their small size (80 - 250 nm), fenestrations act as excellent size-exclusion filters against cells and debris. The plasma passes through fenestration to the extracellular space of Disse underneath and finally to the hepatocytes. To date, our knowledge of the structure and function of fenestrations is marginal despite having important implications for hepatic metabolism, liver disease and aging. The problem arises from both a lack of cell-surface markers for fenestrations and their small size - generally below the optical diffraction limit - which has limited their morphological descriptions to atomic force microscopy and electron microscopy. These techniques, however, provide relatively low throughput, and a limited array of functional probes and markers. Structured illumination microscopy (SIM) is an exciting fluorescence technique that circumnavigates the diffraction limit while allowing multiple imaging channels, higher throughput, and the use of autofluorescence of fluorophores. Here, we will discuss our most recent SIM observations of LSEC fenestrations which have important implications for their function and structure.

Exploring single-cell dynamics with fast localization microscopy

Z. Huang, Britton Chance Ctr. for Biomedical Photonics (China); S. Zeng, Q. Luo, Huazhong Univ. of Science and Technology (China)

Localization microscopy holds superior performances in revealing dynamic processes in living cells by providing the ability to visualize biological structures with unprecedented spatial resolution in a large sample area, but its widespread use in biology is thus far mainly hindered by the slow image speed. Here we will present our recent progress in the development of a fast localization microscope for exploring single-cell dynamics. First of all we will introduce a new method, termed MA Liang (after a traditional Chinese folktale “Ma Liang and his Magic Brush”) for “maximum likelihood algorithm encoded on a Graphics Processing Unit (GPU),” for real-time processing of experimental images even from fast EMCCD cameras working at full frame rate without compromising localization precision or field of view. Then we will explore the potential of the MA Liang method for constructing a fast localization microscope, where the entire image analysis routine could be finished immediately after data acquisition. Finally we will present our latest results in using this newly developed localization microscopy for studying molecular dynamics and interactions inside living cells.

Time-gating for improved resolution in single molecule-based superresolution imaging

J. J. Han, P. M. Goodwin, A. P. Shreve, J. H. Werner, Los Alamos National Lab. (United States)

Super-resolution (SR) fluorescence microscopy techniques based on single molecule photoactivation and localization rely on determining the central position of a single, activated fluorescent dye molecule to a much higher accuracy than the width of its diffraction-limited image. Thompsonson et al. (Biophys. J. 2002, 82, 2775-2783) found that localization accuracy is limited by both the finite number of photons emitted by a single fluorophore prior to bleaching and by the background photons or photoelectrons also measured in the fit window. Of these two factors degrading localization accuracy, the background, in most cases, is the factor that limits localization accuracy. Moreover, for super-resolution microscopy based upon photoswitchable fluorophores, the background can be dominated by yet to be activated molecules present in the field of view. Here we show that the background from these unactivated molecules can be suppressed tremendously via time-gating the fluorescence emission (i.e., only processing photons that arrive at a fixed delay after a short excitation laser pulse). This tremendous decrease in background enables two-dimensional super-resolution images to be obtained with greater spatial resolution than would be obtainable in the absence of time-gating. This increase in spatial resolution is demonstrated using nanostructured thin film samples densely labeled with caged fluorescein. In the future, the background suppression afforded via time-gating will be essential for extending SR imaging based upon single molecule localization to z-depths comparable to confocal imaging.
Multicolor laser source for STED microscopy

G. Keaton, M. J. Leonardo, K. Monro, M. W. Byer, M. Martinez, Mobius Photonics, Inc. (United States)

We report on a pulsed laser source whose wavelength can be switched between 585 nm, 600 nm, and 615 nm. The pulses are 1 ns, 10 MHz, and the pulse energies are 10 to 40 nJ. The laser source uses a laser diode and a series of Yb-doped fiber amplifiers to generate a super-gain and, then, converted into the visible using MgO-doped periodically poled lithium niobate (PPLN). In general, the spectrum of such Raman-shifted light is too broad to be efficiently frequency-converted by PPLN. To overcome this problem, we have used narrow band fiber Bragg gratings to create a dual-wavelength fiber Raman laser. The 1060 nm light is launched into a length of passive fiber, where the Raman effect generates the first Stokes line. This (broad spectrum) light then synchronously pumps the fiber Raman laser, which supports the second and third Stokes lines simultaneously. Either of these lines can be frequency doubled, or the two lines can be frequency summed, to create any of three visible wavelengths. The PPLN crystal accordingly has three poling regions, and the color produced can be selected simply by indexing the crystal. The final output is suitable for high speed STED microscopy.

STED Microscopy - New Developments in Confocal Superresolution

H. F. Gugel, A. Giske, Leica Microsystems CMS GmbH (Germany)

STED microscopy enables imaging of biological samples with resolution not limited by diffraction anymore. It provides new insights in various fields of biology, e.g. membrane biology, neurobiology and physiology. It’s three-dimensional sectioning ability allows the recording of high resolution image stacks. Furthermore, dynamic processes can be recorded at video rate. We present new developments in STED microscopy. Multi-color imaging of biological samples with superresolution and different laser configuration are shown. A comparison of different setup configurations and their performance is presented.

Superresolution microscopy techniques and applications: the use of STORM technology to study cellular details at the molecular level

S. A. Schwartz, Nikon Instruments Inc. (United States)

No abstract available

Buffer-controlled photoswitching microscopy using standard organic fluorophores

F. Koberling, V. Buschmann, PicoQuant GmbH (Germany); S. Fore, PicoQuant Photonics North America, Inc. (United States); S. van de Linde, M. Sauer, S. Wolter, Julius-Maximilians-Univ. Würzburg (Germany); M. Heilemann, Univ. Bielefeld (Germany); R. Erdmann, PicoQuant GmbH (Germany)

The interest in super-resolution microscopy techniques has dramatically increased in the last years due to the unprecedented insight into cellular structure which has become possible [1]. In all camera-based techniques, such as Stochastical Optical Reconstruction Microscopy (STORM), direct STORM (dSTORM) and Photo-activation localization microscopy (PALM), the dye-sensor-molecules are switched between a bright and a dark state, which is generally achieved using 2 different wavelengths for excitation and photoswitching. Many organic fluorophores exhibit intrinsic dark states with a lifetime which can be tuned by adjusting the level of oxidants and reductants in the buffer, thereby allowing to reversibly switch individual fluorophores between an on- and off-state using just a single excitation wavelength [2].

We exploited this redox-level adjusted photoswitching behaviour for high-resolution imaging on a setup based on an inverse microscope coupled with ultrasensitive CCD camera detection. In order to quickly control the quality of the measurement, we used real-time computation of the subdiffraction-resolution image [3]. This greatly increases the applicability of the method, as image analysis times are greatly reduced.

these equations. The speed with which the image series can be obtained can be a problem for the microscopy of living cells. Challenges include pattern-switching speeds, optical efficiency, wavefront quality and fringe contrast, fringe pitch optimization, and polarization issues. We will review some recent developments in 3D-SIM hardware with the goal of super-resolved z-stacks of motile cells.

7905-52, Session 8

Molecular orientation affects localization accuracy in superresolution far-field fluorescence microscopy

J. Engelhardt, Deutsches Krebsforschungszentrum (Germany); J. Keller, Max-Planck-Institut für biophysikalische Chemie (Germany); P. Hoyer, M. Reuss, Deutsches Krebsforschungszentrum (United States); T. M. Staudt, Deutsches Krebsforschungszentrum (Germany); S. W. Hell, Max-Planck-Institut für biophysikalische Chemie (Germany)

We investigate the cooperative effect of molecular tilt and defocus on fluorophore localization by centroid calculation in far-field superresolution microscopy based on stochastic single molecule switching. If tilt angle and defocus are unknown, the localization contains systematic errors up to about ±125 nm. When imaging rotation-impaired fluorophores of unknown random orientation, the average localization accuracy in three-dimensional samples is typically limited to about ±32 nm, restricting the attainable resolution accordingly.

7905-30, Poster Session

Superresolution saturated structured illumination microscopy system: theoretical aspects and real life

D. Fixler, A. Gur, Z. Zalevsky, Bar-Ilan Univ. (Israel)

To beat this resolution limitation, that is, to overcome the classical Abbe's resolution limit, several approaches have been proposed along the years. Recently, two distinct conceptual strategies have overcome light's diffraction barrier, allowing the analysis of biological structures at the super resolution level. One strategy was conceived in the context of laser-scanning microscopy that directly minimizes the size of a scanned focal point, as in stimulated emission depletion (STED) fluorescence microscopy. Another method that we will concentrate in our talk suggests using saturated structured illumination microscopy (SSIM).

From the theoretical aspect using SSIM one may achieve resolution far beyond the diffraction limit by using the nonlinear dependence of the fluorescence emission rate on the illumination intensity. We will present simulation results supporting this statement. But to our knowledge, no experimental demonstration considering biological samples has been published yet using the SSIM method. In other words, only simulations and fluorescent beads have been used to demonstrate the concept of nonlinear structured illumination wide field microscopy. In our talk we present experimental results showing the advantages and the problems when using fluorescence nonlinear effect in SSIM super resolution system. We will discuss the nonlinear phenomenon which is related to fluorescence process and conclude that, contrary to the initial theoretically expected unlimited resolution limit, the nonlinear effect is inhibited by a competition process involving quenching, bleaching and saturation. This is the reason why all the reported results published up-to-date present only simulation or fluorescence beads samples.

7905-39, Poster Session

STAT3 oncogenic functionality inhibition: observation at single-molecule level

B. Liu, A. Bahram, M. Avadisian, P. T. Gunning, C. C. Gradinaru, Univ. of Toronto Mississauga (Canada)

Signal-Transducer-and-Activator-of-Transcription 3 (STAT3) protein plays an important role in the onset of cancers such as leukemia and lymphoma. In this study we want to present a novel therapeutic modality for targeting the oncogenic motility of STAT3 proteins in human diseases. We have designed a cholesterol-based protein membrane anchor (PMA) to tether STAT3 to the phospholipid bilayer of cell membrane and thus inhibit unwanted transcription at the cell nucleus. Using fluorescence techniques such as fluorescence correlation spectroscopy (FCS) and fluorescence anisotropy (FA), we have examined PMA-STAT3 interactions at bulk level; the dissociation constant of PMA-STAT3 was determined in the order of sub-micro molar. We have also demonstrated the membrane localization of STAT3 via PMA by encapsulating TMR labelled STAT3 within giant lipid vesicles. The efficiency and the stability of anchoring the protein in the liposome bilayer were addressed via quantitative epi-fluorescence imaging.

We also probed the STAT3:PMA:Membrane interactions by incubating TMR-STAT3 on top of a PAMC bilayer (containing the PMA) supported on a microscope coverslip. Epi-fluorescence images showed that majority of STAT3 molecules were firmly anchored to the membrane. These studies successfully demonstrated that PMA-induced protein localization is a conceptually viable therapeutic strategy with STAT3 as our model. Ongoing single-molecule experiments will address in depth the molecular mechanism of inhibition and the movement of the ligand-protein complex in the membrane bilayer.

We have developed an assay to entrap, on average, a single pair of PMA and STAT3 molecules in nanovesicles and studied their transient interactions on (sub)millisecond time scale using single molecule Fluorescence Resonant Energy Transfer (smFRET). We envisage that successful application of PMAs in tumors containing aberrant STAT3 activity will be of significant therapeutic importance. Thus, further studies to determine the biochemical and biological utility of PMA-STAT3 interactions are ongoing. Our work will provide valuable information for engineering new small molecule chemo-preventative therapeutics to eradicate haematological malignancies.

7905-40, Poster Session

Single-molecule fluorescence of fast-flowing molecules in microfluidic devices

H. Li, Univ. of Cambridge (United Kingdom)

Single molecule fluorescence experiments are now widely used to detect and characterise biological molecules. In order to rapidly investigate rare analytes down to femtomolar concentration, we have developed a simple experimental set-up to perform single molecule fluorescence on molecules under fast flow up to 10 cm s⁻¹, using flow focussing in a microfluidic device. Dual labelled DNA molecules are excited and detected on the microsecond timescale when they are flowing faster than 1 cm.s⁻¹. Photophysical effects, such as occupation of triplet states and cis/trans photoisomerisation of cyanine dyes that occur on the millisecond timescale are therefore not observed in our measurements. Fluorophores exhibit enhanced brightness (by up to seven-fold) and two colour brightness ratio histograms, commonly used in the analysis of TCCD and FRET data, are narrowed by ∼30%. These properties should improve the capacity of single molecule detection to resolve rare sub-populations and encoded molecules for use in multiplexed biomolecular assays. By encapsulating the molecules in water droplets in oil we can also perform single molecule experiments on protein unfolding. This is a simple way to obtain significant improvements in single molecule fluorescence experiments and perform studies under non-equilibrium conditions.
New insights into the molecular structure of mRFP and EYFP by single-molecule SERS spectroscopy

K. Elgass, S. Peter, A. J. Meixner, F. Schleifenbaum, Eberhard Karls Univ. Tübingen (Germany)

SERS on biological samples and even in living cells using metallic nano-particles has been established in the past decade as a competing method to the well-known fluorescence microscopy approach using autofluorescent proteins (AFPs) for the investigation of intracellular processes.

Raman spectra provide information about the molecular structure of the investigated molecules by probing the vibrational energies of the molecular bonds. However, due to the high complexity of Raman spectra from large molecules such as proteins, it is essential to know the Raman fingerprint of the investigated proteins and possible changes in the fingerprint due to environmental changes. Spectral jumping of Raman bands is well-known as typical single molecule behavior. Consequently, averaged SERS spectra exhibit a less defined band structure than single molecule SERS spectra [2]. For a more facilitated interpretation of the obtained spectra as well as for avoiding intrinsic averaging it therefore is suggestive to investigate single AFPs to gain insight into the local physico-chemical environment and individual molecular characteristics.

We present for the first time single molecule SERS imaging and spectroscopy of the autofluorescent proteins mRFP and EYFP and we compared the molecular fingerprints of mRFP, GFP and EYFP. We observed a Raman band which seems to be specific for red emitting chromophores and several EYFP-specific bands. Additionally, single molecule SERS spectra of EYFP revealed a cross-correlation of two double-bands which can be assigned to the neutral and the anionic form of EYFP. This finding opens up possible applications of EYFP-single-molecule-SERS as a highly sensitive probe for local pH-variations.


Methods to quantify dose-dependent repair kinetics for these DNA-PKCS protein variants upon exposure to gamma-rays. We show show that repair kinetics for gamma irradiation are not the same as those obtained with laser-induced DNA damage. Importantly, the developed methods are highly generalizable and could be used to quantify the kinetics of other DNA damage sensing, signaling, and repair proteins in the living cell.

Fluorescent cyclic voltammetry of immobilized amicyanin: toward single-molecule fluorescent detection


Amicyanin from Paracoccus versutus is a type 1 blue copper protein. This was labeled with a fluorescent dye, which allows for sensing and visualization of protein dynamics. It is shown how chemical redox switching in bulk can be monitored by labeling amicyanin with ATTO647N. The labeled amicyanin was about 94% less fluorescent in the oxidized than in the reduced state. Labeled apo-azurin (with no redox center) was used as a control sample and did not show any fluorescence switching. Chemical redox switching at the single molecule level was observed for amicyanin immobilized on glass. Next, amicyanin was immobilized on semi-transparent gold electrodes with self-assembled monolayers (SAMs). We are currently investigating the fluorescent cyclic voltammetry at pH 7.0 for several choices of SAM. On the basis of this novel method of fluorescent voltammetry, fluorescently labeled redox proteins could be used for an extremely sensitive biosensor, with potential for detection of enzyme turnover at the single molecule level.

Nanoplasmonic enhancement of field localization for superresolution imaging: a computational approach

W. Lee, K. Kim, D. Kim, Yonsei Univ. (Korea, Republic of)

Super-resolution imaging has been widely investigated for analyzing nano-scale molecular interactions in live cells. There have been many approaches including STED microscopy, field optical superlens imaging, and photo-activated light microscopy. In this paper, we study the feasibility of sub-diffraction-limited high resolution imaging based on locally amplified evanescent electromagnetic waves, a.k.a. hot spots, excited and localized by surface nanostructures. To understand the nature of the hot spots, we have numerically investigated the effect of structural parameters such as shape, size, and distribution of various nanopatterns on the imaging characteristics and examined the degree of field localization into hot spots and the amount of background noise. We have considered both random and periodic structures, which include metallic nanoislands, nanogratings and arrays of nanoholes and nanoparticles. The numerical models for the nanopatterns were simulated using rigorous coupled-wave analysis within practical parameter ranges in terms of fabrication. Field properties were analyzed as a nanopattern transition from aperiodic to periodic structures. Near-field properties were also calculated as the number of ridges is varied from one (single nanowire) to infinite (nanograting), which shows the formation of significant subwavelength field localization. Structures that can reduce the localized fields on the sidelobe will be discussed. In general, the spatial predictability of field localization is found to be critical in imaging applications. It is expected that the understanding of field localization by way of surface nanostructures will help provide a highly efficient platform for super-resolution imaging.
Using fast flow to study protein unfolding kinetics under non-equilibrium conditions
S. M. Ibrahim, G. Blaser, J. Shim, C. Abell, S. Jackson, D. Klenerman, Univ. of Cambridge (United Kingdom)

Our group has recently described a simple experimental set-up that uses fast flow of analyte molecules in a microfluidic device to achieve an enhanced rate of data acquisition in single molecule fluorescence measurements by up to two orders of magnitude. By rapidly encasing protein molecules in aqueous droplets in oil, which contain a fixed concentration of denaturant, we have been able to study protein folding/unfolding kinetics under non-equilibrium conditions. We applied this method together with our single-molecule fluorescence coincidence detection technique to study the folding landscape of the small 76-residue protein, ubiquitin, and the large blue fluorescent protein labelled with a donor and acceptor fluorescence resonance energy transfer (FRET) fluorophore pair. The measured unfolding and refolding rate constants obtained using this technique are in agreement with both ensemble measurements and our previous single-molecule measurements using a nano-pipette. However by exploiting the faster data acquisition in the microfluidic device it is possible to obtain FRET histograms at different time points in the unfolding or folding process.

Monitoring kinetics and dynamics of DNA double strand break repair proteins
S. Abdissaalam, The Univ. of Texas at Arlington (United States)

No abstract available.

Superresolution imaging of HIV-1 cell-to-cell transmission
G. P. McNerney, NSF Ctr. for Biophotonics Science and Technology (United States); B. Dale, Mount Sinai School of Medicine (United States); D. L. Thompson, NSF Ctr. for Biophotonics Science and Technology (United States); B. K. Chen, Mount Sinai School of Medicine (United States); T. R. Huser, NSF Ctr. for Biophotonics Science and Technology (United States) and UC Davis Medical Ctr. (United States)

HIV-1 uses multiple mechanisms to evade the human immune system. Here, we investigate the elusive method of cell-to-cell viral transmission via the virological synapse (VS). When a HIV-1 expressing CD4+ T lymphocyte engages another CD4+ T lymphocyte, a VS can form which both strengthens their adhesion and aids viral assembly for release towards the acceptor cell. That cell then rapidly takes up several newly formed viral particles over a short period of time through a largely uncharacterized pathway. This was quantitatively described after (1) the creation of a fluorescent, replication competent, and fully infectious clone of HIV termed HIV Gag-IgFP and (2) the use of rapid time-lapse fluorescence microscopy. The genetically encoded green fluorescent protein (GFP) was crucial for observing this phenomenon because it allowed us to avoid having to isolate viral particles in order to fluorescently tag them. Detailed analysis of the seamless time-lapse tracking data was limited by the limited resolving power of conventional light microscopy (~215 nm) compared to the HIV-1 virion diameter (~130 nm). Structured illumination microscopy (SIM) is a technique that circumnavigates this limit (to ~115 nm) so we therefore used SIM to study the VS mediated transmission of HIV-1. Furthermore, for this application, SIM’s multichannel capability, 3D imaging, short imaging time, and short reconstruction time made SIM a very attractive option over other super-resolution techniques. Here we review recent results from this fresh new look at HIV-1 transmission.

Fractal-like structures for single-molecule brightness and stability improvement
E. Apicella, Consiglio Nazionale delle Ricerche (Italy); Z. K. Gryczynski, R. Luchowski, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); T. Shtoyko, The Univ. of Texas at Tyler (United States); S. D’Auria, Consiglio Nazionale delle Ricerche (Italy); P. Sarkar, S. Raut, J. Borejdo, I. Gryczynski, Univ. of North Texas Health Science Ctr. at Fort Worth (United States)

We describe a method for monitoring the single molecules fluorescence performance in the confocal microscopy technique. We present fractal-like structures substrate that highly improves dye brightness and stability. We report experiments with the dye labeled proteins at low concentrations down to picomolar to see well separated bright spots. Described experiments compared two model immunoassays based on the deposition of rabbit IgG on silver structure. Under standardized conditions we observed stability and brightness enhancement only for those dyes which were immobilized to the surface using only primary antibody. The model immunoassay with primary (nonlabelled) and secondary antibody (labeled) showed the similar intensity as for bare glass substrate. Furthermore, based on scattered/back reflected light it was possible to show that improvement of the fluorescence parameters comes from the places with fractal-like structures. Fractal-like substrate should find a wide application for single molecule studies where the longer time for their observation is demanded.

Fluorescence correlation based studies with solvatochromic dye LDS 798 to characterize size and composition of lipid-based drug delivery vehicles
P. Sarkar, R. Luchowski, S. Raut, A. G. Lacko, I. Gryczynski, Z. K. Gryczynski, Univ. of North Texas Health Science Ctr. at Fort Worth (United States)

Styryl-11 or LDS 798 is an aminostyryl quimolinum dye that has near infra red emission spectra. It’s spectral, radiative and fluorescence decay properties are heavily dependant on the environmental polarity and viscosity. Its quantum yield and fluorescence lifetime increases in hydrophobic environment, but it can dissolve well in aqueous solution. We have used this property of LDS 798 to label hydrophobic nanoparticles designed for drug delivery and characterized their dimensions by studying their diffusion profile by correlation spectroscopy. Using FCS, we were able to detect concentrations less than nanomoles in ideal conditions. Also, we were able to determine whether the particles were carrying the drug (loaded) or empty by measuring the spectral properties and correlation pattern of LDS 798. This concept can be used as a complementary technique to light scattering and transmission electron microscopy for characterizing particle sizes. The NIR absorption and emission of LDS also open possibilities for deep tissue in-vivo applications to monitor/track particles in blood and tissue.
7905-50, Poster Session

**Lifetime-based discrimination between spectrally matching vis and NIR emitting particle labels and probes**

K. Hoffmann, Bundesanstalt für Materialforschung und -prüfung (Germany); T. Behnke, Deutsches Zentrum für Luft- und Raumfahrt e.V. (Germany); U. Resch-Genger, Bundesanstalt für Materialforschung und -prüfung (Germany)

Current security and health concerns require robust and efficient strategies for the detection of multiple analytes or events in parallel. Accordingly, there is an increasing need for simple multiplexing strategies that enable the simultaneous analysis of different targets. An attractive alternative to spectral multiplexing, which relies on fluorescent labels excitable at the same wavelength, yet sufficiently differing in their emission spectra, presents lifetime multiplexing [1]. This strategy has been recently exploited for the discrimination between quantum dot labels and organic dyes revealing multi-exponential decay kinetics and their quantification based upon “pattern-matching” in the lifetime domain. Meanwhile, we succeeded in further expanding the lifetime multiplexing to nanometer-sized particle labels and probes absorbing and emitting in the visible (vis) and near-infrared (NIR) spectral region. Here, we present a first proof-of-principle of this approach for both a pair of VIS- and a pair of NIR-fluorescent particles (25 nm; 100 nm), each loaded with a single dye. The dyes were chosen to reveal similar or even matching absorption and emission spectra, yet different fluorescence lifetimes.

Examples for the lifetime-based distinction between pairs of fluorescent nanoparticles in solution and in cells were presented, despite their complex decay kinetics, thereby underlining the potential of lifetime multiplexing for the life sciences and bioanalysis.

References:


7905-51, Poster Session

**Direct volume-measurement of unlabeled proteins in solution using inverse-FCS**

T. Sandén, R. Wyss, C. Santschi, Ecole Polytechnique Fédérale de Lausanne (Switzerland); S. Wennmalm, Royal Institute of Technology (Sweden); H. Vogel, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Inverse-Fluorescence Correlation Spectroscopy (iFCS) is a recently developed modification of standard FCS that allows analysis of particles without labeling. The particles do not need to generate a signal, instead a signal is generated by the solution surrounding the particles. Particles diffusing through the FCS-detection volume displace a fraction of the surrounding solution, causing transient dips in the detected signal. The dips give a direct, and more accurate measure of the particles’ volume, compared to methods that rely on estimating the diffusion coefficient. Here we show that measurements in reduced detection volumes allow iFCS-analysis of protein molecules.
Theoretical studies of floating-reference method for NIR blood glucose sensing

Z. Shi, Y. Yang, H. Zhao, W. Chen, R. Liu, K. Xu, Tianjin Univ. (China)

Non-invasive blood glucose monitoring using NIR light has been suffered from the variety of optical background that is mainly caused by the change of human body, such as the change of temperature, water concentration, and so on. In order to eliminate these internal influence and external interference a so called floating-reference method has been proposed to provide an internal reference. From the analysis of the diffuse reflectance spectrum, a position has been found where diffuse reflectance of light is not sensitive to the glucose concentrations. Our previous work has proved the existence of reference position using diffusion equation. However, since glucose monitoring generally use the NIR light in region of 1000-2000nm, diffusion equation is not valid because of the high absorption coefficient and small source-detector separations. In this paper, steady-state high-order approximate model is used to further investigate the existence of the floating reference position in semi-infinite medium. Based on the analysis of different optical parameters on the impact of spatially resolved reflectance of light, we find that the existence of the floating-reference position is the result of the interaction of optical parameters. Comparing to the results of Monte Carlo simulation, the applicable region of diffusion approximation and higher-order approximation for the calculation of floating-reference position is discussed at the wavelength of 1000nm-1800nm, using the intralipid solution of different concentrations. The results indicate that when the reduced albedo is greater than 0.93, diffusion approximation results are more close to simulation results, otherwise the high order approximation is more applicable.

An effective method based on reference zone for glucose sensing at 1100-1600 nm

J. Zheng, Y. Yang, K. Xu, Tianjin Univ. (China)

Non-invasive blood glucose sensing by near-infrared spectroscopy is easily interrupted by the strong background variations compared to the weak glucose signals. In this work, according to the distribution of diffuse reflectance intensity at different source-detector separations, a method based on a reference zone and a measuring zone, where the diffuse reflectance intensity is insensitive and most sensitive to the variation of glucose concentration, respectively, is applied. And the data processing method based on the information of two zones is investigated to improve the precision of glucose sensing. Based on the Monte-Carlo simulation in 5% intralipid model, the corresponding optical probe is designed which includes two detecting zones: a reference zone located in 1.3-1.7 mm and a measuring zone located in 1.7-2.1mm. Using the probe, the in vitro experiment with different glucose concentrations is conducted in the intralipid solution at 1100-1600nm in order to build the partial least-square (PLS) model. As a result, compared to the PLS model built by the signal of the measuring zone, the root mean square error of calibration (RMSEC) and root mean square error of cross validation(RMSECV) of the corrected model built by two detecting zones reduces by 43.73%, and 37.04% respectively.

Discriminant analysis of milk adulteration based on infrared spectroscopy and pattern recognition

R. Liu, G. Lv, B. He, K. Xu, Tianjin Univ. (China)

Since the beginning of the 21st century, the issue of food safety is becoming a global concern. It is very important to develop a rapid, cost-effective, and widely available method for food adulteration detection. In this paper, infrared spectroscopy techniques and pattern recognition were applied to study the qualitative discriminant analysis method. The samples were prepared and adulterated with one of the three adulterants, urea, glucose and melamine with different concentrations. First, the spectral characteristics of milk and adulterant samples in mid-infrared (MIR) and near-infrared (NIR) regions were analyzed. Then, pattern recognition methods were used for qualitative discriminant analysis of milk adulteration. Soft independent modeling of class analogy (SIMCA), Bayes discriminant analysis, and partial least squares discriminant analysis (PLSDA) were used to construct discriminant models, respectively. Furthermore, the optimization method of the model was studied. The best spectral pretreatment methods and the optimal band were determined. In the optimal conditions, PLSDA models were constructed respectively for each type of adulterated sample sets (urea, melamine and glucose) and all the three types of adulterated sample sets. Results showed that, the model achieved 93.2% and 92.6% discriminant accuracy respectively in NIR and MIR regions, in the classification of different adulterated and unadulterated milk samples. Thus, it can be concluded that infrared spectroscopy and PLSDA can be used to identify whether the milk has been adulterated or not and the type of adulterant used.

An investigation of the effect of in-vivo interferences on Raman glucose measurements

B. Shim, H. Oh, J. Oh, Y. Ku, M. Kim, Y. Yang, D. Kim, H. Eum, S. Cho, D. R. Miller, LG Electronics Inc. (Korea, Republic of)

Raman spectroscopy is a promising technology for noninvasive blood glucose monitoring because of its good selectivity for the glucose molecule. The low sensitivity of the Raman signal however makes it difficult to quantify the concentration of glucose directly from the Raman spectra. To solve this, statistical methods such as PCA (principle component analysis) and PLS (partial least square) are traditionally used. These statistical methods general work very well and give highly accurate results, provided there is no interference. In the in-vivo case however, there are many interferences such as the inhomogeneity of tissue, physiological changes, and denaturation by the light source. This study investigates the effect of in-vivo interferences on Raman glucose measurements.

In this study, a high throughput dispersive Raman system was constructed with an 830nm multimode laser, a multiple conductor optical fiber bundle, and a back-illuminated CCD. A simply phantom was devised, which is comprised of a plastic cuvette equipped with a human fingernail window and glucose doped human serum used as the sample. To test the inhomogeneity of tissue samples, different sites of the phantom were exposed to the laser. In the case of denaturation, tests were conducted under two laser power densities: low (3.7mW/mm²) and high density (110mW/mm²). To simulate the physiological change, gelatin phantoms of varied concentration were investigated. The result study indicates that the dominant interferences for Raman in-vivo glucose
measurements are the inhomogeneity of the tissue and the denaturation by the laser power density. The next phase for this study will be the design of a high SNR Raman system which affords a low power density laser sample illumination as well as larger volumetric illumination to mitigate the effects of tissue inhomogeneity.

7906A-20, Poster Session

Mapping skin using Raman spectroscopy
A. E. Villanueva-Luna, J. Castro-Ramos, S. Vazquez-Montiel, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico); A. Flores-Gil, Univ. Autónoma del Carmen (Mexico); J. A. Delgado Atencio, C. M. Ortiz-Lima, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico)

In this work we carried out a comparison and localization of the skin Raman spectra. To carry out measurements were made in regions of Raman fingerprint for different skin conditions such as diabetics and retinopathy. In addition to the skin spectra overlap in a comparative way. We took 10 volunteers with different skin colors, body parts sampled were the palm and dorsum of the hand. The excitation wavelength used for this work is 785 nm, a spectrometer with 6 cm-1 resolution and the spectral region used ranges from 300 to 1800 cm-1. We use Matlab to overlap and compare the differences between Raman spectra. We found spectral variations that are caused by difference of surface skin, such as acne and moles. This work is useful because it helps identify potential undesirable behavior of the epidermis.

7906A-21, Poster Session

Prospects of glycemic marker detection using drop coating deposition Raman spectroscopy
N. C. Dingari, I. Barman, J. W. Kang, C. Kong, R. R. Dasari, M. S. Feld, Massachusetts Institute of Technology (United States)

Diabetes mellitus, characterized by the defective regulation of blood glucose, is one of the leading causes of morbidity and mortality worldwide. Given the lack of appropriate therapy alternatives, acute and chronic health complications such as diabetic coma and retinopathy may occur in the absence of glucose monitoring. In addition to blood glucose monitoring, glycated hemoglobin (HbA1c, formed by the non-enzymatic glycosylation of hemoglobin exposed to high levels of blood glucose) has been extensively employed as one of the reliable long-term markers of glycemic control. While several assay techniques are currently in use for analyzing HbA1c levels, these lack ideal sensitivity and specificity, especially in the presence of hemoglobin and uremia.

We propose the application of Raman spectroscopy for sensitive and robust detection of HbA1c. While surface enhanced detection provides an attractive option for detection of minute amounts of analytes, the lack of reproducibility and quantification leads us to a (non-enhanced) pre-concentration based method of detection, namely drop coating deposition Raman (DCDR). In DCDR, Raman spectra are collected from the centers of droplet by the deposited analytes from a drying drop, whose formation is governed by contact line pinning, solvent evaporation and subsequent capillary flow. In this talk, we present the first results of DCDR-based detection of HbA1c. We also highlight the significant differences between HbA1c and hemoglobin features, setting the stage for accurate quantification. In addition, it is shown that the DCDR spectra of these analytes are representative of their hydrated states rather than the solid form.

7906A-22, Poster Session

Comparative study of optical activity in chiral biological media by polar decomposition and differential Mueller matrices analysis
N. Ortega-Quijano, F. Fanjul-Vélez, I. Salas-García, J. L. Arce-Diego, Univ. de Cantabria (Spain)

The introduction of polarimetry in optical imaging of biological tissues provides a powerful method to enhance contrast and specificity in the characterization of anisotropic biological tissues. Moreover, the fact that Mueller calculus can deal with partially polarized light and depolarizing media enables to analyze the strong effect of scattering in light propagation through biological tissues.

In this work, we propose a polarimetric analysis based on differential Mueller matrices. This analysis is not affected by the order in which the effects take place within the medium. We apply it to the study of optical activity in chiral and turbid biological media, in particular to phantoms consisting of a solution of glucose mixed with an aqueous suspension of polystyrene microspheres. The results obtained by Lu-Chipman polar decomposition and by differential Mueller matrices analysis are compared. The results obtained by the polarimetric analysis proposed in this work are in good agreement with those obtained by polar decomposition, with the advantage that differential Mueller matrices provide additional information to further develop polarimetric analysis in a robust way.

7906A-23, Poster Session

Probing Orientation and rotation of red blood cells in optical tweezers by digital holographic microscopy
N. Cardenas, The Univ. of Texas at Arlington (United States); L. Yu, Nanoscope Technologies LLC (United States); S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Interaction of red blood cells (RBC) with optical tweezers has been found to differ under varied physiological (e.g. high osmolarity) and pathological (malaria parasite infection) conditions as compared to its normal conditions. Earlier, we reported difference in rotation of trapped RBC for detection of malaria infection. Disk-like RBC (in isotonic or hypertonic solution) when trapped in optical tweezers get oriented in the vertical plane to maximize interaction with trapping beam. However, classical bright field, phase contrast or epi-fluorescence microscopy cannot confirm its orientation and led to ambiguous conclusions such as folding of RBC during trapping by some researchers. Now, by use of digital holographic microscopy (DHM), we achieved high axial sensitivity that confirmed orientation of trapped red blood cell. Further, DHM enabled quantitative phase imaging of RBC under hypertonic condition. Dynamic changes of rotating RBC (in hypertonic solution) under optical tweezers at different trapping laser power were evaluated by use of DHM. The deviation from linear dependence of rotation speed of RBC on laser power was attributed towards deformation of RBC shape at higher laser power (or speed).
In-vivo particle image velocimetry of the blood flow using the confocal laser scanning microscope

H. Lee, W. Kim, S. Choi, C. Park, Kyungpook National Univ. (Korea, Republic of)

In the previous meeting (Photonic West 2010), we reported “the blood cell-based Particle Image Velocimetry (PIV)” in a micro-tube using the Confocal Laser Scanning Microscope (CLSM). In that study, we employed blood cells as tracing particles, while previous microscopy-assisted PIV required exogenous micro/nano particles as the tracing particles.

In this study, we are reporting the application of the “the blood cell-based PIV” for the measurements of blood stream in a live animal. We used video-rate confocal laser scanning microscopy to observe the motion of blood cells in capillaries of live mouse ear. The video-rate CLSM allowed us to acquire images at the rate of 30 frames per second. The optical sectioning ability of the CLSM makes it possible to image the blood stream at different depths of the blood vessel. The acquired images were used to perform PIV, thus providing the blood velocity profile in capillaries of mouse ear. The velocity near the center of the vessel was measured to be a few hundred µm/sec and the velocity near the wall was about tens of m/sec. The clinical applications of the in vivo confocal PIV are discussed based on the current study.

Real-time monitoring of optical rotation with wavelength dependence by spectroscopic polarized modulation

Y. Otani, Utsunomiya Univ. (Japan); T. Wakayama, Saitama Medical Univ. (Japan); M. Tanaka, Atago Co., Ltd. (Japan); M. Chujo, Tokyo Univ. of Agriculture and Technology (Japan)

It has been mainly used for the concentration measurement of the saccharide and amino acid in chemical field, and sugar and glucose concentration in a field of food and chemical industry. In addition, it is also applied to determine the molecular structure of the amino acid elaborated protein. This report is to propose a real-time monitoring of optical rotation with wavelength dependence for optical rotation (optical activity). Its system is consisted on a polarizer, a birefringence plate, an achromatic quarter wave plate, an analyzer and optical activity (optical activity). Its system is consisted on a polarizer, a birefringence plate, an achromatic quarter wave plate, an analyzer and optical activity (optical activity). Its system is consisted on a polarizer, a birefringence plate, an achromatic quarter wave plate, an analyzer and optical activity (optical activity). The intensity of spectroscopic polarized light is detected by a polarimeter. It is modulated as sinusoidal wave along wave number. The optical rotatory dispersion of the sample is analyzed by Fourier transform method. The properties of optical rotatory dispersion of standard optical rotator made by quartz plate are shown as experimental demonstrations.

Improved calibration procedure for laser Doppler perfusion monitors

I. Fredriksson, Linköping Univ. (Sweden) and Perimed (Sweden); F. Salomonsson, Perimed AB (Sweden); M. Larsson, T. Strömberg, Linköping Univ. (Sweden)

Commercial laser Doppler perfusion monitors are calibrated using the perfusion value, i.e. the first order moment of the Doppler power spectrum, from a measurement in a standardized microsphere colloidal suspension under Brownian motion. The measured perfusion value depends strongly on several parameters of the measurement object that are difficult to keep constant with adequate accuracy, such as the suspension concentration, temperature and the microsphere size distribution.

Variations in the reference suspension can have a significant influence on the reference perfusion value and thus on all measured values. For example, deviation in the temperature, sedimentation or aggregation of the motility standard, or small vibrations of the suspension beaker, all have a large effect on the calibration value. These variations only affect the frequency content of the measured Doppler power spectrum and not the total power. Therefore, by calibrating the system against the zero order moment of the Doppler power spectrum instead of the first order, the calibration procedure will be independent of optical properties (concentration, size distribution etc.), temperature and movements during the procedure.

This improved calibration procedure, including the signal processing required for high quality zero order moment calculations, is described and demonstrated in this paper. The difference in the calibration constant given by measuring on several scattering liquids with a wide range of scattering properties and temperatures is only a few percent. For the conventional calibration procedure, this variation corresponds to the error introduced by merely a 1°C variation of a reference liquid.

Flow cytometry of blood using spectrally encoded confocal microscopy

L. Golan, L. Minai, D. Yelin, Technion-Israel Institute of Technology (Israel)

Flow cytometry of blood samples provides statistical information on cell populations and is a valuable technique for clinical diagnosis of various diseases. Online monitoring of blood cells flowing in vivo in subsurface vessels could be useful for real-time diagnosis of certain life-threatening conditions. Such applications often require imaging of the cells based on their endogenous contrast and avoid the use of externally applied fluorescent markers.

In this work, we demonstrate a high resolution imaging technique based on spectrally encoded confocal microscopy (SECM), which allows imaging of large quantities of cells flowing in parallel through large diameter vessels, using compact imaging probes. Our flow cytometry system contained an imaging probe comprising of a diffraction grating and a high numerical aperture objective lens, which encodes linear spatial locations by wavelengths from a broadband light source. When flowing cells cross the spectral line, the scattered light is detected by a high speed spectrometer, forming a two dimensional image of the entire flowing cell population. The confocal reflectance images provide information on cell size, shape, orientation, as well as on the flow velocity profile across the vessel. Our bench-top system demonstrated sub-cellular resolution imaging of red blood cells flowing at 1 mm/s through a 0.25 mm² flow chamber, with a few micron depth of focus which enabled resolving single cells within a rapid, high-density flow.

The imaging probe could be made compact with no moving parts, does not require electrical power, and is connected to the light source and detection system only by a single optical fiber. This new concept could be useful for non invasive in vivo flow cytometry and as a bedside device for real-time monitoring of hematological disorders.

An optical device employing multiwavelength photoplethysmography for non-invasive in-vivo monitoring of optically active nanoparticles

G. J. Michalak, P. Adhikari, Louisiana Tech Univ. (United States); J. A. Schwartz, G. P. Goodrich, Nanospectra Biosciences, Inc. (United States); D. P. O’Neal, Louisiana Tech Univ. (United States)
Nanoparticles have emerged as a popular tool in the treatment of cancer. Increasing numbers of researchers are developing multifunctional nanoparticles which incorporate biologically and optically active compounds to aid in drug delivery, imaging contrast, and active tumor targeting. These particles are often manufactured using several compounds resulting in a complex architecture, which can be quantified ex vivo by conventional metrology and chemical assays. However, the in vivo behavior of these particles is difficult to predict because of the complex mechanisms of action in any organism. We present a non-invasive optical device capable of measuring the circulating concentration of optically active nanoparticles. The device employs photoplethysmography, a technique used to measure small volume changes in biological tissue using the change in transmission or reflection of light. Similar to the pulse oximeter, the pulse photometer uses multiple wavelengths of visible and near infrared light to interrogate the pulsatile arterial blood and determine its spectral characteristics and therefore its composition. The device was developed to aid in the batch testing of manufactured nanoparticle lots in a murine model and is used to monitor long-circulating nanoparticles and blood oxygen saturation in real-time, with or without anesthesia. This device has the potential to replace conventional ex-vivo assays providing real-time information about the in vivo pharmacokinetics of these nanoparticles. As human trials employing gold nanoshells progress, we hope to employ this technique to provide clinical feedback in conjunction with nanoparticle-assisted thermal therapies.

7906A-04, Session 1

Optimizing source detector separation for an implantable perfusion and oxygenation sensor

T. J. Aki, T. J. King, R. Long, Texas A&M Univ. (United States); J. S. Baba, Oak Ridge National Lab. (United States); M. J. McShane, Texas A&M Univ. (United States); M. N. Ericson, Oak Ridge National Lab. (United States); M. A. Wilson, Univ. of Pittsburgh (United States) and VA Pittsburgh Healthcare System (United States); G. L. Coté, Texas A&M Univ. (United States)

Each year thousands of patients are added to the waiting list for liver transplants. The first 7-10 days after transplant have proven to be the most critical in patient recovery and it is hypothesized that monitoring organ viability signals in this period can increase patient and graft survival rates. An implantable sensor to monitor the organ perfusion and oxygenation signals following surgery is being developed by our group. The sensor operates based on measuring diffuse reflection from three Light Emitting Diodes (735, 805 and 940 nm). In this work the optimal source detector spacing to maximize signal level is investigated. Monte Carlo simulations provided signal levels and corresponding penetration depths as a function of separation between the optical source and detector. To verify these results, we constructed a phantom with optical properties similar to liver tissue in the range of 700 to 1000 nm and perfused it with dye combinations mimicking the optical properties of different blood oxygenation states. Data collected from this in vitro setup were compared to the modeling results. Both results indicated a rapid increase in the optical signal with increasing distance. Through further analysis, it was found that there exists an optimal range of source detector spacing, between 2 and 3 mm, in which the blood signal from the simulated portal vein was maximized. Overall, these results are being used to guide the placement and configuration of our probe for in vivo animal studies.

7906A-05, Session 2

Automated on-chip semen analysis using a handheld lensfree holographic microscope

T. Su, A. F. Erlinger, D. K. Tseng, A. Ozcan, Univ. of California, Los Angeles (United States)

Semen analysis is an important routine that is widely practiced in laboratories for evaluating male fertility and for preparing artificial insemination. However, there is currently no automated technology that can investigate both sperm concentration and motility on a compact and lightweight platform. To provide a solution to this challenging task, here we demonstrate automated semen analysis using a lensfree on-chip microscope. This compact holographic microscope weighs ~46 grams and does not require any lenses, lasers or other bulky optical components to achieve phase and amplitude imaging of sperms over ~24 mm2 field-of-view with a numerical aperture of ~0.2. Using this wide-field lensfree on-chip microscope, each semen sample is imaged for ~10 seconds, capturing a total of ~20 consecutive holographic frames. Digital subtraction of these consecutive holographic frames enables automated quantification of the count, speed and the dynamic trajectories of the moving sperms, while summation of the same frames permits counting of the immobile sperms. The accuracy of this automated platform is verified by analyzing human semen specimens and the results show a close match to the manual counts made with a traditional bright-field microscope. Such a compact and light-weight automated semen analysis platform running on a wide-field lensfree on-chip microscope is especially important for fertility clinics, personal male fertility tests, as well as for field use in veterinary medicine such as in stud farming and animal breeding applications.

7906A-06, Session 2

Multiframe processing-based subpixel resolving optofluidic microscope for on-chip cell imaging

S. A. Lee, G. Zheng, S. Yang, C. Yang, California Institute of Technology (United States)

We report on the implementation of a subpixel resolving optofluidic microscope (SROFM) based on multiframe pixel super resolution algorithm. The proposed device utilizes microfluidic flow to deliver specimens directly across a CMOS image sensor to generate a sequence of low-resolution (LR) projection images, limited by the pixel size of the sensor. LR sequence, where each frame provides a unique “look” of the object with relative subpixel motion between subsequent frames, is used in conjunction with the pixel super resolution algorithm to reconstruct one high resolution (HR) image, in which feature sizes beyond the Nyquist rate of LR frames are visible. This approach allows us to eliminate the aperture arrays employed in current OFM systems and provide a simple on-chip microscopy design that consists simply of a microfluidic channel on a commercial 2D sensor chip. Effectively, our SROFM taking advantage of over-sampling in the time domain to compensate for under-sampling in the spatial domain, for which the resolution is limited by the sensor pixel size. We demonstrate the capability of the device by imaging the microspheres, protist Euglena gracilis, and Entamoeba invadens Cysts with sub-cellular resolution. Our prototype device can image a 0.5 micrometer microsphere with the full with half maximum of 0.91 microns, achieving 7-times resolution enhancement from 3.2-micron pixels. We also show that a HR video can be reconstructed based on the LR sequence, and thus, the dynamic-interaction between the fluid and the sample, such as the in-plane and out-of-plane rotation of the sample, can be monitored in HR.

7906A-07, Session 2

Detection of bacteria with thin wetting film lensless imaging

C. P. Allier, G. Hienard, V. Poher, J. Dinten, Lab. d’Electronique de Technologie de l’Information (France)

Recent studies have demonstrated the ability of Raman spectroscopy and FT-IR spectroscopy to detect and identify single bacteria within heterogeneous mixture. However, these techniques require a careful positioning of the illumination beam onto the bacteria within 1-3 μm,
which can hardly be achieved in sample volume of few ml with low concentration of analytes (100-1000 bacteria/ml). We propose a solution based on two modules in line, a sample preparation module connected to a spectroscopy identification module. The first module concentrates the bacteria from ml to µl, evaporates it on a microscope slide and provides the identification module with the pre-localization of the bacteria. The identification module can then scan the objects of interest only, reducing drastically the total analyses duration and presumably increasing the sensitivity.

This paper focuses on the second step of the sample preparation, namely the detection and localization of µm sized micro-objects in few µl sample. We describe a new lensless imaging technique. Micro-objects are revealed by liquid micro-lenses created during sample evaporation that focalise the incident light onto the sensor. Standard CMOS sensor is able to detect micro-objects, e.g. E.coli and Bacillus subtilis bacteria and 1 µm latex beads, with signal to noise ratio of 45±10. An overall sensitivity is drastically improved by liquid micro-lenses compared with reference fluorescence microscopy images are achieved. This novel modality will be used as a pre-positioning tool prior to Raman spectroscopy.

7906A-08, Session 2

**Novel instrumention of multispectral imaging technology for detecting tissue abnorunity in point-of-care health care**

D. Yi, Sunnybrook and Women’s Health Sciences Ctr. (Canada); F. Wang, Georgia Institute of Technology (United States); L. Kong, The Ctr. for Assistive Technology and Environmental Access (United States)

Multispectral imaging is becoming a new powerful tool in a wide range of biological and clinical studies by adding spectral, spatial and temporal dimensions to tissue abnorunity and the underlying biological processes. A traditional standard spectral imaging system includes two physically separated major components: a band-passing selection device (such as liquid crystal tunable filter and diffraction grating) and a scientific-grade monochrome camera, and is expensive and bulky.

Recently micro-arrayed narrow-band optical filter mosaic was invented and successfully fabricated to reduce the size and cost of multispectral imaging devices in order to meet the clinical requirement for medical diagnostic imaging applications. However it is challenging to integrate and place the micro filter mosaic to the targeting focal plane, i.e., the imaging sensor, of an off-shelf CMOS/CCD camera. This paper presents the methods and results of integrating such a miniaturized filter with off-shelf CMOS imaging sensors to produce handheld real-time multispectral imaging devices for the application of early stage pressure ulcer (ESPU) detection. Unlike conventional multispectral imaging devices which are bulky and expensive, the resulting handheld real-time multispectral ESPU detection can produce multiple images at different wavelengths with a single shot, therefore eliminates the image registration procedure required by traditional multispectral imaging technologies.

7906A-09, Session 2

**Needle-probe techniques for trachea identification**

W. C. Warger II, J. A. Gardecki, E. Namati, M. J. Suter, B. E. Bouma, G. C. Velmahos, G. J. Tearney, Massachusetts General Hospital (United States)

The current standard of care for bedside percutaneous tracheostomy requires two trained clinicians, one to puncture the trachea and insert the cannula and another to confirm intratracheal placement with bronchoscopy. Bronchoscopy introduces additional risks and complications, adds substantial cost, and cannot be offered in the field. In addition, paratracheal insertions can still occur despite bronchoscopy if blood and secretions prevent adequate visualization. A promising alternative would be a needle-based technique that could identify the trachea and confirm placement within the lumen to allow a single operator to perform the procedure in any setting.

We have collected data from freshly excised swine and sheep trachea to determine the feasibility of several optical diagnostic techniques that could be miniaturized within a hand-held needle-probe, including optical frequency domain imaging (OFDI), spectral encoding endoscopy (SEE), Raman spectroscopy, laser speckle imaging, and autofluorescence. The refractive index contrast between the hyaline cartilage rings and the surrounding tissues provide morphologic information within OFDI and SEE images. The biochemical properties from the collagen within the cartilage rings and the adventitial fat within the connective tissue can be probed with Raman spectroscopy. The biomechanical properties of the cartilage and the surrounding soft tissue can be differentiated with laser speckle imaging. The ratio of autofluorescence at three wavelength bands can approximate a proportion of collagen to NADH. The successful battery-operated miniaturization of these techniques individually or in combination could provide an inexpensive point-of-care instrument for single-operator procedures both at the bedside and in the field.

7906A-10, Session 3

**Understanding the mechanism and optimizing a competitive binding fluorescent glucose sensor**

B. M. Cummins, M. V. Pishko, E. E. Simanek, G. L. Coté, Texas A&M Univ. (United States)

Effective management of glucose for diabetic patients requires the frequent measurement of blood glucose levels. For compliance purposes, many groups have noted the attractive possibility for optical modalities to interrogate sensors embedded within tissue. Our lab group is currently developing a fluorescence sensing assay between the lectin Concanavalin A and highly structured glycosylated dendrimers to be sensitive to varying levels of glucose. Previously, this chemistry has elicited as much as a 100% increase in fluorescence intensity across the physiological ranges of glucose from 0-600 mg/dL. However, due to its multivalent nature, the exact mechanism behind the sensing has not yet been well understood. Therefore, to further explore the binding mechanics behind the chemistry, this work presents experiments relating the absorption, scattering, fluorescence lifetime and fluorescence polarization values of the assay to varying concentrations of glucose. Upon determining the aggregative nature of the assay and the associated scattering, the sensing chemistry was optimized to enhance the repeatability and decrease the response time of the sensor by varying the level of glycosylation and generation of the dendrimer, the degree of saccinylaation of the Concanavalin A, and the degree of labeling.

7906A-11, Session 3

**Non-invasive in-vivo glucose sensing using an iris-based technique**

A. J. Webb, B. D. Cameron, The Univ. of Toledo (United States)

Physiological glucose monitoring is important aspect in the treatment of individuals afflicted with diabetes mellitus. Although invasive techniques for glucose monitoring are widely available, it would be very beneficial to make such measurements in a noninvasive manner. In this study, a New Zealand White (NZW) rabbit animal model was utilized to evaluate a developed iris-based imaging technique for the in vivo measurement of physiological glucose concentration. The animals were anesthetized with isoflurane and an insulin/dextose protocol was used to control blood glucose concentration. To further help restrict eye movement, a developed ocular fixation device was used. During the experimental time frame, near infrared illuminated iris images were acquired along with corresponding discrete blood glucose measurements taken with a
handheld glucometer. Calibration was performed using an image-based Partial Least Squares (PLS) technique. Independent validation was also performed to assess model performance along with Clarke Error Grid Analysis (CEGA). Initial validation results were promising and show that a high percentage of the predicted glucose concentrations are within 20% of the reference values.

7906A-12, Session 3

**Advancement in polarimetric glucose sensing: simulation and measurement of birefringence properties of cornea**

B. H. Malik, G. L. Coté, Texas A&M Univ. (United States)

Clinical guidelines dictate that frequent blood glucose monitoring in diabetic patients is critical towards proper management of the disease. Although, several different types of glucose monitors are now commercially available, most of these devices are invasive, thereby adversely affecting patient compliance. To this end, optical polarimetric glucose sensing through the eye has been proposed as a potential noninvasive means to aid in the control of diabetes. Arguably, the most critical and limiting factor towards successful application of such a technique is the time varying corneal birefringence due to eye motion artifact. We present a spatially variant uniaxial eye model along with a geometric ray tracing scheme to serve as a tool towards better understanding of the cornea's birefringence properties. The potential of using a dual-wavelength polarimeter to overcome corneal birefringence under both index-matched and -unmatched environments shall be discussed. The simulations show that index-unmatched coupling of light is spatially limited to a smaller range when compared to index-matched situation. Polarimetric measurements on rabbits' eyes indicate relative agreement between the modeled and experimental values of corneal birefringence. In addition, the observed rotation in the plane of polarized light for multiple wavelengths demonstrates the potential for using a dual-wavelength polarimetric approach to overcome the noise due to time-varying corneal birefringence. These results will ultimately aid us in the development of an appropriate eye coupling mechanism for in vivo polarimetric glucose measurements.

7906A-13, Session 3

**The use of optical polarimetry as a non-invasive in-vivo physiological glucose monitor**

A. J. Webb, B. D. Cameron, The Univ. of Toledo (United States)

There is a need to effectively and accurately monitor physiological glucose levels in individuals afflicted with diabetes mellitus. One promising noninvasive technique involves the use of optical polarimetry, in which the eye is commonly used as the sensing location. Since glucose is a chiral molecule, it has the ability to rotate plane polarized light by an amount that is proportional to glucose concentration. It has also been shown that glucose levels in the aqueous humor of the eye correlate well to those of blood. Therefore, we will report on an in vivo study that is conducted using a New Zealand White (NZW) rabbit model in conjunction with a custom developed Faraday-based optical polarimeter with sub-milidegree resolution. All animals used in this investigation were anesthetized with isoflurane and an insulin/dextrose protocol was used to control blood glucose concentration. A polarized laser light (632.8nm HeNe) signal was coupled through the anterior chamber of the eye using a custom designed ocular apparatus. System calibration was performed through measurement of the detected optical polarimetric signal and corresponding discrete blood glucose measurements taken with a handheld glucometer. Reference blood glucose samples were also measured using a YSI 2300+ glucose analyzer. The study results show that physiological glucose can be predicted with error levels on the order of 15% and correlation analysis indicate that the time delay between blood and aqueous humor glucose levels is on the order of 5 minutes.

7906A-14, Session 3

**In-vivo interstitial glucose characterization and monitoring in the skin by ATR-FTIR spectroscopy**

N. Skrebova Eikje, MC Professional OÜ (Estonia)

Successful development of real-time non-invasive glucose monitoring would represent a major advancement not only in the treatment and management of patients with diabetes mellitus and carbohydrate metabolism disorders, but also for understanding in those biochemical, metabolic and (patho-)physiological processes of glucose at the molecular level in vivo.

Here, ATR-FTIR spectroscopy technique has been challenged not only for in vivo measurement of interstitial glucose levels, but also for their non-invasive molecular qualitative and quantitative comparative characterization in the skin tissue. The results, based on calculated mean values of determined 5 glucose-specific peaks in the glucose-related 1160-1000 cm-1 region, showed intra- and inter-subject differences in interstitial glucose activity levels with their changes at different times and doses of OGTT, while raising questions about the relationships between interstitial and blood glucose levels.

In conclusion, the introduction of ATR-FTIR spectroscopy technique has opened up an access to the interstitial fluid space in the skin tissue for interstitial glucose characterization and monitoring in vivo. Though interstitial versus blood glucose monitoring has different characteristics, it can be argued that accurate and precise measurements of interstitial glucose levels may be more important clinically.

7906A-15, Session 3

**The high-quality spectral fingerprint of glucose captured by Raman spectroscopy in non-invasive glucose measurement**

J. Oh, S. Cho, H. Oh, Y. Ku, B. Shim, M. Kim, Y. Yang, D. Kim, H. Eum, D. R. Miller, LG Electronics Inc. (Korea, Republic of)

Current personal blood glucose monitoring devices have been developed greatly in terms of technical limitations. However, the pain associated with finger pricking still is cited as the main reason many diabetics do not monitor themselves regularly. The elimination of pain and increased patient convenience continue to motivating scientists to develop a noninvasive blood glucose monitoring device, even in the face of significant technical challenges.

Many techniques have been studied various forms of optical and electrical spectroscopy. An optical spectroscopy has the inherent advantage of directly measuring glucose levels through molecular absorption and scattering. Another benefit of using an optical approach is unique nature of a spectral fingerprint for the target analyte, glucose, which is shown in the form of a calibration vector, a product of the partial least-squares (PLS) method. Many research groups claimed successful demonstrated noninvasive in-vitro and phantom measurements in terms of Clark error grid analysis. However in the absence of a PLS calibration vector, it is not known whether such glucose predictions were based on the target glucose molecules or on other interfering substances or uncontrolled experimental parameters.

In this study, we constructed a high throughput dispersive Raman system with 830nm multimode laser, multiple optical fiber bundle, and back-illuminated CCD. In-vitro tests as well as in-vivo pilot tests were performed and calibration vectors were collected. Correlation coefficients between a pure glucose spectrum and calibration vectors were calculated over 0.8 consistently. Results of this study include a spectral fingerprint for glucose which was noninvasively obtained and highly discriminated from interfering spectra using Raman spectroscopy.
Phantoms as standards in optical measurements

R. J. Nordstrom, National Institutes of Health (United States)

As optical technology progresses through the translational research pipeline, phantoms are becoming more important as verification tools to demonstrate proper performance of the devices before clinical studies. Because of the wide range of optical methodologies, there can be no single phantom that is useful for all modes of imaging, but protocols for phantom use should be organized to stand as standards. This paper discusses the features that phantoms must have to be considered as standards for optical measurements.

Fabrication and characterization of phantoms made of polydimethylsiloxane (PDMS)

A. E. Villanueva-Luna, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico); A. Santiago-Alvarado, Univ. Tecnologica de la Mixteca (Mexico); J. Castro-Ramos, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico); B. I. G. Licona-Moran, Univ. Tecnologica de la Mixteca (Mexico); S. Vazquez-Montiel, J. A. Delgado Atencio, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico)

This work describes the fabrication process and characterization of Phantoms made from a base of PDMS, and a mixture of different chemical substances such as melanin, glucose, glycerol and cholesteryl. We vary the concentration of the components, to detect the presence of the components. In Characterization we measured their optical properties, Raman spectrum, roughness and thickness of the samples. The characterization was used a profilometer and Raman spectrometer. The experimental results are presented, and in particular a comparison of Raman spectra obtained with the Raman spectra of blood, finding a correlation of results with compounds from the blood.

Poly(vinyl alcohol) cryogel, multi-layer artery phantoms for optical coherence tomography

C. Bisaillon, G. Campbell, V. Pazos, C. de Grandpré, G. Lamouche, National Research Council Canada (Canada)

We present recent progress made in the development of multi-layer artery phantoms using a poly(vinyl alcohol) cryogel (PVA-C) matrix. PVA-C provides more realistic mechanical properties than other usual phantom materials like silicone or polyurethane because its elastic profile (stress/strain) can be more similar to soft tissues. It also has good potential for multimodal phantoms as it is already used in the fabrication of phantoms in ultrasound and MRI imaging. However, obtaining specific and homogeneous optical properties in PVA-C represents quite a challenge because many composition and processing parameters influence the resulting cryogel. We previously reported a strategy to vary the optical properties of PVA cryogels by adjusting the number of freeze-thaw cycles (FTC) and adding scattering particles and an absorbent. Recent achievements include the determination of the parameters needed to replicate the characteristic OCT signal of the three layers of coronary arteries: the intima, the media and the adventitia. It also includes the fabrication of PVA multi-layer phantoms by an over-molding technique. The elastic profile of the phantom is characterized by tensile tests and demonstrates strain hardening. The mechanical properties obtained are compared to values for coronary arteries found in the literature. Different strategies are implemented to further refine both the optical properties and the mechanical properties. Furthermore, alternative fabrication techniques are explored to improve the uniformity of multi-layer wall dimensions. Finally, potential use of these phantoms will be discussed. Native fabrication techniques are explored to allow a better control on the shape of the phantoms. Finally, potential use of these phantoms will be discussed.

Accurately characterized optical tissue phantoms: how, why and when?

J. Bouchard, I. Veilleux, I. Noiseux, O. Mermut, INO (Canada)

Optical tissue phantoms are very important tools for the development of biomedical imaging applications. A phantom’s optical and physical properties will generally be considered as the true values against which instruments results can be compared to determine their performances. Determining and optimizing the level of accuracy of the technique used to characterize phantoms is, therefore, of critical importance. Calibration phantoms of good and known absolute accuracy are key tools in ensuring instrument standardization and, therefore, long term consistency of the data they produce. Accessing the level of accuracy of phantoms’ properties is also relevant to traceability aspects mandated by FDA regulations for the design and manufacturing of medical devices. Here, basic metrology concepts and terminology will first be reviewed to clarify the distinction between subsets of phantoms, for example, phantoms for accuracy and precision goals vs phantoms for mimicking the real tissue. A review of the literature on the subject of characterization accuracy of tissue simulating phantoms will then be presented. The latest results for error estimation of a time-resolved transmittance characterization technique will be presented. The requirements for accuracy will then be discussed with respect to the various end goals that need to be achieved when using tissue simulating phantoms during the development, verification or validation phase of a clinical application development. Although the discussion will be presented in the context of bulk diffuse optical tissue phantoms, many of the basic concepts presented here are applicable to other types of phantoms.
scattering and absorption, homogeneity, tissue-like refractive index and durability have been extensively investigated. Absorption is included by adding a green dye and scattering by adding TiO2 or SiO2 particles. Optical coherence tomography measurements demonstrated a linear dependence of the attenuation coefficient with scatter concentration. Tissue often possesses structural inhomogeneity. Layers of different cell types can be formed, cavities containing fluid and blood vessels can disrupt uniform tissue structures, all resulting in a structural complex geometry. It is therefore paramount that structural variations such as layers or inclusions can be incorporated in the phantom. A thin layered phantom configuration enables evaluation of diagnostic modalities which use fitting procedures for quantitative determination of optical properties in vivo such as reflectance spectroscopy and optical coherence tomography.

Toward a reference standard for tissue phantoms

P. Di Ninni, F. Martelli, G. Zaccanti, Univ. degli Studi di Firenze (Italy)

A reference standard for tissue phantoms, i.e., a phantom with well known and stable optical properties, reproducible, and easy to be found, would be very useful for many applications based on measurements of diffused light. Although many tissue-equivalent phantoms have been proposed to our knowledge none of them has been characterized sufficiently well to be suggested as a reference standard. Here it is suggested the use of Intralipid 20% as reference standard for the diffusive medium and the use of Indian ink as reference standard for the absorbing medium. Measurements of optical properties carried out at visible and NIR wavelengths on samples of Intralipid 20% showed a high stability and small batch-to-batch variations: measurements have been carried out using samples from nine different batches with expiry dates spreading over ten years. For the reduced scattering coefficient the values measured at l = 632.8, 750, and 831 nm show maximum deviations from the average values of 2.2, 1.1, and 1.4% respectively. Furthermore, the absorption coefficient of Intralipid 20% differs only slightly from that of water. For Indian ink from different batches and different brands we observed that while absorption and extinction coefficients have a large variability, the single scattering albedo shows weak variations. Therefore, the absorption coefficient of ink can be obtained from measurements of extinction coefficient. Samples obtained from the same batch show identical optical properties and the diluted ink remains stable for at least one year. We stress that it is very important to apply ultrasound before using ink.

Novel phantom materials for use in optical coherence elastography

B. F. Kennedy, F. Blume, The Univ. of Western Australia (Australia); C. Li, Curtin Univ. of Technology (Australia); R. A. McLaughlin, The Univ. of Western Australia (Australia); X. Lou, Curtin Univ. of Technology (Australia); D. D. Sampson, The Univ. of Western Australia (Australia)

Optical coherence elastography (OCE) is an imaging technique which uses optical coherence tomography (OCT) to measure tissue strain. OCE has shown potential to provide additional contrast not present in OCT images. It is important that OCE systems are validated using a tissue-mimicking phantom prior to imaging tissue. An ideal OCE phantom should accurately mimic the optical and viscoelastic properties of tissue whilst fulfilling the practical requirements of long lifetime, ease of fabrication and biocompatibility. No phantom material proposed to date simultaneously satisfies all of these requirements. This can cause misleading results and loss of efficiency due to time-consuming fabrication. In this work, we present two types of phantom based on novel materials. These materials are fibrin and poly(2-hydroxyethyl methacrylate) (pHEMA). Fibrin is a naturally occurring protein in humans that provides structural support for blood clots. It is formed from the protein fibrinogen by proteolysis induced by the enzyme thrombin. pHEMA is a synthetic polymer hydrogel that has been commonly used in vision correction devices including contact lenses and intraocular lenses, and more recently as a cornea replacement and socket prostheses. We describe the fabrication process for these phantoms, detailing the mechanism used to vary the optical scattering and viscoelasticity in both cases. We compare the optical and viscoelastic properties of these novel phantom materials to those of the more commonly used room-temperature vulcanizing silicone and to those of tissue. We describe the advantages/disadvantages of each phantom and discuss the key points which should be considered when using these materials.

Spatial distributions of optical and acoustic properties and correlations with temperature in cyclically frozen-thawed poly(vinyl alcohol) gel breast phantoms

D. Piras, W. Xia, M. Heijblom, W. Steenbergen, Univ. Twente (Netherlands); T. G. van Leeuwen, Academisch Medisch Ctr. (Netherlands); S. Manchur, Univ. Twente (Netherlands)

Tissue mimicking phantoms are important for the evaluation of performances of imaging systems. In photoacoustics, phantoms need to accomplish both optical and acoustic properties of soft tissues. PVA (Polyvinyl alcohol) gels have been widely used to simulate those tissue properties. Gels are formed by cyclically freezing-thawing (FT) PVA solutions, which causes the formation of the cross-linked gel, with pores caused by the freezing of water phase. Pores are then responsible for refractive index fluctuations causing the observed scattering properties. The sizes and morphologies of pores, and therefore the optical properties, are expected to be linked to the rate and extent of FT. When making large phantoms as for breast imaging photoacoustic systems, temperature gradients will exist which could result in spatially distributed inhomogeneous pore sizes and therefore inhomogeneous optical and acoustic properties. This might cause over- or underestimation of the imaging system performances.

We investigated the effects on spatial distribution of optical and acoustic properties of FT and the resulting temperature differences in large phantoms. These properties were compared with PVA microstructure using scanning electron microscopy, studies of which so far have been limited to small scale samples. Temperatures at the phantom surface and in the center during the entire FT cycle and with varying cycle times were sensed using thermocouples. Significant temperature differences between PVA phantom surface and bulk have been found during FT, and we correlated the resulting changes in reduced optical scattering coefficients, speed of sound and acoustic attenuation with the temperature differences.

Photothermal OCT imaging of 1210nm laser irradiated agarose tissue phantoms with nanoparticles

O. D. Ayala, A. S. Paranjape, L. L. Ma, K. P. Johnston, The Univ. of Texas at Austin (United States); R. V. Kuranov, The Univ. of Texas Health Science Ctr. at San Antonio (United States); T. E. Milner, The Univ. of Texas at Austin (United States)

Optical Coherence Tomography (OCT) is a rapidly growing technique for non-contact high resolution imaging of tissue structures. A current
motivation in OCT imaging is to provide specific and high contrast imaging of selective tissue components. We describe experimental results for measurement of thermo-elastic expansion of laser excited tissue phantoms containing near infrared absorbing nanoparticles we call ‘nanorose’. Infrared absorbing nanorose absorb incident laser energy in NIR and cause thermo-elastic expansion in the tissue phantoms. Agarose tissue phantoms containing dextran coated metallic nanoroses capable of absorbing 1210nm light are prepared at various concentrations. These tissue phantoms are then irradiated with a 1210nm wavelength diode laser modulated at 200Hz. Thermo-elastic expansion in tissue phantoms is detected using swept source photothermal Optical Coherence Tomography at 1328nm. Transmission measurements (1210nm) of nanorose tissue phantoms presented linear light attenuation as nanorose concentration was increased. Although previous studies using 800nm light excitation and swept source photothermal OCT detection have demonstrated an absorption cross section of (31 ± 5) x 10^-11 cm^2 in Polyvinyl alcohol (PVA) tissue phantoms containing nanorose, depth penetration is limited. Light excitation at 1210nm was implemented since fatty acids have a higher absorption coefficient at 1210nm and increased penetration depth into tissue is achieved due to reduced tissue scattering at this wavelength. A greater penetration depth and higher absorption of fatty acids may improve specific targeting of nanoroses by 1210nm laser irradiation.

7906B-36, Session 3

3D solid tissue phantoms for combined diffuse reflectance, intrinsic fluorescence and Raman spectroscopies

J. R. Weber, Z. I. Volynskaya, Z. Yaqoob, Massachusetts Institute of Technology (United States); S. McGee, A. Saha, Case Western Reserve Univ. (United States); R. R. Dasari, Massachusetts Institute of Technology (United States); M. Fitzmaurice, Case Western Reserve Univ. (United States)

Solid 3-D tissue phantoms have been designed and fabricated for simulation of breast tissue optical properties for Multimodal Spectroscopy (MMS). MMS includes three forms of spectroscopy: Diffuse Reflectance Spectroscopy (DRS), Intrinsic Fluorescence Spectroscopy (IFS), and Raman Spectroscopy. The tissue phantoms contain scattering and absorbing agents for the DRS measurement, a fluorescenting agent for the IFS measurement and Raman-active inclusions. The optical properties are in a background matrix of gelatin, which can be poured into layers, cut into three-dimensional shapes, and can support embedded structures. Raman active calcium hydroxyapatite inclusions of sizes ~0.5-2mm are embedded within the surrounding gelatin matrix, allowing the phantoms to simulate the type of calcifications found in breast tissue that may associate with malignancy. The surrounding optical absorption, scattering and fluorescence properties are chosen to match those measured previously in normal and malignant breast tissue with MMS. These phantoms will be used to study the sensitivity of the Raman signal to changes in tissue optical properties and probe location with respect to inclusions. This work will ultimately help us understand our Raman sensitivity to calcifications for more accurate diagnosis of breast lesions during core needle biopsy.

7906B-41, Session 3

System-independent assessment of OCT axial resolution with a ‘bar chart’ phantom

R. C. Chang, National Institute of Standards and Technology (United States); A. Agrawal, U.S. Food and Drug Administration (United States); C. Stafford, National Institute of Standards and Technology (United States); M. Connors, U.S. Food and Drug Administration (United States) and Univ. of Maryland (United States); J. Pfefer, U.S. Food and Drug Administration (United States); J. Hwang, National Institute of Standards and Technology (United States)

We present a novel optical phantom approach as a unique testbed for the characterization of optical coherence tomography (OCT) axial resolution and contrast. Based on a combinatorial methods approach from polymer science, we have established a multilayered “bar chart” in depth, consisting of layers of light-scattering microspheres with intervening layers of transparent silicone. Varying the diameter of the microspheres and the thickness of the silicone layers permits different spatial frequencies, from 10 to 200 mm-1, to be replicated in the axial dimension of the phantom. This range of spatial frequencies covers the expected range of clinical OCT axial resolution and therefore provides a means of determining the axial modulation transfer function. Because the phantom’s dimensions are accurately known independent of the OCT system, no information about the system’s spatial calibration is required. We have evaluated this novel phantom on several different OCT platforms, including time- and spectral-domain systems having different operating wavelengths.

7906B-37, Session 4

Optical tissue phantoms: realistic absorption across the spectrum

I. Noisieux, I. Veilleux, S. Leclair, J. Bouchard, J. Osouf, O. Mermut, INO (Canada)

Optical tissue phantoms are used to mimic the optical properties of tissues. For the skin, the absorption coefficient (µa) is mostly dominated by the melanin. For example, in several biomedical applications, oxygen concentration is the most important target to monitor. Hence, phantoms should mimic both the flat µa response of the bulk tissue and also the oxy/deoxy-hemoglobin response over a continuous broad region of the spectrum. Typical tissue phantoms rely on the addition of an absorber component having a narrow absorption peak near the target wavelength. Addition of a second absorber can be used to adjust absorption at a second wavelength but the behavior at the intermediate wavelengths is often unsatisfactory in terms of mimicking tissue. Narrow peak absorbers cause strong variations in µa in such phantoms and this especially limits their utility for multilambda usage. This also clearly limits applications requiring a realistic absorption spectrum. Using an absorber imparting a flat absorption profile across the visible-NIR spectrum enables a more realistic response, and also provides a baseline to which additional spectral features could be added. Using carbon black, we have developed tissue phantoms having mostly a flat absorbance in the spectral region between 600 and 850 nm. By adding additional absorbers, we have also developed phantoms that mimic both the oxy- and deoxy- hemoglobin spectra over a continuous spectral range.

7906B-38, Session 4

Development of a skin phantom of the epidermis and evaluation by using fluorescence techniques


Pharmaceutical drugs applied to the skin have to overcome the natural barrier function of the stratum corneum (SC) the outermost layer of the epidermis. The SC consists of corneocytes embedded in a lipid matrix. The main components of this matrix are fatty acids, triglycerides, cholesterol and ceramides. As epidermis tissue is difficult to separate and to handle we develop a skin phantom that resembles the lipid matrix of the SC and the residual epidermis including corneocytes. The main intent is to achieve optical properties similar to the skin. Spectroscopic and microscopic methods are used for the evaluation.
We investigate the influence of the naturally occurring changes of the pH-value in the skin that varies from about 4.6 to 7.4 and the changing polarity of the skin environment on fluorescent dyes. By changing the phantoms ingredients we reproduce different skin conditions. The phantom may be adjusted to pathological situations in the skin.

Furthermore, the phantom is used to incorporate fluorescent dyes and fluorescent-labeled drugs to perform calibration measurements in widefield and laser-scanning microscopes. Having a calibration function for a labeled drug it is possible to quantify the drug penetration rates to compare drug carrier systems (DCS). The phantom is advantageous for creating well defined slides of a certain thickness and defined fluorescence dye concentration compared to native porcine or human skin. Miscellaneous fluorescent dyes in combination with drugs and DCS can be tested in a comparative skin penetration study.

7906B-39, Session 4

Phantoms of fingers with various tones of skin for LLLT dosimetry

M. V. Pires de Sousa, E. M. Yoshimura, A. L. O. Ramos, A. C. Magalhães, M. T. Saito, M. C. Chavantes, L. R. dos Santos, Univ. de São Paulo (Brazil)

Due to the great number of new clinical applications of Low-Level-Laser-Therapy (LLLT), the development of precise, stable and low coast solid phantoms of skin, fat, muscle and bone becomes extremely important. The aim is to find the best combination of matrix, absorber and scatterers, which simulates skin, fat, muscle and bone tissues to build LLLT phantoms. Eight kinds of cylindrical phantoms simulating human fingers were constructed and tested.

Matrixes of polyester resins and paraffin were used with various concentrations of dyes and scatterers (Al2O3 nanoparticles) to adjust the optical parameters. A CCD camera was used to obtain transmission and scattering images of the phantoms, and of swine tissues and volunteer’s fingers illuminated by lasers (diode 635 and 820 nm, and HeNe, 633 nm).

The light fluence transmitted through the sample form Gaussian shaped profiles. Light scattered at 90 degrees shows an intensity profile with a steep growth followed by an exponential attenuation. The comparison of these two kinds of profiles for phantoms and swine tissue was used to evaluate the concentrations that better simulate different kinds of tissues. The phantoms were constructed with the materials thus obtained. Dyed resin with 10% concentration of Al2O3 particles simulates quite well the inner part of a finger (bone, muscle and fat tissues); paraffin with varied concentrations of dyes simulate skin in various tones. The outcomes of this study point to a reliable tool to aid clinicians with LLLT dosimetry.

7906B-40, Session 4

Hyperspectral image projection of a pig kidney for the evaluation of imagers used for oximetry

D. W. Allen, M. Litorja, J. Hwang, National Institute of Standards and Technology (United States); K. J. Zuzak, The Univ. of Texas at Arlington (United States) and Digital Light Innovations (United States)

Hyperspectral image projection applied to optical medical imaging can provide a means to evaluate imager performance. This allows repeated viewing of unique surgical scenes without the need for costly experiments on patients. Additionally, the generated scene can be well characterized and used repeatedly as a standard by many different sensors at different times and locations. This paper describes the use of hyperspectral images of a pig kidney in which the artery is clamped and unclamped, altering the oxygenation in the tissue. The scene of the kidney is projected with the full spectral content allowing the oxygenation status of the tissue to be observed and evaluated spatially. The change in the oxygenation of the projected scene provides several points on an oximetric scale. The oximetric scale can then be realized using other imagers under test. The potential measurement uncertainties associated with the imager under test can then be estimated.
Theoretical and experimental comparison of tissue phantoms using white-light spectroscopy
K. A. Popov, T. P. Kurzweg, Drexel Univ. (United States)

Optical biopsies have been proposed to provide a minimally invasive approach for detection and monitoring pre-cancerous conditions. White light spectroscopy has been used for optical biopsies as processed backscattered light can reveal the nucleus size of cells. In our previous work, we have shown nuclear size estimation through spectroscopy in the visible spectrum. More detailed results were discovered when the scattering vs. wavelength spectra was converted into the Fourier domain. In this paper, we compare our experimental results against different theory abstractions. The first order Rayleigh-Deby-Gans model simplifies backscattering theory to a first order Bessel function. These first order models represent single scattering events. High-order models, or Mie scattering models, are used to model the effect of single and multiple scattering, as well as the absorption and the optical contrast of the scatterers. Through these theoretical studies, we analyze the spectrum details of in terms of morphology dependent resonances (MDRs), of higher and lower orders. Experimental comparisons will be made to each of these theoretical models, in both the spatial and frequency domain. The experimental results will be gathered with different wavelength resolutions, detailing the higher order backscattered results. In addition, we will look at different wavelength regions, of approximately 45 nm, centered at 500, 550, 600, and 650 nm, which will help classify the scatterer size.

Measuring the transport mean free path, anisotropy coefficient, and shape of the phase function with low-coherence enhanced backscattering spectroscopy
V. M. Turzhitsky, A. J. Radosevich, J. D. Rogers, N. N. Mutyal, V. Backman, Northwestern Univ. (United States)

There are very few existing techniques that can measure properties of the phase function from biological tissue. Most of these methods, such as the integrating sphere technique, require sectioning the sample into thin slices in order to allow for transmission measurements. Low-coherence enhanced backscattering is a technique that has recently shown promise for tissue characterization due to its sensitivity for length scales that are much smaller than the transport mean free path. This novel method employs partial spatial coherence illumination coupled with spectrally resolved detection to obtain wavelength-dependent enhanced backscattering information. The resulting signal is sensitive to the transport mean free path ($\lambda^*$) and the anisotropy coefficient ($g$) of the scattering media when $\lambda^*$ is much greater than the spatial coherence length ($L_{sc}$). In this work, we will apply a general model for tissue scattering that is based on the Whittle-Matern correlation function. The resulting two-parameter phase function is then validated by applying the Born approximation, is linked to physical properties of the scattering medium. We then evaluate the accuracy of several methods for solving the inverse problem and obtaining $g$ and $\lambda^*$ from the scattering media by simulating an LEBS signal at varying $L_{sc}$ that can be obtained from an existing instrument. The results are then validated with a tissue phantom that closely mimics the tissue-relevant phase function.

Nanoscale nuclear architecture for cancer diagnosis by spatial-domain low-coherence quantitative phase microscopy
P. Wang, R. K. Bista, D. Y. Lo, W. E. Khalbuss, W. Qiu, K. D. Staton, L. Zhang, Univ. of Pittsburgh (United States); T. A. Brentnall, Univ. of Washington (United States); R. E. Brand, Y. Liu, Univ. of Pittsburgh (United States)

Alterations in nuclear architecture are the hallmark diagnostic characteristic of cancer cells. Despite the discovery of numerous molecular biomarkers, the microscopic examination of morphological changes in the cell and tissue, particularly alterations in nuclear structure, is still a widely practiced method to diagnose the cancer. However, this approach is not efficient enough to detect subtle structural changes and consequently is often challenging to make a definitive diagnosis of malignancy due to limited availability of human cell samples and morphological similarity with certain benign conditions. In this work, we show that the increased heterogeneity in nanoscale nuclear architecture quantified by spatial-domain low-coherence quantitative phase microscopy (SL-QPM), is more sensitive for the identification of cancer cells than conventional cytopathology. In a proof-of-concept experiment with an animal model of intestinal carcinogenesis - APC/Min mouse model, the increased nuclear heterogeneity is observed in cytopologically normal-appearing intestinal epithelial cells from the APC/Min mice with the presence of tumors. We further demonstrate the detection of malignancy in cytoplogically indeterminate cells from cancer patients in human cytology specimens of colorectal and pancreatic cancers. The determination of nanoscale nuclear architecture using this simple and practical optical instrument is a significant advance towards cancer diagnosis. This technique may provide a new capability for elucidating the mechanism of malignancy and correlating functional and molecular parameters with malignancy associated structural changes, which in turn may help for better prognosis and management of cancer patients.

Quantitative spectroscopic imaging using dark field microscopy
A. Nadort, D. J. Faber, T. G. van Leeuwen, Academisch Medisch Ctr. (Netherlands)

Attempts to perform quantitative spectroscopic measurements on biological tissues are numerous, but lack accuracy due to the unknown photon migration paths within the scattering tissue. Optical methods such as spatially or time resolved diffuse reflectance spectroscopy utilize elegant ways to make estimates of the photon paths to perform quantitative absorption. However, these methods do not produce and utilize information on structural details within the biological tissue. We present an alternative method based on dark field microscopy to produce images with both structural and spectral information of biological tissue. This configuration has proven to generate high quality images of subsurface structures, such as the microcirculation. To succeed in quantitative spectroscopy we have commenced to model light propagation in the multiple scattering dark field regime.

The dark field configuration is achieved by a ring-based illumination with a central imaging pathway, consequently only the multiple scattered light will reach the CCD detector. To model the optical paths we make use of tissue simulating optical phantoms with controlled scattering and absorption properties. We used homogeneous (scatterer + absorber solutions) and heterogeneous (solid scattering structures with small flow channels) optical phantoms. We combined Monte Carlo simulations of
optical path length distributions within the image and Lambert-Beer's law of absorption to produce an optical model for dark field spectroscopy. Our results on controlled phantoms show that it is possible to perform quantitative spectroscopy on images obtained using a dark field microscopy configuration. We will proceed to more complex biological tissues and in-vivo situations in the future.

7907-05, Session 1
Effect of clearing agents on scattering coefficient and anisotropy of scattering of dermis studied by reflectance confocal microscopy
S. L. Jacques, R. Samatham, K. G. Phillips, Oregon Health & Science Univ. (United States)
The mechanism of action of clearing agents to improve optical imaging of mouse skin during reflectance-mode confocal microscopy was tested. The dermal side of excised dorsal mouse skin was exposed for 1 hr to saline, glycerin, or 80% DMSO, then the clearing agent was removed and the dermis placed against a glass cover-slip through which a confocal microscope measured reflectance at 488 nm wavelength. An untreated control was also measured. The axial attenuation of reflectance signal, R(z_f) versus increasing depth of focus z_f, behaved as R = ρ exp( μ z_f 2 G), where ρ is tissue reflectivity and μ is attenuation [cm^-1]. The factor 2G accounts for the in/out path of photons, and the numerical aperture of the lens. The ρ,μ data were mapped to values of scattering coefficient (μs [cm^-1]) and anisotropy of scattering (g). Images showed that glycerin significantly increased the g of dermis from ~0.7 to about 0.99, with little change in the μs of dermis at ~300 cm^-1. DMSO and saline had only slight and inconsistent effects on g and μs.

7907-06, Session 1
A comparative study of 3×3 and 4×4 Mueller matrix decomposition methods for polarimetric characterization of complex tissue-like turbid medium
N. Ghosh, A. Banerjee, Indian Institute of Science Education and Research (India); M. F. G. Wood, M. A. Wallenberg, I. A. Vitkin, Univ. of Toronto (Canada)
We have recently developed and validated a 4 ×4 Mueller matrix decomposition method for extraction and quantification of individual, intrinsic polarimetry characteristics from complex tissue-like turbid medium. Initial biomedical application of this promising approach was explored for monitoring of myocardial tissue regeneration following stem cell therapy. Note that in comparison with the 4 ×4 Mueller matrix (that involves both linear and circular polarization measurements), the 3 ×3 Mueller matrix measurement (that involves linear polarization measurements alone) may be more amenable for many practical biomedical applications involving spectroscopic and imaging polarimetry. We have thus investigated the efficacy of the 3 ×3 Mueller matrix decomposition method to delineate individual intrinsic polarimetry characteristics in complex tissue-like turbid media exhibiting simultaneous optical scattering and polarization effects (common tissue-polarimetry effects are depolarization, linear birefringence and optical activity). The applicability of this 3 ×3 Mueller matrix decomposition method is based on the assumption that the depolarization of linearly polarized light due to multiple scattering (the dominant cause of depolarization in tissue) is independent of the orientation angle of the incident linear polarization vector. We have thus conducted detailed experimental and theoretical studies to investigate the validity of this assumption and its dependence on the sample polarization properties and on scattering and detection geometry effects. Experimental studies were conducted using a high-sensitivity polarization modulation / synchronous detection experimental system on optical phantoms having controlled sample polarizing properties. The phantoms were developed using polyacrylamide as a base medium, with polystyrene microspheres to create turbidity, sucrose to induce optical activity, and mechanical stretching to cause linear birefringence. Theoretical studies were carried out using a polarization sensitive forward Monte Carlo (MC) model capable of simulating all the simultaneous scattering and polarization effects (scattering and polarization properties similar to the experimental phantoms). The details of the results of these validation studies of the 3 ×3 Mueller matrix decomposition method (its efficacy to quantify individual polarimetry characteristics and its domain of validity) will be presented and initial application of this approach for quantification of tissue structural anisotropy will be discussed.

7907-07, Session 1
Phase function of biological soft tissues for the complete solid angle
R. Michels, Univ. Ulm (Germany)
With the help of a solution of the transport equation it is possible to calculate the light propagation in biological tissue quite precisely if the exact phase function of the scattering tissue is known. The phase function of most structured tissues depends on the two scattering angles (polar and azimuthal angle) and the incident direction of the light. Even though the use of the complete phase function is crucial for a precise calculation of the light propagation and is the only way to understand e.g. the anisotropic light propagation in structured tissue, most commonly phase functions are used which depend only on one scattering angle. This simplification is most likely due to missing measurements of more realistic phase functions of biological tissues. We present the goniometric measurements of the phase function of different porcine soft tissues for the whole solid angle and different incident directions. Furthermore we present a simple, three parametrical model which describes the phase function of structured biological tissue versus the incident direction and both scattering angles for the use in solutions of the transport equations like Monte Carlo simulations.

7907-08, Session 1
Monte Carlo simulation of photon migration in turbid random media based on the object-oriented programming paradigm
I. Meglinski, A. Doronin, Univ. of Otago (New Zealand)
Based on the Object-Oriented Programming (OOP) paradigm we introduce a new concept of Monte Carlo (MC) modelling of photon migration in turbid scattering medium. Features of OOP such as data abstraction, encapsulation, modularity, polymorphism and inheritance allow creation of objects defining the key features of optical radiation (e.g. wavelength, polarization, coherence, etc.), medium properties, probes configuration and their intercourse. This approach increases the efficiency of code manageability and provides superior opportunities to generalise the MC technique for a unify use in various of applications. The use of NVIDIA CUDA technology gives an access to the graphic libraries specially designed to enable an optimal calculation of algebraic functions with the parallel throughput. This accelerates an average modelling time up to 100 times or more.

7907-10, Session 2
Optical sectioning with HiLo microscopy
J. Mertz, D. Lim, T. Ford, K. Chu, Boston Univ. (United States)
I will describe a new method of obtaining optical sectioning with a standard wide-field fluorescence microscope. The method, called HiLo imaging, involves acquiring two images, one with structured illumination and another with uniform illumination.
HiLo imaging is fast, robust, provides a user-defined depth of field, and works with both fluorescent and non-fluorescent samples. Moreover, it is generalizable to a variety of illumination and imaging configurations. Demonstrations will be presented using speckle or grid pattern illumination, in different configurations such as endomicroscopy, macroscopy, and light sheet illumination microscopy.

7907-11, Session 2

Laser speckle imaging in the spatial frequency domain using Monte Carlo

T. B. Rice, A. Mazhar, S. D. Konecky, D. J. Cuccia, A. J. Durkin, B. J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

Laser Speckle Imaging (LSI) is a fast, noninvasive method to obtain relative flow changes in a dynamic light scattering system. As a step toward more quantitative measurements, we use multiple techniques to model the speckle contrast of coherent light using intensity correlation based on single scattering, diffusion, and Monte Carlo. The time scales in which the approximation methods break down is explored. In this regime, for times much greater than the correlation time, Monte Carlo is used to provide accurate correlation predictions. Next, Spatial Frequency Domain Imaging (SFDI) is incorporated into the Monte Carlo model, which uses sinusoidal projection patterns to gate photon path length and increase information content. One major challenge for LSI is depth averaging of the correlation content. SFDI allows for depth sensitive measurements because the penetration of the photons depends on the spatial frequency of the projection pattern. Depth sectioning of the speckle correlation is performed and analyzed using this technique. Finally, incoherent SFDI is used to measure both absorption and scattering. By inserting these properties a priori into the Monte Carlo speckle model, quantitative Brownian motion and flow measurements are achieved.

7907-12, Session 2

Effects of combined ordered and unordered motion in laser speckle contrast imaging

S. J. Kirkpatrick, Michigan Technological Univ. (United States); D. D. Duncan, Portland State Univ. (United States)

Laser speckle contrast imaging and analysis usually assumes a priori a decorrelation model based either upon ordered (Lorentzian line shape) or un-ordered (Gaussian line shape) motion. However, in many actual, in vivo situations, this is probably unlikely and there is some combination of ordered and un-ordered motion that gives rise to the observed speckle motion in the imagery. An example of this would be when using LSCI to assess cranial blood flow. In this scenario, one could envision a blood vessel, characterized by ordered motion being in the same field of view as the surrounding brain tissue which may be characterized by un-ordered (random) motion. Clearly, at least two separate statistical models for inferring the correlation time and subsequently flow velocities are required. Several authors in the past have address this issue in a variety of ways. One approach is to view Gaussian and Lorentzian behaviors as limiting cases, and the actual local behavior is some combination of the two. The actual local behavior, then, may be described by a convolution of the two line shapes (Voigt line shape). However, in the scenario envisioned, different portions of the LSCI images may be best described by one, or even all of the statistical models. Herein, we address through numerical simulations the issue of multiple behaviors in a single field of view in LSCI and provide suggestions on how to allow for discrimination between different behaviors in a single LSCI image.

7907-13, Session 2

Cellular dimensionality in dynamic light scattering

R. An, K. Jeong, J. J. Turek, D. D. Nolte, Purdue Univ. (United States)

We have developed motility contrast imaging (MCI) as a coherence-domain volumetric imaging approach that uses sub cellular dynamics as an endogenous imaging contrast agent of living tissue [1]. Fluctuation spectroscopy analysis of dynamic light scattering from three-dimensional tissue has identified functional frequency bands related to organellar transport, membrane undulations and cell movements. However, dynamic light scattering from conventional two-dimensional cell culture shows significant differences and much lower sensitivity to sub cellular dynamics compared with three-dimensional tissues.

In this paper, we track the behavior of dynamic light scattering as we bridge the gap between the two extremes of 2D cell culture and 3D tissue spheroids. In a backscattering geometry, we capture speckle from 2D cell culture consisting of isolated cells or planar rafts of cells on cell-culture surfaces. The motility contrast is weak in this limit. As the cellular density increases to cover the surface, the motility contrast increases, especially as the cells become crowded or have multiple layers. As environmental perturbations (temperature, gravity) or pharmaceuticals (anti-mitotic drugs) are applied, the fluctuation spectral response becomes more dramatic as the dimensionality of the cellular aggregations increases. A key question we address is what contributions to the motility contrast, and to the response to cellular perturbations, arise from the changing cellular dimensionality, and what contributions arise from the increasing optical thickness of the targets.


7907-14, Session 2

Endoscopic laser speckle contrast imaging system using a fibre image guide

L. Song, D. S. Elson, Imperial College London (United Kingdom)

There are several challenges when fibre image guides (FIG) are used for endoscopic speckle acquisition: cross talk between fibre cores, FIG fixed pattern noise, reducing the probe diameter and low sensitivity due to the decreased speckle numbers through the FIG. In this paper, an endoscopic laser speckle contrast analysis system (ELASCA) based on a leached FIG and customized image processing program is presented. Different methods of acquiring LASCA images through leached FIGs were investigated including the effect of changing the number of speckles per fibre, defocusing the FIG image onto the CCD and processing speckle images with masks and Butterworth filters to deal with the FIG fixed pattern and noise from the cladding. A phantom consisting of intralipid suspension pumped at varying speed through a network of small channels to simulate blood capillaries was illuminated by a fibre delivered laser (130mW, 660 nm). Images with a field of view of 10 mm diameter were acquired through a micro lens, transferred through the FIG and recorded by a CCD after a magnification system. Speed ranges from 0 to 2 mm/s were tested. The experimental results showed that this system could detect speed changes and the sensitivity of the defocused speckle images increased. In contrast to the currently reported ELASCA results, this system can both give a map of the observed area and the temporal change in flow. An additional benefit is the small size of the FIG, which is compatible with current endoscopic instrument channels and will allow additional surgical applications.
Fourier-transform light scattering of cells and tissues

G. Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Recently we have introduced Fourier transform light scattering (FTLS) as a novel experimental approach that combines optical microscopy, holography, and light scattering for studying inhomogeneous and dynamic media. FTLS relies on quantifying the optical phase and amplitude associated with a coherent image field and propagating it numerically to the scattering plane. Because it detects all the scattered angles (spatial frequencies) simultaneously in each point of the image plane, FTLS can be regarded as the spatial equivalent of Fourier transform infrared spectroscopy (FTIR), in which all the temporal frequencies are detected at each moment in time. Full angular information, limited only by the microscope objective, is obtained from extremely weak scatterers, such as a single micron-sized particles, single cell nucleus, or single neuronal axon.

We derived two mathematical relationships between quantitative phase images of thin inhomogeneous media slices and the scattering parameters of the bulk, i.e. scattering mean free path, is, and anisotropy factor, g. The latter turns out to be inversely proportional to the square of the phase shift and g is related to the phase gradient. These formulas, referred collectively to as the scattering-phase theorem, allow for mapping large cross-sections of tissues in terms of scattering properties and may offer a straightforward experimental alternative to simulations of tissue scattering. This quantitative phase imaging based approach operates without fitting or iterative procedures. The knowledge of is and g has great impact on predicting the outcome of a broad range of scattering experiments on large samples. Further, we mapped human prostate biopsies in terms of their scattering properties and found excellent correlation between areas of strong scattering and cancer.

Studies using dynamic FTLS (dFTLS) on particles undergoing Brownian motion demonstrated that quantitative diffusion information in agreement with predictions by the Stokes-Einstein equation can be extracted without the typical need for particle tracking. We present experimental measurements of angular light scattering from single red blood cells. We also quantified intracellular mass transport in neurons, glia and microglia cells, which revealed a combination of diffusion and deterministic intracellular transport. In sum, we believe that static and dynamic FTLS may make in the future a broad impact both in basic sciences and clinical applications.

Laser interference fringe tomography: a novel 3D imaging technique for pathology

F. Kazemzadeh, T. M. Haylock, L. M. Chifman, A. R. Hajian, B. B. Behr, A. T. Cenko, J. T. Meade, Univ. of Waterloo (Canada); J. Hendrikse, Tornado Medical Systems (Canada)

Laser interference fringe tomography (LIFT) is within the class of optical imaging devices designed for in vivo and ex vivo medical imaging applications. LIFT is a very simple and cost-effective three-dimensional imaging device with performance rivaling some of the leading three-dimensional imaging devices in ophthalmology. Like optical coherence tomography (OCT), it measures the reflectivity as a function of depth within a sample and is capable of producing three-dimensional images from optically scattering media. Unlike OCT, LIFT has the potential capability to produce high spectral resolution, full-color images. The optical design of LIFT along with the planned iterations, improvements, and miniaturization are presented and discussed in addition to the theoretical concepts. Preliminary imaging results of the device are shown.
High-speed simulation of skin spectral reflectance based on an optical path-length matrix method and its application
I. Fujiwara, S. Yamamoto, M. Yamauchi, Chiba Univ. (Japan); K. Ogawa-Ochiai, Chiba Univ. Hospital (Japan); T. Nakaguchi, N. Tsumura, Chiba Univ. (Japan)

In this research, we propose an optical path-length matrix method for high-speed simulation of photon migration in a human skin. The optical path-length matrix is defined as the probability density distribution of optical path-length in the skin. Generally, Monte Carlo simulation is used to simulate a skin reflectance, since it can simulate the reflectance accurately. However, it requires a huge computation time, thus this is not easily applicable in practical imaging system with large number of pixels. On the other hand, the proposed path-length matrix method achieves the simulation in a short time. The skin model was assumed to be two-layered media of the epidermal and dermal layers. For obtaining the path-length matrix, photon migration in the model without any absorption was simulated only once by Monte Carlo simulation for each wavelength, and the probabilistic density histograms of the optical path-length at each layer were acquired and stored in the optical path-length matrix. Skin spectral reflectance for arbitrary absorption can be calculated easily by accumulating all combination of an element in the above pre-computed path-length matrix and absorption coefficient based on the Beer-Lambert law. The proposed method was compared with the conventional Monte Carlo simulation among the wavelength 400-700nm, 61 bands with every 5nm. The computational time of the proposed method was approximately 10 minutes; while the conventional method was 15 hours. In addition, the error margin of the proposed method was approximately less than 1.6%. This method was applied to skin spectral image analysis for skin chromophore quantification.

Retention of indocyanine green as a potential marker for optical detection of blood brain barrier disruption
A. Ergin, Boston Univ. (United States); S. Joshi, M. Wang, Columbia Univ. (United States); I. J. Bigio, Boston Univ. (United States)

Osmotic disruption of the blood brain barrier (BBB) by intraarterial mannitol injection is often used to enhance delivery of chemotherapeutic drugs to brain tissue. The disruption is affected by a number of factors, and variations can have a profound impact on regional delivery. We report an optical method to monitor BBB disruption in real time. Optically measured brain tissue concentrations of indocyanine green (ICG), and Evan’s blue (EB) enable the quantification of BBB disruption after BBB disruption. Using the optical pharmacokinetics technique, a variation of diffuse reflectance spectroscopy, we were able to track in vivo brain tissue concentrations of ICG and EB in New Zealand white rabbits before and after barrier disruption. EB penetrates the BBB only when the barrier is breached and was also used to assess the distribution of disruption on post mortem examination.

In animals that demonstrated successful disruption, the optical measurements exhibited an increase in retained ICG concentrations compared to those without BBB disruption. Brain tissue concentrations of EB and the brain:plasma EB partition coefficient progressively increased during the period of observation.

This study shows the feasibility of optical monitoring of BBB disruption, a method than can help improve intraarterial delivery of chemotherapeutic drugs.

Near-infrared scattering imaging of depolarization waves in a rat hypoxic brain model and its application to assessment of brain tissue reversibility
S. Kawauchi, S. Sato, Y. Uozumi, H. Nawashiro, M. Ishihara, M. Kikuchi, National Defense Medical College (Japan)

Light scattering signal, which is sensitive to cellular/subcellular structural integrity, is a potential indicator of tissue viability in brain, because metabolic energy is used in part to maintain the structure of the cells. We previously observed a unique triphasic scattering change (TSC) at a certain time after ischemia or hypoxia for rat brains by fiber-based diffuse reflectance measurements. The TSC coincided with cerebral ATP exhaustion and it was shown to be associated with anoxic depolarization (AD). Hypoxia-reoxygenation experiments under spontaneous respiration showed that when reoxygenation was started before TSC, all rats survived, while no rats survived when reoxygenation was started after TSC. Survival was probabilistic when reoxygenation was started during TSC, indicating that TSC can be regarded as the critical time zone for cerebral resuscitation. A question arose here: what determined the reversibility of brain tissue? We thought that spatiotemporal behaviors of scattering waves might relate to the probabilistic survival. In this study, we performed near-infrared scattering imaging of rat brain during hypoxia followed by reoxygenation. About 2 min after starting hypoxia, scattering wave was generated focally in the bilateral outermost regions in the cortex and spread toward the midline at ~6 mm/min. When reoxygenation was started before the leading edge of scattering wave reached the middle point of the hemisphere, the tissue was reversible. When scattering wave further spread on the cortex, reoxygenation did not save the brain. These suggest that the coverage of scattering wave on the cortex determines the reversibility of brain tissue after hypoxia.

Quantification of field carcinogenesis in isolated colonocytes via partial wave spectroscopic microscopy: novel means of colorectal cancer (CRC) screening
D. Damiania, Northwestern Univ. (United States); H. Roy, Evanston Hospital (United States); H. Subramanian, Northwestern Univ. (United States); M. Dela Cruz, Evanston Hospital (United States); Y. Zhu, V. Backman, Northwestern Univ. (United States)

Colon cancer is one of the leading causes of cancer related deaths in United States. Although flexible sigmoidoscopy, colonoscopy etc. are commonly used to screen for colon cancer, they are often limited in their application, expensive or intrusive. Particularly, the cytology/histopathology based on light microscopy, used as gold standard for colon polyp classification, has diffraction-limited resolution and hence cannot detect changes in the cell/tissue nano-architecture. However, we had earlier reported the development of partial wave spectroscopic microscopy (PWS) technique to measure the nano-scale morphological changes during early cancerization within cells. PWS offers sub-diffractional sensitivity by quantifying the nanoscale refractive index fluctuations within cells in terms of intracellular disorder strength (Ld) based on mesoscopic light transport theory. Using concept of field carcinogenesis, we report statistically significant gradient increase in Ld by probing the ‘cytologically normal’ appearing rectal cells from controls to patients harboring non-advanced adenoma (%Difference = 40%, P < 0.03), advanced adenoma (%Difference = 132%, P < 0.01), and having genetic mutation, Hereditry non-polyposis colorectal cancer (%Difference = 200%, P < 0.02). We also demonstrate PWS results from isolated colonocytes from feces samples of azoxymethane (AOM) treated rats and age-matched saline-treated controls. We report statistically significant
increase in disorder in these colonocytes after 5-week (Effect-size = 42%, P < 0.025), 8-week (Effect-size = 48%, P < 0.02), 10-week (Effect-size = 66%, P < 0.005) and 18-week (Effect-size = 70%, P < 0.001) AOM-treatment. This screening approach is minimally-intrusive and highly patient-compliant. Overall, PWS can quantify nano-architectural alterations in histologically-normal colonocytes.

7907-24, Session 4

Optical screening for lung cancer using epithelial cells obtained from buccal mucosa (cheek cells)

H. Subramanian, Northwestern Univ. (United States); H. Roy, Northshore Univ. Health System (United States); D. Damania, M. Shah, L. Cherkezyan, P. Pradhan, V. Backman, Northwestern Univ. (United States)

Lung cancer is the leading cause of cancer related death in United States. However, current screening techniques for detecting lung cancer are limited due to the cost-effectiveness, suboptimal efficacy or poor resolution. Traditional light microscopy or histopathology which is used popularly for studying cell/tissue micro-architecture cannot probe cell-nanoarchitecture which can be altered at the early stages of carcinogenesis due to diffraction-limited resolution (~300 nm). Earlier, we reported the development of a novel optical imaging technique, partial wave spectroscopic (PWS) microscopy, which statistically measures the nanoscale refractive-index fluctuations within a cell in a quantifiable parameter, namely, disorder strength (Ld). For screening lung cancer, our approach was to investigate easily accessible cheek cells based on emerging genetic/epigenetic data that suggests that the buccal epithelium is altered in lung field carcinogenesis. We performed PWS analysis on microscopically normal buccal epithelial brushings from smokers with and without lung cancer (n=135). Ld was significantly (>50%) elevated in patients harboring lung cancer compared to neoplasia-free smokers. The performance characteristic was excellent with an area under the receiver operator characteristic curve >0.80 and was equivalent for both disease stage (early versus late) and histologies (small cell versus non-small cell lung cancers). This novel approach provides a proof of concept that interrogation of buccal mucosa using PWS may potentially lead to a minimally-intrusive pre-screen technique that is clinically easy to perform and cost-effective.

7907-25, Session 4

Imaging fluorescence and broadband reflectance of breast pathologies in situ

A. M. Laughnhey, V. Krishnaswamy, Dartmouth College (United States); W. A. Wells, Dartmouth Hitchcock Medical Ctr. (United States); O. M. Conde, Univ. de Cantabria (Spain); K. Paulsen, B. W. Pogue, Dartmouth College (United States)

A k-Nearest Neighbor (k-NN) classifier trained using parameters extracted from the elastic scattering spectrum, automated diagnosis of benign and malignant breast pathologies in situ with a sensitivity and specificity of 91% and 77% respectively. Performance of the classifier was validated on >21,000 spectra from emerging breast (invasive and in-situ) cancers. Parameter selection potentially improve specificity, the scanning spectroscopy system has been modified to probe broadband (450-800nm) absorption, scattering and fluorescence in localized tissue volumes. The scanning-beam architecture employs a telecentric, dark-field illumination and confocal detection to image fields up to 1.5x1.5cm. The sampling spot size (100µm lateral resolution) confines the volume of tissue probed to within a few transport pathlengths so that multiple-scattering effects are minimized and simple empirical models may be used to analyze spectra. Initial phantom studies demonstrate a linear response between recovered absorption and scattering parameters and the relative chromophore and scattering concentrations respectively. The measurement geometry minimizes the effects of intrinsic absorption on recovered fluorescence, but the fluorescence rate increases with scattering due to increased backscattering of the excitation light. Therefore, calibration curves for fluorophore concentrations are made over a physiologically-relevant range of scattering. Blue light excitation (405nm) is used to detect red fluorescence from Protoporphyrin IX (PpIX) molecules in turbid phantoms over the range of optical properties encountered in tissue. Preliminary reflectance and autofluorescence spectra from breast tissues are acquired and parameterized according to their chromophores, fluorophores and scattering centers. Probing complimentary light-tissue interactions is expected to improve sensitivity to disease and overall efficacy of the classifier.

7907-26, Session 4

Guiding biopsy of dysplasia in Barrett’s esophagus during endoscopy with polarized light-scattering spectroscopy

L. Qiu, D. Pleskow, R. Chuttani, E. Vitkin, L. Guo, A. Sacks, J. Goldsmith, M. Modell, Harvard Medical School (United States); E. Hanlon, Dept. of Veterans Affairs (United States); I. Itzkan, L. T. Perelman, Harvard Medical School (United States)

The incidence of esophageal cancer is increasing more rapidly than any other type of cancer in the United States. However, almost 100% of cases occur in patients with Barrett’s esophagus (BE), a condition in which metaplastic columnar epithelium replaces the normal squamous epithelium of the esophagus. Although BE is an otherwise benign complication of esophageal reflux, it affects approximately 3 million Americans. Of all patients with BE, a small portion eventually develop high grade dysplasia (HGD) which usually appears right before esophageal cancer. So diagnosing HGD is a first priority job for BE screening. However, standard-of-care screening relies upon visual endoscopy and a prescribed pattern of biopsy, which selects only a tiny fraction of the affected esophageal tissue for pathological examination and has a low probability of detection, because dysplasia is highly focal and visually indistinguishable. Previously we demonstrated how the physics of light scattering by small particles could reveal pre-cancer cellular changes. That demonstration used a fiber optic probe illuminating one square millimeter of tissue. Searching the entire area of a diseased esophagus with such a probe is unfeasible. Now, we have developed an optical system with polarizing spectroscopy which can perform rapid optical scanning and multispectral imaging of the entire esophageal surface and can present a diagnosis in near real time. By detecting and mapping suspicious sites in esophageal epithelium during screening endoscopy, this system has enabled guided biopsy of invisible, precancerous dysplasia for the first time.

7907-27, Session 4

Wavelet-based multifractal detrended fluctuation analysis of light scattering spectra from normal and cancerous human cervical tissues

J. Soni, Indian Institute of Science Education and Research (India); J. M. Jagtap, Indian Institute of Technology Kanpur (India); S. Ghosh, H. Purwar, Indian Institute of Science Education and Research (India); A. Pradhan, Indian Institute of Technology Kanpur (India); P. K. Panigrahi, N. Ghosh, Indian Institute of Science Education and Research (India)

Elastically scattered light from biological tissue contain rich morphological and functional information of potential biomedical importance. Both the angular and wavelength dependence of the scattered light from tissue can be analyzed to extract and quantify subtle morphological changes taking place during progression of a disease like...
cancer, and thus may be exploited as a sensitive tool for early diagnosis of cancer. In order to explore this, we have analyzed the fluctuations in the elastic scattering spectra recorded from normal and cancerous human tissues, using wavelet decomposition approach. The elastic scattering spectra (wavelength 400 nm - 800 nm) were recorded at varying scattering angles (10 deg. - 150 deg.) from normal and cancerous tissue sections resected from human uterine cervix, and were subjected to the wavelet-based multifractal detrended fluctuation analysis. The wavelet-based analysis revealed clear signature of self-similar behaviour in the spectral fluctuations of light scattering from the tissues. For this analysis, the wavelets belonging to the Daubechies (Db) basis was used to isolate the local polynomial trend and to characterize the local fluctuations over and above the polynomial trend. Thus obtained light scattering spectral fluctuations from both normal and cancerous tissues showed multifractal behaviour \([F_q (s) = s^{\beta(q)}]\), with the degree of this multifractality being considerably weaker in the cancerous tissues as compared to their normal counter parts. This multifractal behaviour in the elastic scattering spectral fluctuations has been identified to originate from the multi-scale self-similar nature of local refractive index fluctuations in the tissue. The self-similar characteristics of the spectral fluctuations was further confirmed by the Fourier domain analysis which also showed power law behaviour \([1/ f^{(2H+1)}, H = \text{the Hurst exponent}]\), confirming the consistency of the wavelet-based multifractal detrended fluctuation analysis. The details of these results will be presented and their implications for diagnosis of cancer will be discussed.

Variations in the optical scattering properties of skin in murine animal models

K. Calabro, Boston Univ. (United States); A. Curtis, J. Galarneau, T. Krucker, Novartis Institutes for Biomedical Research, Inc. (United States); I. J. Bigio, Boston Univ. (United States)

In the work presented here, the optical scattering properties of mouse skin are investigated in depth with the use of Elastic Scattering Spectroscopy (ESS). In particular, sources of variation that lead to experimental error are identified and examined. The thickness of the dermal layer of the skin is determined to be the primary source of variation due to its high concentration of collagen. Specifically, gender differences in skin thickness are found to cause increases in the reflectance and scattering coefficient value by a factor of two for males as compared to females. Changes in the hair growth cycle are found to influence scattering strength not only due to changes in skin thickness, but also from melanin collection in hair follicles. To better isolate scattering signals, tissue from exsanguinated mice is used, thus reducing absorption effects from hemoglobin. Because direct and/or indirect measurement of mouse skin is common in the development of novel biomedical optics techniques (optical biopsy, molecular imaging, in vivo monitoring of glucose/blood oxygenation, etc.), the purpose of this work is to identify sources of experimental variation that may arise in these studies, such that care can be taken to avoid or compensate for their effects.

Influence of cellular precancerous structural changes on macroscopic light scattering optical properties

N. N. Mutyal, A. J. Radosevich, Northwestern Univ. (United States); A. Tiwari, Evanston Hospital (United States); Y. Stypula, V. M. Turzhitsky, J. D. Rogers, R. Wali, Northwestern Univ. (United States); H. Roy, Evanston Hospital (United States); V. Backman, Northwestern Univ. (United States)

In our previous studies we have shown that Low-Coherence Enhanced Backscattering (LEBS) Spectroscopy demonstrated differences in the optical properties of histologically-normal tissue in the initial stages of colorectal carcinogenesis. The objective of this study is to elucidate how these optical differences are caused by micro- and nano-architectural changes in tissue that, in turn, are brought about by genetic and epigenetic alterations. Specifically, we elucidated the actual changes in the components of tissue which might be responsible for the differential optical properties. We studied animal and cell line models: isolated colonocytes pellet from the AOM-treated rat model of colon carcinogenesis, control human adenocarcinoma HT29 cell line, Csk-knockdown shRNA transfected HT29 cells and EGFR-knockdown HT29 cells (these three cell lines exhibit differential neoplastic aggressiveness despite being microscopically similar). We found that LEBS was sensitive to the structural differences of these microscopically similar cells. This partly explains the optical alterations previously observed in tissue. In order to investigate the molecular mechanisms driving the phenomenon, we studied the expression profile of genes implicated directly or indirectly in cytoskeletal regulation in the colorectal tissues from saline versus AOM-treated rat. Our data suggest that a number of genes regulating the metastatic potential were up regulated and genes being described as tumor suppressor in metastatic cancer were down regulated in cells despite being microscopically normal. Additionally, to understand the effect of the cytoskeleton in determining the changes in the structural and optical properties of cells, we used pharmacological disruption of the cytoskeleton and found that the difference in the optical markers is negated by this action, suggesting cytoskeletal involvement. In conclusion early structural intra cellular precancerous changes can be detected by optical markers measured with LEBS.

Documenting cellular morphology of circulating tumor cells using differential interference contrast-based quantitative phase imaging

K. G. Phillips, J. C. Gladish, J. E. Aslan, S. L. Jacques, Oregon Health & Science Univ. (United States); P. Kuhn, The Scripps Research Institute (United States); O. J. T. McCarty, Oregon Health & Science Univ. (United States)

Circulating tumor cell (CTC) enumeration and characterization has the potential of providing insight into the aggressiveness of cancer growth and metastasis in cancer patients and may assist in therapeutic decisions regarding diagnosis, staging, and treatment. Owing to their ultra-low concentration in blood and the difficulty of their detection, currently little quantitative information is known about the physical properties of CTCs. In this study we document the thickness distribution and cellular organization of CTCs based on quantitative phase imaging. CTCs are first identified using a fiber-optic array scanning technology (FAST) to identify CTCs in peripheral blood draws from colon cancer patients based on the immunofluorescent labeling of cells on a glass substrate. Subsequent to FAST identification, quantitative phase imaging of CTCs is achieved through phase retrieval based on the transport of intensity (TI) model of differential interference contrast (DIC) imaging. A comparison of CTC physical properties to peripheral blood cells is made.

Tissue scattering properties from organelle to organ scales

H. Ding, X. Liang, Z. Wang, S. A. Boppart, G. Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Light scattering from tissues has evolved as a dynamic area of study and attracted extensive research interest, especially due to the potential it offers for in-vivo diagnosis. The main challenges in applying such methods to the clinic stem from the insufficient knowledge of the tissue
optical properties and their large variability across samples. The direct measurement of these scattering parameters is extremely challenging. Therefore, often, simulations such as Monte Carlo and finite difference time domain, are used iteratively instead. In response to this challenge, we have proposed recently a novel method to extract the scattering properties of bulk tissues from quantitative phase imaging (QPI) of slices. We spatially map the tissue slices in terms of the scattering parameters, i.e., $I_s$ and $g$. We illustrate this approach with measurements across different organs from rat models.

The scattering-phase theorem recently derived in our lab provides a mathematical relationship between $I_s$ and $g$ on one hand, and the statistics of phase shift distribution associated with a thin tissue slice, as measured via QPI. Our method provides fast and spatially resolved access to tissue scattering mean free path $I_s$ and anisotropy factor $g$ from quantitative phase images of thin tissue slices. This quantitative phase imaging based approach operates without fitting or iterative procedures. The knowledge of $I_s$ and $g$ has great impact on predicting the outcome of a broad range of scattering experiments on large samples. Our method allows building up an exhaustive database, where various tissue types, healthy and diseased, will be fully characterized in terms of their scattering properties, from microscopic (organelle) to macroscopic (organ) spatial scales.

**7907-33, Session 5**

**Rapid analysis of white blood cells with diffraction imaging flow cytometry**

X. Hu, J. Q. Lu, East Carolina Univ. (United States); Y. Feng, Tianjin Univ. (China)

In a major hospital, one to two thousands of complete blood count (CBC) are ordered daily in which enumeration of white blood cells (WBC) plays an important role for diagnosis of various diseases. Up to 20% of CBC are flagged as abnormal and blood films are prepared for manual review of WBC. The diverse morphology of WBC makes it very challenging to draw consistent diagnosis even for specialists. We have recently developed a diffraction imaging flow cytometer method which can be used to rapidly acquire diffraction image data from single flowing cells. In this method, a coherent laser beam is used to interrogate single flowing cells carried with a laminar flow through a jet-in-fluid flow chamber which allows acquisition of high contrast diffraction images from the cell using a microscope objective. In addition, we have developed a database of 3D morphology of WBC with the confocal imaging method. We will present a wide range of modeling and experimental results to demonstrate the strong correlation between the features of the diffraction image data and 3D morphological features of the cells. In particular, we will present results obtained with primary WBC and cultured WBC derived from leukemia patients in terms of their 3D morphology reconstructed from confocal images and features of image texture extracted from diffraction data.

**7907-34, Session 5**

**Time-lapsed integrated Raman- and angular-scattering microscopy of immune cells**

D. W. Shipp, A. J. Berger, Univ. of Rochester (United States)

Integrated Raman- and Angular-scattering Microscopy (IRAM) combines two light scattering techniques to make chemical and morphological measurements of intact, single cells without the use of external labeling. In previous work, IRAM has successfully differentiated between two types of immune cells (lymphocytes and granulocytes). IRAM can also identify activated CD8+ T cells based on changes in relative chemical content and on the size and number of sub-micron-diameter organelles. The IRAM system has recently been improved to allow the same cells to be measured repeatedly over extended periods of time. A single computer program now controls the microscope stage, a flip mirror to switch between illumination modes, and CCDs for Raman, elastic and bright field image acquisition. After the user has identified several locations of interest, these locations are visited sequentially several times and each type of data is recorded upon each visit. Trials on polystyrene beads show consistent Raman spectra (within 5% in band ratios) and extracted diameters (within 15 nm) over several measurements.

Unlike polystyrene beads, however, cellular samples will undergo various chemical and morphological changes. IRAM will be able to detect these changes in real time. This enables unique studies of the chemical and structural changes within a single cell as it experiences processes such as mitosis, apoptosis, or immune cell activation. The ongoing applications of IRAM to biological processes in single cells will be discussed.

**7907-35, Session 5**

**Intrinsic optical signal imaging of glucose-stimulated physiological responses in the insulin secreting INS-1 x-cell line**

Y. Li, W. Cui, X. Wang, F. Amthor, X. Yao, The Univ. of Alabama at Birmingham (United States)

INS-1 cells express many important features of the pancreatic islet x-cells, and thus provide a popular mimic model for studying diabetes related pathological changes of x-cells in the islets of Langerhans. Recently, we demonstrated the feasibility of near infrared (NIR) imaging of intrinsic optical signals (IOSs) in stimulus activated INS-1 cells. During the experiment, continuous illumination of NIR light was used for recording fast IOSs and image sequences were recorded at a speed of 10 frames per second. A small dose of high concentration glucose medium was added as a physiological stimulus. Fast imaging sequences disclosed rapid IOSs tightly correlated with the glucose-stimulation. All cells displayed robust IOSs approximately 1 second after the glucose stimuli were introduced, and the signals reached their peaks values within 5 seconds. High resolution images revealed both positive and negative IOSs from the stimulus activated INS-1 cells. While cell boundaries were mainly dominated by positive IOSs, cell interiors displayed both strong negative and weak positive responses. In conclusion, we demonstrated IOS imaging of glucose-stimulated physiological activities in INS-1 cells. High-speed imaging sequences disclosed fast IOSs that have patterns and time courses comparable to x-cell electrical activities, indicating potential tight correlations between the fast IOSs and electrophysiological responses. We anticipate that further investigations of stimulus-evoked IOSs correlated with islet activates will lead to a new methodology for noninvasive, functional evaluation of islets of Langerhans.

**7907-36, Session 6**

**Optical property measurement with 3D-OCT to differentiate soft tissues**

L. Scolaro, B. R. Klyen, R. A. McLaughlin, The Univ. of Western Australia (Australia); S. L. Jacques, Oregon Health & Science Univ. (United States); D. D. Sampson, The Univ. of Western Australia (Australia)

This study presents measurements of the optical properties of soft tissue with 3D-OCT imaging, with the aim of enhancing tissue differentiation. We have utilized a single-scattering model of OCT to extract the tissue reflectance profile described by two optical parameters, the attenuation coefficient $\mu$ and local reflectivity $\rho$. We outline a method for calibrating and correcting the OCT reflectance profile to take into account system modulation factors due to the confocal gate and depth scanning of the reference arm. To achieve this, we measure the confocal function $F(z)$ and reference scan function $S(z)$ for our system. The final model for OCT reflectance is described by $R(z) = S(z) F(z) \rho(z-\eta \mu)$, where $z$ is the axial location and $\eta$ is the position of the tissue surface. We have recorded 3D-OCT images for chicken muscle, chicken skin, bovine muscle and lamb kidney. A first-order polynomial fit is used to...
approximate single scattering for the corrected log reflectance profiles obtained. The fitted parameters are further processed to extract two optical properties, the scattering coefficient \( \mu_s \) and the anisotropy of scattering g. We show that application of this two-parameter model to soft tissues provides quantitative differentiation of tissue type, enhancing discrimination of the tissue compared to using only OCT reflectance values. The final aim of this study is to improve characterization of tissue pathologies and as a first step we have shown that automated application of our quantification method can differentiate soft tissue types.

7907-37, Session 6

**Optical coherence tomography speckle decorrelation for detecting cell death**

G. Farhat, Univ. of Toronto (Canada) and Sunnybrook Health Sciences Ctr. (Canada) and Ontario Cancer Institute (Canada); A. Mariampillai, Univ. of Toronto (Canada) and Ontario Cancer Institute (Canada); V. X. D. Yang, Ryerson Univ. (Canada) and Sunnybrook Health Sciences Ctr. (Canada); G. J. Czarnota, Sunnybrook Health Sciences Ctr. (Canada); M. C. Kolios, Ryerson Univ. (Canada) and Univ. of Toronto (Canada)

Speckle in optical coherence tomography (OCT) images results from light backscattered by multiple scatterers within a resolution volume. The intensity of speckle is dependent on the number, size, optical properties and spatial distribution of scatterers. Imaging of living cells and tissues produces changes in the speckle pattern due to the motion of subresolution scatterers. During apoptosis, cells undergo a series of morphological changes leading to the fragmentation of the cell on very short time scales. We hypothesize that the rate of intracellular motion will vary between viable cells and those undergoing apoptosis. We have investigated the ability to detect cell death by applying a speckle decorrelation analysis method using OCT. Acute myeloid leukemia cells were treated with cisplatin, a chemotherapeutic agent known to induce apoptosis, for 24 hours and subsequently centrifuged to create a tightly packed cell sample. A control sample was simultaneously prepared using untreated cells from the same batch. Optical coherence tomography images were acquired of cell samples at a rate of 50 frames per second. Pixel intensities were plotted as a function of time and time-correlation curves generated for each sample. Apoptosis was confirmed in the treated cells by hematoxylin and eosin, as well as TUNEL staining. Results indicated a 20% faster decorrelation rate in the apoptotic cell sample compared to the untreated cells. This novel study demonstrates that the rate of intracellular motion as measured by OCT speckle decorrelation can be used to detect cell death.

7907-38, Session 6

**Determining size, shape, and orientation of non-spherical scatterers using the fiber optic interferometric two-dimensional scattering (FITS) system**

M. G. Giacomelli, Y. Zhu, J. Lee, A. Wax, Duke Univ. (United States)

Angle-resolved low coherence interferometry (a/SCI) is a light scattering technique that has shown promise as a method of detecting neoplasia and analyzing cellular structure both in vivo and as a research tool. Combined with Mie theory and the T-matrix, a method for simulating scattered fields from non-spherical scatterers, we have previously demonstrated that a/SCI is capable of obtaining quantitative nuclear structural information from epithelial and sub-epithelial sites in studies of cells, animals and human tissues. Recently we have improved upon past techniques by developing a novel fiber optic low coherence interferometer based on a hybrid Michelson-Sagnac geometry that combines the depth resolution of optical coherence tomography with polar and azimuthal angle-resolved and polarization sensitive scattered field measurements. By scanning over a wide range of solid angles and resolving polarization, a wealth of information is available for inverse analysis. To exploit this, we present a T-matrix based inverse analysis procedure that recovers the size, spheroidal aspect ratio, and orientation of both individual and ensemble scatterers. We extend analysis to aligned and randomly oriented ensemble scatterers allowing the study of cultured cells. Using micro-spheroidal phantoms and cells, we show that the technique achieves subwavelength accuracy in determining size and shape and provides unambiguous inverse fits over a large range of possible scatterer geometries. Finally, we demonstrate the ability of the system to distinguish between similar scatters in different orientations.

7907-39, Session 6

**Fourier-domain low-coherence interferometry for detection of early colorectal cancer development in the AOM rat model**

F. E. Robles, Y. Zhu, Duke Univ. (United States); J. Lee, S. Sharma, The Hanner Institutes for Health Sciences (United States); A. Wax, Duke Univ. (United States)

We present Fourier domain low coherence interferometry (fLCI) applied to the detection of preneoplastic changes in the colon using the ex-vivo azoxymethane (AOM) rat carcinogenesis model. fLCI measures depth resolved spectral oscillations, also known as local oscillations, resulting from coherent fields induced by the scattering by the front and back surfaces of cell nuclei. The depth resolution of fLCI permits nuclear morphology measurements within thick tissues, making this technique sensitive to the earliest stages of precancerous development. To achieve depth resolved spectroscopic analysis, we use the dual window method, which obtains simultaneously high spectral and depth resolution, and yields access to the local oscillations. Further, similar to Fourier domain optical coherence tomography, fLCI signals can be processed to yield cross sectional images of samples, thereby enabling co-registration of the structural information with the spectroscopic analysis. In this study, forty rats were randomized into groups of ten, where three groups received intraperitoneal (IP) injections of AOM, once per week, for two consecutive weeks. The remaining group received saline by IP and served as the control group. At 4, 8, and 12 weeks after the completion of the dosing regimen, the colon tissue was analyzed. The results show highly statistically significant differences between the AOM-treated and control group samples. Further, the results suggest that fLCI may be used to detect the field effect of carcinogenesis, in addition to identifying specific areas where more advanced neoplastic development has occurred. A complete analysis of the animal study will be presented.

7907-40, Session 6

**Cell death monitoring using quantitative optical coherence tomography methods**

G. Farhat, Univ. of Toronto (Canada) and Sunnybrook Health Sciences Ctr. (Canada) and Ontario Cancer Institute (Canada); V. X. D. Yang, Ryerson Univ. (Canada) and Sunnybrook Health Sciences Ctr. (Canada); M. C. Kolios, Ryerson Univ. (Canada) and Univ. of Toronto (Canada); G. J. Czarnota, Sunnybrook Health Sciences Ctr. (Canada)

Cell death is characterized by a series of predictable morphological changes at the cellular level. It has been shown that optical coherence tomography (OCT) imaging is sensitive to these structural changes [1, 2]. We present a method to detect cell death using quantitative parameters extracted from OCT backscatter spectra and images. Cell death was induced in acute myeloid leukemia cells under four conditions: treatment with cisplatin or a single 80Gy dose of radiation to induce apoptosis,
treatment with colchicine to induce mitotic arrest and growth factor withdrawal to induce oncotic/necrotic cell death. Cells were centrifuged 24 hours after treatment to generate tightly packed cell samples and OCT data was collected in the form of complex interference fringe signals. Normalized backscatter spectra obtained by calculating the Fourier transform of the OCT signals and normalizing by the spectrum obtained from a reference phantom were used to calculate the integrated backscatter. Statistical analysis of pixel intensities were performed by fitting a generalized gamma distribution to histograms of the OCT envelope and extracting the scale and shape parameters of the best fit curves. Estimates of the attenuation coefficient were obtained from averaged OCT depth profiles. Our results indicate these parameters are sensitive to structural changes, which were confirmed histologically, with differences in integrated backscatter ranging from 30% to 80% between treated and viable cells. This study shows the potential for using this method to detect and differentiate modes of cell death.


7907-41, Session 6

Lidar-like equation model for optical coherence tomography signal solution

M. M. Amaral, M. P. Paele, E. Landulfo, N. U. Wetter, A. Zanardi de Freitas, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

The objective of this work was to develop a LIDAR-like equation model to analyze the measured Optical coherence tomography (OCT) signal and determine the total extinction coefficient of a scattering sample. OCT is an interferometric technique that explore sample backscattering feature to acquire in depth cross-section images using a low coherence light source. Although, almost of the OCT applications are intended to generate images for diagnostic, similar to histological images, but the backscattering signal carries much more information. The backscattering problem is similar to those found on LIDAR (Light Detection And Ranging) problem, this similar situation indicate a path that should be followed to solve the OCT problem. To determine the total extinction coefficient three inversion methods was used: the slope, boundary point and optical depth methods solutions, each algorithm were implemented on LabVIEW environment. These algorithms were used to analyze the OCT signal of ceramic and resin. For optical depth method is necessary to know the sample transmittance and it was measured with an integrating sphere. Different layer combination of this material was implemented and images of total extinction coefficient variation along the optical path were obtained in order to evaluate the potential of this technique to differentiate structures with different optical properties. The sample optical characteristics extracted from OCT signal can be use as an additional quantitative method to help clinical diagnoses when applied on biological tissues among others.

7907-42, Session 6

Spatially resolved measurements of dynamic light scattering by Fourier domain OCT

M. Hagen-Eggert, Medizinisches Laserzentrum Lübeck GmbH (Germany); D. Hillmann, P. Koch, Thorlabs GmbH (Germany); G. Hüttmann, Univ. zu Lübeck (Germany)

A method to make spatially resolved dynamic light scattering measurements with a Fourier-domain OCT system is presented. Fluctuations of signal intensity and phase, which are caused by Brownian motion are analysed by autocorrelation function similar to DLS measurements. Based on a ultrafast Fourier-domain OCT system, this method can determine quantitatively diffusion properties, like the hydrodynamic diameter or the diffusion-constant of colloidal suspensions with high depth resolution at particle sizes ranging from 20 nm to a few microns. The experimental setup consists of a Thorlabs spectral radar OCT system equipped with a high power SLD from Superlum. As measurement probe a fiber with a FC-PC connector, for which the polished fiber end worked as the reference mirror, or specially designed miniature interferometer were used. Performance of this technique is demonstrated with polystyrene particle suspensions and compared to measurements of a conventional DLS device. Furthermore, the capability of making spatially resolved measurements with micrometer resolution will be demonstrated. Therefore we used samples of spatially separated particle suspensions and mixtures of different particles, where the separation of the spectra was investigated successfully. Applications may be found in the measurement of particle size distributions of inhomogeneous samples, time dependent changes of particle-compositions or diffusion properties at boundary surfaces. Additionally, the method has the capability to become a useful benefit in clinical diagnostics, especially in ophthalmology, where the molecular compositions and pathological changes of anterior eye components could be detected.

7907-43, Poster Session

Images of depolarization power and retardance to study stages of dysplasia in human cervical tissues

J. M. Jagtap, M. Mozumder, P. Shukla, A. Pradhan, Indian Institute of Technology Kanpur (India)

It is well known that morphological and biochemical changes occur in biological tissues during the development of early tumors. Most tumors are curable provided they are detected at an early stage. We have earlier reported that Mueller Matrix light scattering has the potential to discriminate normal and precancerous human cervical tissue using a mean arithmetic value for depolarization. However there was no ideal cut-off value for depolarization and retardance for different grade of dysplasia. Extending the study further by analyzing the images with Principal Component Analysis (PCA), promising results have been obtained. We report here the measurements taken in ex vivo biopsy slides for retardance which shows significant differences by using the PCA. Grading of dysplasia through the depolarization power of the epithelial layer is displayed by the covariance map. The changes noticed in the stromal region near basal layer of the cervical tissue through the retardance parameter, where histopathology falls short, shows a promising way to confirm the disease. PCA performed on mean value of retardance and the covariance matrix of the depolarization power images has shown that there is a distinction between the stages of dysplasia. It may be worth looking at such changes to improve sensitivity of detection at early stages of cancer as well as discrimination of grades of dysplasia by using this as a supplementary technique to histopathology.

7907-44, Poster Session

Estimation of chromophore concentrations with diffuse optical spectroscopy in the near-infrared wavelength range up to 1600 nm

R. Nachabe, B. H. W. Hendriks, M. B. van der Mark, M. van der Voort, A. E Desjardins, Philips Research Nederland B.V. (Netherlands); H. J. C. M. Sterenborg, Erasmus MC (Netherlands)

Diffuse optical spectra were acquired on phantoms and animal tissues in the near infrared wavelength range up to 1600 nm by using a two-fiber optical probe with a fiber distance separation of 2.5 mm. The spectra were analyzed by a fit-model derived from diffusion theory. In this fit-model, measured absorption coefficients of pure water and lipid are used to estimate the concentration of these chromophores in phantoms and animal tissues. The results show that the lipid concentration in the phantoms, with different values of the reduced scattering coefficient, can be determined within 5% accuracy.
Image reconstruction using measurements in volume speckle fields formed by different wavelengths

N. V. Petrov, Saint-Petersburg State Univ. of Information Technologies, Mechanics and Optics (Russian Federation); M. V. Volkov, Saint-Petersburg State Univ. (Russian Federation); A. A. Gorodetsky, V. G. Bespalov, Saint-Petersburg State Univ. of Information Technologies, Mechanics and Optics (Russian Federation)

Phase retrieval methods afford simple setup requiring no reference beam, and they using in many applications, including x-ray imaging, deformation analysis, optical microscopy of phase object. A single-beam multiple-intensity reconstruction (SBMIR) method of phase retrieval using intensity measurements in a volume speckle fields and wave propagation equation was demonstrated recently. In comparison with other phase retrieval techniques, such as Gerchberg-Saxton and Yang-Gu algorithms, that have constraint that the test object is known to be either a phase-only or amplitude only, SBMIR-technology can be used both for amplitude and/or amplitude-phase objects both in transmission and reflection ways. Here we present innovative modification of this method, using multiple wavelength speckle patterns recorded in one or several distances. Wave propagation equation can be used as one describing wavefront variation due to wavelength change at a fixed distance. Use of three RGB wavelengths radiation, corresponding to spectral response of CCD matrix and DCRaw image converter, allowing to extract the spectral information from Bayer filter channels in documental mode with extended dynamic range, yields thee diffraction patterns from one exposure. We present wavefront retrieval method using intensity measurements in volume speckle fields formed by several wavelengths. This method has a fast convergence and allows registering up to three diffraction speckle patterns at one shot. Both numerical models and experimental results are presented.
7908-30, Poster Session

Development of optical immunosensors and their application to the analysis of human bone morphogenetic protein-7 (BMP-7)

C. Kim, J. I. Rhee, Chonnam National Univ. (Korea, Republic of); O. Sohn, B&P Tech Co., Ltd. (Korea, Republic of)

Bone morphogenetic protein-7 (BMP-7) induces bone formation and renders it to a protein of pharmaceutical importance. Immuno-optical sensors have been developed and applied to determine the concentrations of BMP-7. Hydrophilic CdSe/ZnS quantum dots (QDs) were synthesized and conjugated to the antibody of BMP-7. A 96-well microtiter plate and an optical fiber of 2 mm diameter have been used to immobilize the QDs-conjugated antibody. The fluorescence intensity was measured with a multifunctional microplate reader and a fluorescence detector (M-FOS) at excitation and emission wavelengths of 480 nm and 600 nm. Limit of detection was 0.0-1.0 ng/mL with a 96-well microtiter plate and 0.0-10.0 ng/mL (R2=0.979) with an optic fiber immunosensor. The optic fiber immunosensor has been also applied to a sequential injection analysis for the automatic determination of BMP-7.

7908-31, Poster Session

A novel high-sensitive miniaturized optical system for fluorescence detection

M. Yao, J. Fang, Louisiana Tech Univ. (United States)

Fluorescence based detection system is widely used in the field of biochemistry and medicine due to their high accuracy, sensitivity and selectivity. Using this technology to develop the sensor, lab-on-a-chip and miniaturized detection system has been presented in a lot of papers. However the assembling size and sensitivity of the optical pass or system are the main bottlenecks for miniaturized portable system. In this report, we present a high sensitivity and miniaturized fluorescence detection system especially for the applications of lab-on-a-chip, portable bio-detection system and point-of-care diagnostic system.

The system presented in this paper integrated a low-power and high intensity LED as a light source, all necessary optical components (lenses, filters and a dichroic mirror) and a photodiode with preamplifier into one package about 2 cm × 2 cm × 2 cm. The integrated photodiode-preamplifier features a large active area with extremely high sensitivity with low noise. It is ideal configuration for fluorescence detection and low-light-level medical diagnostic apparatus. The prototype has been tested using fluoresce dye 5-Carboxyfluorescein (5-FAM) dissolved in solvent DMSO (Dimethyl Sulfoxide) and diluted with DI water in various ratios as the testing solution samples. Resolution approximation method is accepted to evaluate the sensitivity of system. The testing results prove a remarkable sensitivity at pico-scale molar around 1.08 pM/L. This sensitivity range should meet the most of bio-detection requirements. This cost-effective detection system, described in this paper, can be easily integrated with the microchip, portable fluorescence detection device and system for biological, chemical, medical and point-of-care applications.

7908-32, Poster Session

Nanometric ion sensing using near-field ratiometric fluorescence sensing

E. Wajnryt, A. Lewis, P. Hamra, The Hebrew Univ. of Jerusalem (Israel); C. Lewis, Nanonics Imaging Ltd. (Israel)

A nanometric measurement of ionic concentrations at distances extending from nanometers to microns from a charged surface immersed in solution is described. AFM and NSOM techniques were combined using NSOM’s nanometric light confinement abilities for optical pH sensing. AFM allowed for knowledge/control of the distance between the optical pH-meter and investigated surface. A ratiometric method of fluorescence sensing was used. The measurements provide important experimental underpinnings to long established solution structure theories, eg. those of Debye and Gouy-Chapman.

7908-34, Poster Session

Development of biosensor for CRP detection using nanoporous structure

O. Kim, S. Yeom, H. Yuan, B. Kang, K. Kim, S. Kang, Kyungpook National Univ. (Korea, Republic of)

A highly sensitive and real-time monitoring biosensor detecting C-reactive protein was developed using a nano-porous aluminum anodic oxide layer. The porous structure Al2O3 coated with Ni and Au was used as a sensing membrane. C-reactive protein is a reactant that increases in inflammatory. The device detecting C-reactive protein in precise has been significance in medical. The fabricated device using interferometry and localized surface plasmon resonance (LSPR) phenomenon has high sensitivity compared with a single sensing principal device. To fabricate a sensing membrane, the anodizing method was conducted. With two-step anodizing method, the fabrication was successful in the side of better surface uniformity. The thickness of the layer depends on the anodizing time. Au deposition was conducted to induce the LSPR phenomenon and to immobilize C-reactive protein by SAM method. The sensing system was consisted of optical spectrometer and an optical fiber reflectance probe. The change of refractive index caused by C-reactive protein antibody-antigen reaction on the membrane can be determined by the aforementioned sensor system in real-time. As results, the fabricated biosensor system has high sensitivity, selectivity and lightweight. C-reactive protein can be measured quantitatively. We measured C-reactive protein using developed system, and confirmed its possibility of a medical use.

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7908-01, Session 1

Non-radiative excitation fluorescence: driving biology beyond the diffraction limit

P. Winckler, R. Jaffiol, J. Plain, Univ. de Technologie Troyes (France)

We propose a new active substrate allowing us to drive fluorescence investigations on biological sample at nanometric scale. We demonstrate the potentiality of our technique through two biological relevant applications: imaging of the cell adhesion points and single molecule spectroscopy at high (micromolar) concentration.

For single molecule detection in solution, the issue is to get the probability of finding one molecule in the observation volume significantly inferior to one. This can be achieved through diluting the dye of interest or limiting the detection to a very small volume. Common fluorescence spectroscopy techniques (such as Fluorescence Correlation Spectroscopy, FCS), based on confocal detection, present two important limitations for in vivo studies. First, its requires to work with a nanomolar concentration of dye, very far from the biologically relevant concentration...
(i.e \( \mu \text{m}, \) or higher). On the other hand, the background signal such as auto-fluorescence, is still often important. In order to overcome these limitations, we propose a new approach based on a non-radiative energy transfer, which consist to strongly reduce the size of the observation volume, down to the attoliter range. We present two crucial applications of our technique: one for nano-imaging on living cells, the other on FCS in sub-diffracted volume.

7908-02, Session 1

**Nanoparticle light scattering in bead-based diffraction biosensors**

K. Hayrapetyan, K. Arif, C. A. Savran, D. D. Nolte, Purdue Univ. (United States)

Diffraction-based biosensors act as spatial matched-filters for rapid capture and sensitive detection of molecular biomarkers. Nanoparticle-based diffraction gratings may be a versatile fluorescence or radio-label-free mechanism for high-throughput and early diagnosis of diseases. Optical diffraction biosensors can be microfabricated by photolithographic patterning of a functionalized surface and in-situ assembly of bi-conjugated nanoparticles, such as gold nanoparticles, which can capture target molecules.

We have developed a model based on a combination of Mie theory for scattering from spherical particles and MSDI (Mie Surface Double Interaction). The model operates in the dilute nanoparticle limit, and must consider the relative contributions from different surface-scattering partial waves to best match experiment and exact scattering theory. The application goal of this work is to explore the trade-offs between the sensitivity and throughput among three detection methods: diffraction, imaging and scanning. We use interferometric surfaces to simulate positive/negative quadrature and nodal/antinodal conditions.

Experimentally, we use thermal oxide on silicon with appropriate thermal oxide thicknesses tuned to phase quadrature interferometric conditions and use self-assembled gold nanoparticles. The particles are detectable in the dilute limit through Molecular Interferometric Imaging (MII) and Spinning-Disc Interferometry (SDI) in a heterodyne scattering condition, or through diffraction in a homodyne scattering configuration.

7908-03, Session 1

**Trapping single DNA molecules in solution**

J. C. Woehl, C. A. Carlson, Univ. of Wisconsin-Milwaukee (United States)

The ability to manipulate matter on submicron length scales has revolutionized biophysical research and fueled important scientific and technological advances in past decades. For example, larger dielectric particles can be trapped free in solution by steep electromagnetic field gradients produced by a strongly focused laser beam (optical tweezers). Pushing the limits to the nanometer level, however, has proven challenging. The only known method for trapping fluorescent nanoparticles such as single molecules uses a time-varying DC field in a feedback loop to counteract Brownian motion. This trap, however, can only operate when the particle can be located through fluorescence detection, which is problematic in the case of single molecules where intermittent fluorescence emission is often observed, and requires a complex hardware and software setup.

In this contribution, we will discuss a novel and elegant approach for the trapping and manipulation of single molecules and other particles over extended periods of time: the electrostatic corral. The proposed trapping scheme has distinct characteristics which set it apart from other trapping techniques, such as a trapping efficiency that scales favorably with particle size (down to the single molecule level), a stable potential well that does not require any imaging for particle trapping, and multi-particle trapping capabilities. The feasibility of the corral trap approach will be demonstrated with experiments on micro- and nanoscale particles, with particular emphasis on the trapping of single-stranded DNA molecules.

7908-04, Session 1

**Dual-modality in-vivo imaging for MRI detection of tumors and NIRF-guided surgery using multicomponent nanoparticles**

J. Key, Purdue Univ. (United States); K. Kim, I. C. Kwon, K. Choi, Korea Institute of Science and Technology (Korea, Republic of); D. Knapp, J. F. Leary, Purdue Univ. (United States)

It is challenging to both detect early-stage cancer noninvasively and remove it specifically during surgery. Magnetic resonance imaging (MRI) is one of the best imaging modalities for noninvasive cancer detection because of its high spatial resolution and diverse image information including anatomical, physiological, and molecular information. However, MRI using contrast agents does not have enough sensitivity to delineate tumor margins during surgery. Moreover, since most surgical tools contain metal substances, image-guided surgery is hard to perform with a MR machine using superconducting magnets. Also MR imaging is too slow for real-time guided-surgery. Near infrared fluorescence (NIRF) imaging has recently received great interest for in-vivo imaging due to its low tissue auto-fluorescence, high signal-to-noise ratios, and short image-acquisition times. NIRF imaging can be used to delineate tumor margins during surgery, but current NIRF imaging cannot provide enough penetration depth to detect early-stage cancer deep inside body. To overcome these restrictions we have developed dual-modality in-vivo imaging for MRI detection of tumors and NIRF-guided surgery using multi-component nanoparticles. NIRF dye, Cy5.5, conjugated glycol chitosan nanoparticles (HGC) exhibit excellent tumor targeting ability with NIRF imaging due to an enhanced permeability and retention (EPR) effect and prolonged circulation times. Superparamagnetic iron oxide (SPIO) nanoparticles, as MR contrast agents, were loaded into the nanoparticles, resulting in HGC-SPIO nanoparticles. HGC-SPIO nanoparticles were characterized and are being evaluated in mice by both NIRF and MR imaging. Our results indicate HGC-SPIO nanoparticles have the potential for dual-modality in-vivo imaging with MRI detection of tumors and NIRF-guided surgery.

7908-05, Session 1

**High-precision three-dimensional Position measurement of particles by digital Gabor holography**

M. K. Kim, M. C. Potocoava, L. G. Krzewina, Univ. of South Florida (United States)

Position measurement of optically trapped particles is a very important issue in many applications. The conventional method is to aim a probe laser at the trapped particle and to measure the laser deflection using a position-sensitive photodetector. The method is very effective with position resolution as high as several nanometers. But it is very difficult to obtain simultaneous three-dimensional measurements of the particle position. The purpose this paper is to propose and demonstrate use of digital Gabor holography for simultaneous three dimensional calibration of the particle position with nanometric precision. By modulating the particle position with a dual piezo-driven mount, and analyzing the digital Gabor holography (DGH) image of a 10 um particle, we have unambiguously demonstrated DGH is capable of about 50 nm resolution in all three directions, which is at least an order of magnitude improvement over previous holography-based results. We use the 3D position measurement by DGH in a time-resolved experiment. The base of a liquid medium is shifted with a square wave signal to the piezo-driven base, while a particle in the liquid is held by an optical trap. The relaxation of the particle position is measured to determine the optical trap strength if the viscosity of the liquid is known, or vice versa.

Nanoscale actuation of gold nanorods using kilovoltage radiation enhances the efficacy of radiation therapy in vivo

P. Diagaradjane, A. Deorukkar, S. Krishnan, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States)

Selective photothermal ablation of tumors by optical actuation of molecularly targeted gold nanoparticles has shown promising preclinical results. Despite these convincing results, the limited penetration of light in tissues remains a major challenge in the treatment of bulky and deep-seated tumors that are inaccessible to the irradiating light/laser source, suggesting the need for alternate strategies for real-time clinical translation. Here, we report a novel approach of nanoscale actuation of photoelectron-mediated DNA damage and cell kill by kilovoltage (keV) X-ray radiation of tumor-targeted gold nanoparticles (GNRs). In brief, the incident keV radiation is absorbed by inner shell electrons of high atomic number (Z) gold resulting in the production of photo- and Auger/Coster-Kronig electrons which, in turn, create DNA strand breaks. Engineered GNRs were enveloped in a self-assembled monolayer of poly(ethylene glycol) and decorated with Cetuximab (a monoclonal antibody targeting epidermal growth factor receptor) for tumor-specific delivery. Biological radiation dose enhancement was confirmed by assessment of subcellular compartmental localization of GNR clusters within the cytoplasm, reduced clonogenic survival, and slower DNA repair kinetics. In a subcutaneous HCT116 colorectal cancer model that is known to be resistant to cetuximab (due to K-Ras mutation), this treatment approach (2x1011 conjugated GNRs/mouse i.v.; 6 Gy; 250 keV X-rays) demonstrated significant radiosensitization (time to doubling of tumor volume of 19 days vs. 10 days for untreated GNRs, p < 0.001). These promising results highlight the clinical translation potential of this novel nanoscale actuation strategy, which can be used with conventional radiotherapy regimes for improved therapeutic outcomes.

Spectroscopic characterization of bionanoparticles originating from newly developed self-forming synthetic PEGylated lipids (QuSomes)

R. K. Bista, R. F. Bruch, A. M. Covington, Univ. of Nevada, Reno (United States)

In this work, we have aimed to merge the advantages of nanotechnology and biophotonics in conjunction with vibrational spectroscopic techniques in order to understand the various aspects of new kinds of synthetic bionanoparticles originating from self-forming synthetic biopolymers known as polyethylene glycol (PEGylated) lipids. In particular, two complementary molecular spectroscopic techniques based on thin layer Fourier transform infrared and confocal laser tweezers Raman spectroscopy have been employed for the investigations of newly developed artificial PEGylated lipids trademarked as QuSomes. Here, the lipid labeled GDM-12 has saturated 14 acyl chains whereas GDO-12 is characterized by monounsaturated 18 acyl chains, and GDS-23 is composed of saturated 18 acyl chains in their hydrophobic chain. Similarly, GDM-12 and GDO-12 contain 12 units, and GDS-23 contains 23 units of hydrophobic PEG head groups. In contrast to conventional phospholipids, this novel kind of lipid can form liposomes spontaneously upon hydration, without the input of external activation energy. In addition, fluorescence correlation spectroscopy has been utilized to measure the size distribution of such nanoparticles in suspension as well as scanning electron microscopy has been applied for the imaging purposes. Although such PEGylated lipids show a common spectral pattern, important differences in the spectra have been observed, enabling us to distinguish these different lipids on the basis of characteristic features calculated from the spectroscopic band component analysis. Finally, in this study, detailed spectroscopic results due to the vibrational band assignments and band component analysis corresponding to various functional groups for individual nanoparticles have been analyzed and discussed.

DMSO effects on FRET to dye-labeled DNA in conjugated polymer-based DNA detection

M. Kang, O. K. Nag, T. Kwon, J. Yoo, H. Y. Woo, Pusan National Univ. (Korea, Republic of)

In this study, DMSO effects as solvent were studied in fluorescence resonance energy transfer (FRET) from a cationic polyfluorene copolymer (FHQ, FPQ) to a fluorescein (Fl)-labeled oligonucleotide (ssDNA-FI) in phosphate buffer solution (PBS). Among various organic solvents, dimethyl sulfoxide (DMSO) has attracted considerable interests owing to its intermediate polarity and compatibility with water and biomolecules such as nucleic acids, proteins, carbohydrates, etc. It also has low toxicity compared to THF or NMP, and dissolves both hydrophobic and hydrophilic materials well. Upon addition of DMSO, the optical properties of polymers and the probe dye were substantially modified and the FRET-induced PL signal was enhanced 3.8–37 times, relative to that in PBS. The hydrophobic interaction between polymers and ssDNA-FI is expected to decrease in the presence of DMSO, which induces the weaker polymer/ssDNA-FI complexation with longer intermolecular D-A separation and perturbs the competition between the FRET and PL quenching processes such as photo-induced charge transfer. The gradual decrease in Fl PL quenching with increasing the DMSO content was investigated by measuring the Stern-Volmer quenching constants and PL lifetime of the excited Fl* in polymer/ssDNA-FI (600 ps in PBS and 2.120 ps in 80 vol% DMSO for FHQ/ssDNA-FI) in PBS/DMSO mixtures. The substantially reduced PL quenching would amplify the resulting FRET Fl signal. The signal amplification in real DNA detection was also demonstrated with fluorescein-labeled PNA (probe PNA) in the presence of a complementary target DNA and noncomplementary DNA in aqueous DMSO solutions. This approach suggests a simple way of modifying the fine-structure of polymer/ssDNA-FI and improving the detection sensitivity in conjugated polymer-based FRET bioassays.

Development of carbon-fluorine spectroscopy: an emerging analytical tool for pharmaceutical and biomedical applications

F. Menaa, B. Menaa, O. N. Sharts, Fluorotronics, Inc. (United States)

Carbon-Fluorine Spectroscopy (CFS), aka Fluoro-Raman Spectroscopy (FRS), is a patented platform technology using various methods and a family of devices called PLIRFATM (Pulsed Laser Isochronic Raman and Fluorescence Apparatus) developed by Fluorotronics, Inc. The key feature of this promising and flexible technology is based on the discovery of a characteristic optical signature of carbon-fluorine bond(s) in the fingerprint spectral area of 500 cm-1 and 800 cm-1 allowing detection, characterization, imaging, and measurement of fluoroorganics. The FRS method is ultra-specific, ultra-sensitive, non-destructive, rapid, require no sample preparation, easy to use and therefore, is cost effective. Furthermore, the C-F bond signal is directly proportional to the concentration of the analyte, allowing a quantitative determination of F-labelled compounds. Since the C-F bond is unique in its character, it can be used as an external or internal chemical tag/molecular marker. Furthermore, the C-F label is efficient, soluble, cheaper, smaller, more stable and less toxic than fluorescent dyes, nanoparticles or quantum dot materials. Consequently, FRS can be used for numerous applications. For instance, C-F bonds are often incorporated into pharmaceutical, chemical, and biological molecules as well as in polymers and nano-materials to achieve special properties (e.g. molecular stability, tracing a compound during the production or the synthesis cycle).
is applicable for multiplexing, high throughput analysis of any type of fluoroorganics regardless their physical state.

In this study, we present some of our data and review the most important applications of our patented technology in the pharmaceutical and biomedical fields.

7908-42, Session 1

**Fluorescence single particle tracking for sizing of nanoparticles in undiluted biological fluids**

K. Braeckmans, K. Buyens, W. Bouquet-Geerardyn, C. Vervaet, P. Joye, F. De Vos, Univ. Gent (Belgium); L. Plawinski, L. Doeuvre, INSERM (France); N. N. Sanders, Univ. Gent (USA); J. Demeester, S. C. De Smedt, Univ. Gent (Belgium)

Developing functional nanoparticles is an area of great interest for drug delivery and biomedical imaging. In order to make the transition from the laboratory to a successful and safe product, accurate characterisation of nanomaterials is a prerequisite, not only in simple solvents but also in biological media. One of the most important parameters is the effective size of nanoparticles as it directly influences their in vivo processing and biodistribution. However, due to a lack of suitable sizing methods, systematic studies are missing and hamper efficient development of improved nanoparticulate systems. Here we report on the first study where the aggregation of drug delivery nanoparticles is followed in real time in undiluted whole blood by using fluorescence single particle tracking (ISPT) with maximum entropy analysis. ISPT sizing has the potential to become an important tool in any field where the characterisation of nanomatter in complex fluids is of critical importance for designing improved nanomaterials.

7908-12, Session 2

**Blue fluorescent carbon nanoparticles**

V. Varadarajan, R. Chaudhary, S. K. Mohanty, A. R. Koymen, The Univ. of Texas at Arlington (United States)

Carbon nanoparticles (CNPs) are emerging as very important building blocks for nanotechnology and biomedical applications due to their unique electronic, optical, mechanical and thermal properties. While CNPs having predominantly graphitic structure show fluorescence in green-red spectrum, nanotubes emit in the near-infrared spectrum. Recently, octadecylamine-functionalized detonation-nanodiamonds have shown blue fluorescence similar to natural diamond. These detonation-nanodiamonds can contain impurities and the mechanism behind blue fluorescence cannot be attributed to specific dislocations or nitrogen vacancy point defects. We report optical spectroscopic studies of CNPs prepared using electric plasma discharge method under controlled laboratory environment. Fluorescence spectroscopic measurements showed evident blue fluorescence exhibited by the CNPs. Raman spectroscopy of these CNPs showed a distinct peak at 1330 cm-1 (characteristic of Diamond) and another peak at 1600 cm-1 (graphite band). In biomedical imaging, these CNPs will offer a major advantage over carbon nanotubes and semiconductor quantum dots because of their less toxic nature. Thus, these intrinsically fluorescent CNPs can be functionalized to target cancer cells and fluorescent cells can be irradiated with near-IR laser beam for targeted photothermal therapy.

7908-13, Session 2

**Studying the uptake of QD-transferrin in HeLa cells with structured-illumination microscopy**

D. Chen, G. Xu, Shenzhen Univ. (China)

Cancer is one of the biggest threats to the health of human being. Using specific drug to kill the cancer cells but do harmless to the normal cells at the same time is an ideal way for cancer therapy. Transferrin (TF) is a protein with high affinity for TF receptors, which are overexpressed in most cancer cells. So, TF is an optimal ligand for specifically targeting cancer cells. On the other hand, quantum dots (QDs) are fluorescence labels with board excitation spectrum and narrow emission spectrum, resistance to the photobleaching, and these characteristics make it became a promising fluorescence label in biology. In this paper, QD is linked to TF to form a bioconjugate, which will be recognized by TF receptor on the surface of HeLa cell membrane and internalized by the cell, and such process is recorded with a home-built structured illumination microscopy (SIM). Thanks to the luminescent characteristic of such QD-Tf bioconjugates and the sectioning ability of SIM, the uptake of these bioconjugates by cells is visible and recorded with high resolution.

7908-14, Session 2

**Nanostructured sensors to monitor the microenvironment in wounds**

A. N. Cartwright, Univ. at Buffalo (United States)

No abstract available

7908-15, Session 3

**Phase conjugating nanomirrors: utilizing optical phase conjugation for imaging**

B. G. Yust, D. Sardar, A. Tsin, The Univ. of Texas at San Antonio (United States)

Optical phase conjugation is a nonlinear effect in which light incident upon a nonlinear medium may be conjugated so that the output signal is in the opposite direction of the input, as seen in four-wave mixing. It has been shown that these nonlinear effects may still be seen in various nanocrystals and nanoparticles. Barium titanate (BaTiO3) is a good candidate for phase conjugation on the nano-scale, as four-wave mixing has been shown in nanoparticles of this type. Also, the ability to dope this material with rare earth elements, with strong absorption and emission lines, makes it possible to use these as multi-functional, multi-modal probes for biomedical applications. BaTiO3 nanoparticles are synthesized using a precipitation method and fully characterized through STEM and XRD to obtain morphology. Optical and nonlinear properties are also studied for the visible and near-infrared regions. Gold shells are added to the BaTiO3 core to enhance the electric field within the core, and any optical signal gains are studied. Finally, these particles are used in a four wave mixing setup to optically conjugate scattered light traveling through turbid media, such as tissue, to re-obtain lost image information due to the scattering process.

7908-16, Session 3

**Combined Raman confocal and atomic force microscopy studies of cancerous cells treated with Paclitaxel**

L. Derely, P. Collart Dutilleul, Univ. Montpellier 1 (France); V. Szabo, C. Gergely, Univ. Montpellier 2 (France); F. J. G. Cuisinier, Univ. Montpellier 1 (France)

Paclitaxel interferes with the normal function of microtubule breakdown, induces apoptosis in cancer cells and sequesters free tubulin. Atomic force microscopy (an MFP3D- Asylum Research AFM) in imaging and force mode was used to determine the morphological and mechanical modifications induced on living cells. As this drug acts also on other cell mechanisms it is important to monitor it’s accumulation in the cell compartments. Hence, the intracellular spreading of the drug was followed using a WITEC 300R confocal Raman microscope equipped
with a CCD camera. The studies were performed on living epithelial MCF-7 breast cancer cells. Paclitaxel was added to cell culture media for 3, 6 and 9 hours.

Among the specific Paclitaxel Raman bands we selected the one at 1714 cm\(^{-1}\) because it is not superposed by the spectrum of the cells. Confocal Raman images are formed by monitoring this band, the C-C C-N band and the PO4 band. Paclitaxel slightly accumulates in the nucleus forming patches. The drug is also concentrated in the vicinity of the cell membrane and in an area close to the nucleus where proteins accumulate. Our AFM images reveal that the treated cancerous MCF-7 cells keep the same size as the non treated ones, but their shape becomes more oval. Cell’s elasticity is also modified: a difference of 2 kPa in the Young Modulus characterizes the treated MCF-7 mammary cancerous cell.

Our observations demonstrate that Paclitaxel acts not only on microtubules but also accumulates in other cell compartments (nucleus) where microtubules are absent.

7908-17, Session 3

In-situ formation of microstructures near live cells using spatially structured near-infrared laser microbeam

N. D. Ingle, L. Gu, S. K. Mohanty, The Univ. of Texas at Arlington (United States)

Cellular and axonal migration is important from various physiological aspects including immune-response, neuronal injury-repair and formation of functional cellular-networks during organogenesis. In order to study cellular behavior such as polarization in response to inhibition, physical obstacles have been created by different microfabrication methods. However, none exists for in-situ fabrication of microstructures at desired locations near live cells in culture medium. Here, we report formation of linear microstructures from the culture media in regular petridish near growing cells using spatially-structured near infrared (NIR) laser beam. A tunable Ti: Sapphire laser beam was shaped into an elliptic profile by use of a cylindrical lens and focused using a 20X microscope objective onto the regular cell culture media (DMEM: F12) containing propidium iodide (PI). Irradiation near the desired cell with the NIR laser microbeam for few seconds resulted in formation of an elongated microstructure inside the media at a higher focus. The time to form the microstructure was found to depend on the laser beam power. Based on the kinetics of structure formation and power dependence, this was attributed to radiation pressure induced concentration of materials from the media itself. The microstructure was held by the elongated Gravitino-optical trap and could be repositioned by movement of the sample stage. The microstructure could be brought back to the petridish surface by reducing the laser beam power or switching off the laser beam. Further, orientation of these microstructures was achieved by rotating the same elongated laser beam profile at lower power levels. Multiple microstructures were formed and organized near live cells. This would enable study of response of cells/axons to the immediate physical hindrance provided by such structure formation and also eliminate the biocompatibility requirement posed on artificial microstructure materials. We will present these results and put forward our hypothesis behind the structure formation mechanism.

7908-18, Session 3

Experimental analysis of cross-talk effects between a series of nanohole structures on the same metal film

F. Vasefi, M. Najiminaini, B. Kaminska, J. J. L. Carson, Simon Fraser Univ. (Canada)

Nano-hole arrays are promising optical devices for bio-sensing applications due to their strong enhancement of optical transmission. Transmission enhancement occurs when light incident on the perforated metal film interacts resonantly with a surface plasmon mode, which exists at the interface of the metal and adjacent dielectric medium. Nano-hole arrays have defined maxima and minima in the zero-order transmission spectra, which are determined by the geometry, dielectric constant, and angle of incident light. Fabrication of nano-hole arrays, each of different geometry, on the same metal film results in an optical element with various transmission spectra in different spatial positions that could potentially be applicable to many multispectral bio-sensing applications.

The miniaturization of optical sensing devices requires integration of multiple nano-hole arrays with smaller spacing; however, the effect of plasmonic cross-talk and its effect on spectral transmission are not fully understood. In this work, we experimentally fabricated different sets of nano-hole arrays (each with the area size of 30 µm x 30 µm) with various hole diameter and spacing. The nano-hole arrays were designed to have resonance peaks in near infrared region of the optical spectrum. The nano-hole arrays were fabricated on a Pyrex substrate using Electron Beam Lithography (EBL) followed by deposition of an optically thick gold film. The spectral transmission of each nano-hole array set with different spacing between the arrays was measured and the effect on the transmission peak, resonance wavelength and resonance bandwidth was analyzed.

7908-40, Session 3

Spinning biochips: the development of the BioCD and noninertial microfluidic disks

D. D. Nolte, Purdue Univ. (United States)

Spinning biodisks have advantages that make them attractive for specialized biochip applications such as protein microarrays, biosensors and diagnostic assays. The two main classes of spinning biodisks are microfluidic disks and bio-optical compact discs [1]. Microfluidic biodisks take advantage of non-inertial pumping for lab-on-a-chip uses. BioCDs use spinning-disk interferometry, under the condition of common-path phase quadrature, to perform interferometric label-free detection of molecular recognition and binding.

For multiplexed biomarker detection there is a strong driving force for fluorescence-free assays. We have developed multiple interferometric quadrature classes, such as microdiffraction, in-line, phase contrast and holographic adaptive optics. Thin molecular films are detected with a surface height sensitivity of approximately one picometer. The current generation of BioCD uses a single thermal oxide to establish the phase-sensitive detection of immobilized protein. Because phase from binding analyte is transduced directly to intensity, this interferometric platform also can be used for direct imaging and real-time monitoring using molecular interferometric imaging (M2I).

Small protein spots enable scalability to many spots per disk for high-throughput and highly-multiplexed immunoassays. Immunoassays have been applied to haptoglobin using protein A/G immobilization of antibodies, as well as to the detection (in patient sera) of prostate specific antigen (PSA) for prostate cancer, and CA125 for epithelial ovarian cancer (EOC).


7908-41, Session 3

Functional nanoscale imaging of protein surfaces

P. D. A. Cristea, R. Tuducea, O. Arsenea, Polytechnical Univ. of Bucharest (Romania); D. V. Nicolau, Univ. of Liverpool (United Kingdom)

No abstract available
Three-dimensional polymer nanostructures for applications in cell biology generated by high-repetition-rate sub-15-fs near-infrared laser pulses

M. H. Straub, M. Licht, K. Koenig, M. Afshar, D. Feili, H. Seidel, Univ. des Saarlandes (Germany)

In recent years two-photon photopolymerization has emerged as a novel and extremely powerful technique of three-dimensional nanostructure formation. Utilizing high repetition-rate femtosecond-pulsed lasers sub-micron structural element sizes are readily achieved. As two-photon excitation of the photoinitiator confines the photopolymerization reaction to the centre of the focal spot, complex-shaped three-dimensional structures can be formed using appropriate beam steering and/or nanopositioning systems. Here, we report on the fabrication of three-dimensional arrangements made of biocompatible polymer material, which can be used as templates for cell growth. Using three-dimensional cell cages as cell culture substrates is advantageous, as cells may develop in a more natural environment as compared to conventional planar growth methods. The two-photon fabrication experiments are carried out on a commercial microscope setup. Near-infrared light generated by a sub-15 fs pulsed Ti:Sapphire laser system (centre wavelength 800 nm, bandwidth 120 nm, repetition rate 85 MHz) is focused into the polymer material by a high-numerical aperture oil immersion objective. Due to the high peak intensities sub-NJ pulse energies in the focal spot are sufficient to polymerize the material at structural element dimensions of 100 nm or even below. Therefore, cell cages of sophisticated architecture can be constructed involving very fine features which take into account the specific needs of various types of cells. Ultimately, our research aims at three-dimensional assemblies of photopolymerized structural elements involving sub-100 nm features, which provide cell culture substrates far superior to those currently existing.

Incoherent lensfree imaging on a chip using compressive decoding of nanostructured surfaces

B. Khademhosseini, I. Sencan, G. Biener, T. Su, A. F. Coskun, D. K. Tseng, A. Ozcan, Univ. of California, Los Angeles (United States)

Lensfree on-chip imaging is becoming an important substitute to conventional lens-based microscopy by providing compact and cost-effective microscopes, which are especially important for imaging of microfluidic systems. Although digital-holography can provide powerful solutions for on-chip microscopy, it has limitations for imaging of incoherent objects such as fluorescent samples. To address this limitation, we introduce a lensfree imaging modality that utilizes nanostructured surfaces to achieve sub-pixel resolution imaging of incoherent samples located on a chip. In conventional lensfree on-chip imaging, the point-spread-function is space-invariant (excluding pixelation) and its functional-form is dictated by diffraction. The main function of the nano-structured surface in our technique is to break this space-invariance, i.e., a point-source located at any arbitrary position on the chip will create a different diffraction pattern than others. By registering these diffraction patterns of different points on the nano-structured chip, one can decompose/decode any arbitrary pattern sampled by the sensor-array into a discreet set of incoherent sources located on the structured-chip. To validate the performance of this technique, we created a nano-structured chip using focused-ion-beam milling, which was then calibrated by recording the diffraction patterns created by a tightly-focused spot on its surface, while raster-scanning the focus position. This calibration process is only done once for a given structured-chip, and can then be used to decode any arbitrary object on the chip using compressive sampling algorithms. We validated sub-pixel resolution of this nano-structured chip by resolving two closely spaced incoherent spots achieving a resolution of ~2μm without the use of any lenses.

Silicon-on-insulator nanopillar-array optical sensor

T. Xu, M. Xu, Univ. of Toronto (Canada); N. Zhu, Royal Institute of Technology (Sweden); P. Kumari, Univ. of Toronto (Canada); L. Wosinski, Royal Institute of Technology (Sweden); S. Aitchison, H. Ruda, Univ. of Toronto (Canada)

To fulfill the need for low-loss, high sensitivity, and compactness, our pillar-array optical microcavity sensor consists of a low-loss input silicon waveguide, leading into 277-nm-diameter and 500-nm-tall silicon pillar arrays, and output into another silicon waveguide. This compact pillar-array arrangement allows more than 80% connected void space and at least 30% of the electric field energy of the optical mode in air, overlapping with analytes, and therefore has enhanced sensitivity. We studied the sensor sensitivity for both bulk index change and surface index modification. As a bulk index sensor, for environmental refractive index change of 0.01, a resonance peak wavelength shift of 3.5 nm was measured. As a surface index sensor, the simulations show, for a coating with thickness of 1 angstrom, the resonance wavelength shifts as large as 0.4 nm. Combining with a sharp 0.06 nm wide resonance peak, our pillar-array sensor is able to resolve ultra small bulk and surface refractive index changes caused by biomaterials.

In addition to low-loss, high sensitivity, and compactness, photonic crystal bandgap structures allow us to customize the sensitivity and the resonant quality factor of the sensor to suit individual application needs. Also, the manufacturing protocol is compatible with CMOS microfabrication processes, and therefore, in comparison with other available sensor platforms, is promising to be made in large quantity at low expense.

Demonstration of a reusable plasmonic polymer microarray sensing platform

P. Roche, M. Cheung, S. Taslimi, V. P. Chodavarapu, A. G. Kirk, McGill Univ. (Canada)

High throughput plasmonic sensors are a popular research field, standard surface plasmon resonance (SPR) instruments can achieve high throughput only in imaging configuration. This leads to consideration of pattern substrates for conventional SPR and isolated nanoparticle localized SPR (LSPR) arrays, both of which have some disadvantages. Spot functionalization relies upon mask or pin printing to accomplish density, and this increases the complexity of use and standard operating procedures. Both patterned and nanoparticle arrays assay platforms are also commonly single use, unlike some SPR imaging and multi channel angular sensing SPR approaches. The microarray format proposed here is intended for multiple usage and regenerated, with a simple optical readout method. A plasmonic polymer of exquisite refractive index sensitivity and incorporate glass-like physical and mechanical stability provides the sensing element to the platform. Further, the standard sol-gel chemistry is well understood and amenable to easy covalent functionalization as well as matrix methods such as nitrocellulose. The microarray of polymer spots is deposited on a patterned SU-8 surface isolating each spot. Interrogation is accomplished by evanescently coupled white light interacting with polymer immobilized gold nanorods. Mie scattering from rods is collected in the darkfield using a low cost bespoke inverted microscope. Using this platform, imaging is accomplished in real-time.
7908-24, Session 4

**Improved signal-to-noise detection of single virion using microcavities**

T. Lu, Univ. of Victoria (Canada) and California Institute of Technology (United States); H. Lee, T. Chen, J. H. Kim, California Institute of Technology (United States); S. Herchak, Univ. of Victoria (Canada); K. Vahala, California Institute of Technology (United States)

The resonance wavelength of whispering gallery microcavities (WGMs) shifts when a particle lands on the surface in the vicinity of the confined optical mode; and by monitoring the cavity resonance wavelength one can detect single particles of the size comparable to a single virion. To date, detection of single Influenza A (InA) virion binding to a silica microsphere with Q exceeding one million has been demonstrated by monitoring the corresponding resonance wavelength change. The reported signal-to-noise-ratio in that work was 3:1. In this work, by employing a stable fiber-delay-line reference to monitor the microcavity resonance–wavelength shift in real time, we have reduced the impact of laser frequency jitter noise on our measurement and achieved SNR levels exceeding 30:1 for detection of single InflA virion. This jitter noise results from the external-cavity semiconductor laser used here to probe the microcavity sensor (a high Q microtoroid). The r.m.s. resonance wavelength measurement noise in our experiment is below 0.5 fm. We have also observed binding of polystyrene beads with radii as small as 25nm with SNR levels close to 10:1.

7908-25, Session 4

**Modulation of signal/noise and readout intensity of microfabricated microarray surfaces**

S. Dobrouj, J. Aveyard, D. V. Nicolau, Univ. of Liverpool (United Kingdom)

No abstract available

7908-26, Session 4

**Single-molecule detection of brominated diphenyl ether 47 (BDE 47) using a non-competitive phage anti-immunocomplex assay in nanowells**

J. Han, H. Kim, S. Lakshmana, S. Gee, B. Hammock, I. M. Kennedy, Univ. of California, Davis (United States)

Brominated diphenyl ethers (BDEs) have been widely used as flame retardants in various consumer products. However, concerns have arisen due to possible dispersion of these small compounds in the environment and their adverse effects on human health through dietary intake, breathing or direct contact. Therefore, rapid, simple, high-throughput detection is in great demand. In this study, we propose the microarray integrated with an electrophoretic particle entrapment system (EPES) which enables us to effectively trap the nanoparticles conjugated with biological samples into nanowells. The microarray/EPES is superior to other biosensors using immunoassays in terms of lowering the limit of detection to the femto- or atto-molar level with extremely small amounts of solutions. To detect BDE 47 on the microarray, we used a phage borne peptide which specifically binds to the complex of BDE 47 and anti BDE 47 polyclonal antibody (BDE 47 PAb). This method overcomes the limitations of sensitivity, precision, and on-site monitoring which are typical in conventional competitive assays when applied to small compounds. The nanoweb was patterned onto a layer of PMMA and LOL on a conductive and transparent indium tin oxide (ITO)-glass slide by using e-beam lithography. The suspension of 200 nm-fluorescent (blue emission)-carboxylated polystyrene (PS) particles coated with protein A followed by BDE 47 PAb was added to the chip that was connected to the p-Hoeve voltage. On top of the droplet, another ITO-coated-glass slide was covered and connected to a ground terminal. After trapping the particles into the nanowells, the mixture of different concentrations of BDE 47 and fixed amount of phage peptide labeled with fluorescein isothiocyanate (FITC) was added. Quantification of the phage peptide bound to the analyte from each nanowells of the array was performed by using a single molecule detection system.

7908-27, Session 4

**Nanometric measurement of optical pressure deformation of fluid interface by digital holography**

D. Clark, M. K. Kim, Univ. of South Florida (United States)

Surface deformation on a liquid interface by optical radiation pressure of a cw laser is typically very weak and insufficient to overcome the surface tensions of most fluid interfaces. For this reason, pulsed laser sources or specially prepared fluids with exceptionally reduced surface tension are used to produce observable effect. It is our approach to use the method of digital holographic microscopy to image such deformations with nanometric precision and length scales. The quantitative phase analysis inherent to digital holography yields an imaging method which can easily observe the very slight surface deformations of standard fluid-fluid interfaces by cw optical radiation pressure. We have applied a cw 535 nm laser on methanol (index 1.33) to silicone oil (1.39) interface while monitoring the interface deformation with a digital holography setup with a low-power HeNe laser. The green laser power is varied over a range of zero to 1.5 W. From the resulting phase images, physical deformations were calculated, which were observed to vary linearly with power, up to 1.6 um. The minimum deformation of about 100 nm can be resolved with 100 mW of focused green laser. We ascertain that the observed effect is not of thermal origin by repeating the measurements with different combinations of fluids. Preliminary time-resolved measurements show the effect is faster than milliseconds. Through additional calibration experiments, the measured deformations will be used to calculate surface tension of the interfaces. Improvement of the resolution as small as 10 nm should be possible through systematic improvements of apparatus.

7908-28, Session 4

**Local plasma membrane permeabilization of living cells by nanosecond electric pulses using atomic force microscopy**

G. L. Thompson, Clemson Univ. (United States) and Ball Aerospace Inc. (United States); C. C. Roth, Air Force Research Lab. (United States); A. G. Pakhomov, Old Dominion Univ. (United States); G. J. Wilmink, B. L. Ibe, Air Force Research Lab. (United States)

Numerous studies provide evidence that nanosecond electric pulses (nsEPs) can trigger the formation of nanopores in the plasma membranes of cells. However, the biophysical mechanism responsible for nanopore formation is not well understood. In this study, we hypothesize that membrane damage induced by nsEPs is primarily dependent on local molecular composition and mechanical strength of the plasma membrane. To test this hypothesis, we positioned metal-coated, nanoscale cantilever tips using an atomic force microscope (AFM) to deliver nsEPs to localized areas on the surface of the plasma membrane. Simultaneous fluorescence imaging (FLI) techniques were used to evaluate the magnitude of membrane damage. To approximate pore size, we conducted experiments using fluorophores of various diameters: propidium iodide (~1 nm) and thallium ions (~0.2 nm). In addition, we conducted computational modeling simulations to verify that the
electric field provided by the nsEP is concentrated between the tip and the plasma membrane. The results show that we could effectively deliver nsEPs using the AFM tips. The fluorescence data shows that focused nsEPs cause increases in membrane permeability. Since the changes in membrane permeability are cell-type dependent, this finding suggests that membrane composition is a critical feature in nanoporation mechanisms. In future studies, we plan to elucidate the effect that specific, local molecular structures and compositions have on efficacy of electroporation.

Versatile, high-efficiency tip-enhanced Raman spectroscopy (TERS) instrumentation for end-user applications

N. J. Kolodziejski, D. E. Wolf, Radiation Monitoring Devices, Inc. (United States); Y. Lu, Univ. of Nebraska-Lincoln (United States); R. S. Gurjar, Radiation Monitoring Devices, Inc. (United States)

We present the design principles and performance characteristics of a shear force based Tip-Enhanced Raman Spectroscopy system for end-user applications. Various designs for high-NA reflecting optics are evaluated for their optimization of photon delivery and collection for TERS, while allowing for use with samples of any thickness and opacity. The integration of Au, Ag, and Au-coated W tips with quartz tuning forks into manufacturable units is investigated for q-value yield and plasmonic enhancement factors. Finally we discuss methods to mitigate the overall challenges to the technique becoming routine and user-friendly.
Dithiocarbamates as capping ligands for water-soluble quantum dots

A. R. Clapp, Y. Zhang, Iowa State Univ. (United States)

We investigated the suitability of dithiocarbamate (DTC) species as capping ligands for colloidal quantum dots (QDs). DTC ligands are generated by reacting carbon disulfide (CS2) with primary or secondary amines on appropriate precursor molecules. A biphasic exchange procedure efficiently replaces the existing hydrophobic capping ligands on the QD surface with the newly formed DTCs. The reaction conversion is conveniently monitored by UV-vis absorption spectroscopy. Due to their inherent water solubility and variety of side chain functional groups, we used several amino acids as precursors in this reaction/exchange procedure. The performance of DTC-ligands, as evaluated by the preservation of luminescence and colloidal stability, varied widely among amino precursors. For the best DTC-ligand and QD combinations, the quantum yield of the water-soluble QDs rivaled that of the original hydrophobic-capped QDs dispersed in organic solvents. The mean density of DTC-ligands per nanocrystal was estimated through a mass balance calculation which suggested nearly complete coverage of the available nanocrystal surface. The accessibility of the QD surface was evaluated by self-assembly of His-tagged dye-labeled proteins and peptides using fluorescence resonance energy transfer. DTC-capped QDs were also exposed to cell cultures to evaluate their stability and potential use for biological applications. In general, DTC-capped QDs have many advantages over other water-soluble QD formulations and provide a flexible chemistry for controlling the QD surface functionalization.

Synthesis, properties, and applications of complex nanocrystal heterostructures

L. Manna, Univ. del Salento (Italy)

No abstract available

Size determination of quantum dots with fluorescence correlation spectroscopy

D. Hill, H. Löhmannsröben, Univ. Potsdam (Germany); A. Zulqurnain, W. J. Parak, Philipsps-Univ. Marburg (Germany); N. Hildebrandt, C. Ast, Fraunhofer-Institut für Angewandte Polymerforschung (Germany)

Semiconductor quantum dots (QDs) are highly interesting fluorophores for all kinds of spectroscopic applications. Although their fluorescence properties are well investigated, accurate size determination of QDs is still a problem. TEM techniques can image the inorganic core/shell system of QDs, but size determination of polymer coated QDs is difficult. SEC (size exclusion chromatography) compares the QD size only with standard polymers and their sizes, and is therefore not easy to use on nanoparticles. As QDs are fluorescent, single molecule spectroscopy methods such as fluorescence correlation spectroscopy (FCS) can be used to determine QDs diffusion coefficients and hence their hydrodynamic radii. Moreover, this method for size determination requires only very low concentrations of quantum dots which is a mayor advantage compared to other techniques such as dynamic light scattering.

Within our contribution we present the size determination of commercially available and self-modified QDs and other fluorescent nanoparticles with FCS. The commercial QDs (QD525, QD565, QD605, QD665 and QD705 - purchased from Invitrogen Inc.) have a rather thick polymer shell and are functionalized with either streptavidin, biotin or carboxylic groups. The self-modified QDs consist of the same commercial core/shell QDs and are modified with a polymer shell and several bio-functionalisation groups. Furthermore, FePt nanoparticles doped with fluorescent dyes are used for reference measurements.

For all nanoparticles the diffusion coefficients were measured by FCS and the hydrodynamic radii were calculated according to the Stokes-Einstein equation. The obtained results are in good agreement with the size information provided by Invitrogen Inc. which demonstrates that FCS is an important technique for QD size determination at very low concentrations.

Engineered nanocrystals for imaging, sensing and therapeutics

M. Han, A*STAR Institute of Materials Research and Engineering (Singapore)

Colloidal semiconductor nanocrystals have attracted great attention for their distinguished roles in fundamental studies and technical applications such as biological labeling and optoelectronic devices. In addition to the size-tunable binary or core-shell nanocrystals with different emission colors, great efforts have also been put to develop highly luminescent composition-tunable quantum dots across the whole visible spectrum. Surface-engineered nanocrystals have been used as multifunctional biological nanoprobes for imaging, sensing, diagnostics, controlled release, targeted delivery and therapeutic applications. For example, the photoluminescence of molecularly engineered non-fluorescent quantum dots with iron(III) dithiocarbamates was selectively switched on by nitric oxide from OFF to ON state through controlled energy-transfer process. Such a “turn on” rather than conventional “turn off” mechanism can be used for sensing nitric oxide, which has been found to be synthesized in mammalian cells and triggered an extraordinary impetus for scientific research in all the fields of biology and medicine.

Facile synthesis of FePt nanoparticles for CT/MRI dual-modal molecular targeting imaging contrast agents

C. Chen, National Taiwan Normal Univ. (Taiwan)

This research explored the potential for using FePt nanoparticles as dual contrast property in combined X-ray computed tomography (CT) and magnetic resonance imaging (MRI). We applied water-soluble FePt nanoparticles of 3, 6 and 12 nm in diameter (3 nm-, 6 nm- and
12 nm-FePt) as a dual modality contrast agent for CT/MRI molecular imaging. The cytotoxicity and hemolysis examinations revealed that FePt nanoparticles were excellent in the biocompatibility and hemocompatibility. The bio-distribution analysis showed the highest serum concentration and circulation half-life for 12 nm-FePt, followed by 6 nm-FePt then 3 nm-FePt. Thus, the 3 nm-FePt showed higher brain concentrations. Then, the amounts of FePt nanoparticles in major organs were very sparse after 168hr. Anti-Her2 antibody conjugated FePt nanoparticles demonstrated molecular expression dependent CT/MRI dual contrast effect in the MBT2 cells with high endogenous Her2 expression and its Her2/neu gene knock out counterpart. The CT/MRI contrast effect of 12 nm-FePt outperformed that of 3 nm-FePt. The selective contrast enhancement of Her2/neu overexpression cancer lesions in both CT and MRI was found in tumor bearing mice after tail vein injection of 12 nm-FePt conjugated with anti-Her2 antibody. With respects to the MR images before injection, a reduction of tumor lesion intensity to 51% was observed at 24 hr after injection. On the other hand, a 138% contrast enhancement of the tumor tissue 24 hr after targeting was observed in CT image analysis. The results indicated the potential of FePt nanoparticles to serve as novel multi-modal molecular imaging contrast agents in clinical settings.

7909-51, Session 2

Size and surface chemistry of Au nanoparticles determine doxorubicin cytotoxicity

J. L. Nadeau, H. Chibli, X. Zhang, McGill Univ. (Canada)

Several formulations of gold-doxorubicin conjugates (Au-Dox) have been reported. However, the effects of particle size, lability of the conjugating bond, and specific targeting have not been fully explored. In this work we compare the relative cytotoxicity of 5 nm vs. 2 nm Au-Dox, and explore the effects of polyethylene glycol (PEG) and specific cell-targeting sequences on toxicity in B16 melanoma cells and in mice. We find that Au-Dox does not show increased cytotoxicity over Dox alone unless the particles are small enough to enter the nucleus. Surprisingly, cleavable bonds do not increase effectiveness, with even stably-bonded Au-Dox showing maximum cytotoxicity in less than one hour. Preliminary studies on molecular mechanisms of action implicate reactive oxygen species formation leading to apoptosis as the primary cause of cell destruction, with Dox-resistant cancer cells showing reduced resistance to Au-Dox. These results have important implications for the development of Au nanoparticle-based anticancer agents.

7909-08, Session 3

State-of-the-art toxicological and microscopic assessment of biomedical nanocrystals on the lung in vitro

M. J. D. Clift, P. Gehr, B. Rothen-Rutishauser, Univ. Bern (Switzerland)

Due to the ever increasing production of nanosized materials for a variety of novel applications (e.g. biomedicine), increased research has focused upon understanding the potential toxicity of these nanomaterials. In order to determine the potential toxicity of nanoparticles (NPs), it is essential that, in parallel to biochemical and toxicological testing, their specific route of uptake (if any), as well as their possible and subsequent intracellular localisation is investigated. By using our novel 3D in vitro triple cell co-culture model of the human epithelial airway barrier (containing epithelial cells and macrophages (apical side) and dendritic cells (basolateral side)) which has been shown to mimic the architecture of this structural arrangement in vivo, in combination with a novel air-liquid interface cell exposure system, it is possible to mimic the exposure of NPs to the lung in vitro. Using both conventional and state-of-the art toxicological tests, in addition to light, laser scanning and transmission electron microscopy methods, it has been possible to determine the interaction of both fluorescent (designed core-shell NPs with shell-embedded fluorophores) and electron dense NPs with the in vitro triple cell co-culture. It has been observed that the material of the engineered NPs has a significant influence upon their resultant toxicity, dependant upon the specific exposure method used, although no difference upon their intracellular localisation. The results of these studies show that despite different compositions, specific NPs intended for use in biomedical applications, when exposed realistically (exposure method/concentration/primary contact cells) cause minimal effects to the lung airway tissue in vitro.

7909-09, Session 3

Size- and structure-dependent toxicity of silica particulates

S. Hanada, K. Miyaoi, A. Hoshino, K. Yamamoto, International Medical Ctr. of Japan (Japan)

Nano- and micro-particulates firmly attach with the surface of various biological systems, because their high-specific surface area might interact with surfaces of tissues or cells. In some chronic pulmonary disease such as asbestosis, pneumoconiosis and silicosis, causative particulates, which are accumulated in the lung, will induce chronic inflammatory disorder, followed by poor prognosis diseases such as fibrosis, lung cancer and mesothelioma.

Our group has been studying the biocompatibility of silicon nanoparticles. We assessed in vitro cytotoxicity of silicon nanoparticles and found that the silicon particles were not toxic in the low-concentration region. In this research, we assessed the cytotoxicity of the various kinds of silica particles, including amorphous silica (18 nm, 120 nm and 1400 nm) and crystal silica (1300 nm) in mouse alveolar macrophage cell line culture. After macrophages are phagocytic cells, which uptake inhaled particles and interact with other immune cells by attractants such as chemokines and cytokines.

Their median lethal concentrations (LC50) measured by WST assay depend on particle size and those were related with inflammation response, about which we measured MIP-2. By contrast, production of TGF-beta, which is a fibrosis maker, by addition of crystal silica was much higher than that of amorphous one in their low-concentrations. We assume that TGF-beta production in the low-dose of crystal silica leads to future pulmonary fibrosis and that the difference between amorphous and crystal might be caused by particle shape, oxy-radical generation and low-solubility. We conclude that differences of silica particulate affect cytotoxicity and immune response.

7909-10, Session 3

Bridging the fields of nanoscience and toxicology: nanoparticle impact on biological models

A. Ambrosone, V. Marchesano, L. Mattera, A. Tino, C. Tortiglione, Istituto di Cibernetica Eduardo Caianiello (Italy)

In the emerging area of nanotechnology a key issue is related to the potential impacts of the novel nanomaterials on the environment and human health so that this technology can be used with minimal risk. Specifically designed to combine on a single structure multipurpose tags and properties, smart nanomaterials need a comprehensive
characterization of both chemico-physical properties and adequate toxicological evaluation, which is a challenging endeavor; the in vitro toxicity assays that are often employed for nanotoxicity assessments do not accurately predict in vivo response. To overcome these limitations and gain a deeper understanding of nanoparticle-cell interactions, we have employed cnidarian models, and in particular the freshwater polyp Hydra vulgaris, not opposed to more complex and evolved systems, but to add valuable information, at an intermediate level between early metazoan and vertebrates, on both cytotoxicity and on pollution affecting the environment. By testing nanocrystals of different sizes, core/shell composition and surface coatings, in vivo, at whole animal level, we investigated the impact of their properties on uptake, accumulation, biodistribution, elicitation of behavioural responses. We assessed acute and sublethal toxicity by scoring for alteration of morphological traits, population growth rates, and influence on the regenerative capabilities of Hydra. Furthermore, we investigated the cellular and molecular mechanisms activated by nanoparticles internalization. Thus by using approaches spanning from animal biology to cell and molecular biology we provide an analysis on metal based and semiconductor NC , discussing the crucial role played by the synthesis route and chemical surface on the toxicity for living organisms.

7909-11, Session 4

**Multiplexed solid-phase nucleic acid hybridization assays using semiconductor quantum dots as donors in fluorescence resonance energy transfer (FRET)**

W. R. Algar, U. J. Krull, Univ. of Toronto Mississauga (Canada)

The use of quantum dots (QDs) as donors in fluorescence resonance energy transfer (FRET) provides new opportunities in bioanalysis. In our group, we have used mixed films of QDs and oligonucleotide probes immobilized on optical fibers to demonstrate the potential for novel spectroscopic detection platforms that capitalize on the unique optical properties of QDs and FRET. This presentation will highlight the use of different combinations of CdSe/ZnS QDs and fluorescent dyes as FRET pairs to achieve the two-plex, three-plex, and four-plex detection of target nucleic acid sequences. Multiplexed analyses are possible using a single excitation source and a single substrate, in the ensemble, and via ratiometric signals. Spatial registration or sorting methods, imaging or spatial scanning, and single molecule spectroscopy are not required. These strategies are competitive with established technologies, such as molecular beacons, with nanomolar (picomole) limits of detection, induced by photooxidation of one QD in the dimer is presented and emission spectra reveals the unique dynamic properties of interacting QDs in a dimer. Evidence of transition from coupled to decoupled states induced by photooxidation of one QD in the dimer is presented and described with a model involving multiexciton decay characteristics. Controlled assembly of a fixed number of QDs into clusters and quantitative optical analysis techniques will allow for measurements in understanding the excitonic charge transfer mechanism during the photooxidation of QDs.

7909-13, Session 4

**Time-correlated hyperspectral studies of biexciton characteristics in dimeric colloidal quantum dot quantum dot pairs under photo-oxidation**

J. Hwang, H. Kang, M. L. Clarke, National Institute of Standards and Technology (United States); S. H. DePaoli Lacerta, U.S. Food and Drug Administration (United States); L. F. Pease III, The Univ. of Utah (United States)

Optical properties of photooxidizing single and dimeric CdSe/ZnS core/shell colloidal quantum dot (QDs) are investigated. Single and clustered QDs of dimers, trimers, and tetramers are assembled and preferentially collected using electrospay differential mobility analysis with electrostatic deposition. A multimodal time-correlated hyperspectral confocal microscope capable of simultaneously measuring the time evolution of photoluminescence (PL) intensity fluctuation, PL lifetime, and emission spectra reveals the unique dynamic properties of interacting QDs in a dimer. Evidence of transition from coupled to decoupled states induced by photooxidation of one QD in the dimer is presented and described with a model involving multieexciton decay characteristics. Controlled assembly of a fixed number of QDs into clusters and quantitative optical analysis techniques will allow for measurements in understanding the excitonic charge transfer mechanism during the photooxidation of QDs.

7909-12, Session 4

**Time-resolved and steady-state FRET spectroscopy on commercial biocompatible quantum dots**

D. Wegner, D. Geissler, H. Löhmansröben, Univ. Potsdam (Germany); N. Hildebrandt, Fraunhofer-Institut für Angewandte Polymerforschung (Germany)

Semiconductor nanocrystals (quantum dots - QDs) possess unique photophysical properties making them highly interesting for many kinds of biochemical applications. Besides their use as common fluorophores in spectroscopy and microscopy, QDs are well suited for Förster resonance energy transfer (FRET). Their broad absorption cross-sections and size-tunable absorption and emission spectra offer several advantages for the use of QDs both as FRET donors and acceptors. Therefore, QD-based FRET pairs can be efficiently used as biological and chemical sensors for highly sensitive multiplexed detection. In this contribution we present the use of several commercial biocompatible QDs (Qdot® Nanocrystals - Invitrogen) as FRET donors in combination with commercial organic dyes as FRET acceptors. In order to investigate the FRET process within our donor-acceptor pairs, we used biotinylated QDs and streptavidin labeled with dyes. The well known biotin-streptavidin molecular recognition enables FRET from QDs to the dyes and provides defined distances between them. Steady-state and time-resolved fluorescence measurements were performed in order to investigate the QD-to-dye FRET. Despite the large size of the polymer coated biocompatible QDs our results demonstrate the efficient use of these QDs as efficient donors for steady-state and time-resolved FRET applications in nano-biotechnology.

7909-14, Session 5

**An optical nanoparticle gun**

J. Feldmann, Ludwig-Maximilians-Univ. München (Germany)

Gold nanoparticles combine several chemical, biological and optical advantages. During recent years some possibilities to manipulate gold nanoparticles with light have been investigated also for biological and medical applications. Here we show that light pressure can be used for “shooting individual gold nanoparticles” in aqueous solution. By balancing attracting and repulsive forces we can laser print single gold nanoparticles with an accuracy of several tens of nanometer.
Localized surface plasmon properties of Au nanorings and their diffusion in biotissue

C. Lee, S. Wu, H. Tseng, T. Chi, K. Yang, J. Wang, National Taiwan Univ. (Taiwan); M. Tsai, Chang Gung Univ. (Taiwan); Y. Kiang, C. Yang, National Taiwan Univ. (Taiwan)

At localized surface plasmon resonance (LSPR), coherent scattering and absorption of metal nanoparticles (NPs) are enhanced. Due to its interference (coherence) detection nature, optical coherence tomography (OCT) is a suitable approach for monitoring the LSPR of Au NPs. Hence, resonant Au NPs can be detected by OCT scanning with high sensitivity. Swept-source OCT systems based on sweeping-frequency lasers as the light sources around 1300 nm have been widely built for medical diagnosis. In this paper, aqueous solutions of Au nanorings (NRs) with different LSPR wavelengths are prepared. Their LSPR-induced extinction cross sections at 1310 nm are estimated with OCT scanning of solution droplets on coverslip. The results are reasonably consistent with the data at individual LSPR wavelengths obtained from transmission measurements of Au NR solutions and numerical simulations. Then, the resonant and non-resonant Au NRs are delivered into mouse liver samples for tracking Au NR diffusion in the samples through continual OCT scanning for one hour. With resonant Au NRs, the average A-mode scan profiles of OCT scanning at different delay times clearly demonstrate the extension of strong backscattering depth with time. The calculation of speckle variance among successive OCT scanning images, which can be used to represent the local transport speed of Au NRs, leads to the illustrations of downward propagation and spreading of major Au NR motion spot with time. In a homogeneous bio-tissue like mice liver, the fabricated Au NRs can diffuse down to a depth of several hundred microns within 60 min.

Nanoscale plasmonic resonators with high Purcell factor: spontaneous and stimulated emission

E. M. Goldys, Macquarie Univ. (Australia)

Plasmonic nanoparticles with silver cores and silica shells containing Eu fluorophores near the surface produced by wet chemistry method exhibit reduced fluorescence lifetimes compared with the same fluorophores in free space conditions. These can be interpreted within the Purcell framework which is well known for the spontaneous and stimulated emission of the nanoparticle behave as energy-storing resonators. Surprisingly, the structures show high Purcell factors of over 60, comparable with those observed in high quality semiconductor micropillar cavities. Such high Purcell factors result from very low mode volumes (~ 10 000 nm^3 and comparatively high cavity Q factors (~100). Structures such as those are capable of lasing [ref Noginov], provided a gain medium is introduced into the shell. We present the method and predictions of the lasing wavelengths and lasing threshold. We also demonstrate a simple diagnostic method that can identify the proximity of a given nanoparticle to the lasing threshold. Furthermore, we show that these structures can enhance the electric field by a factor of over 1500 (at 99.9% of threshold gain) and beyond. We discuss the implication of such enhancement for biosensing with these “smart dust” nanoparticles.

Magnetic supracolloidal assemblies for biophysical applications

J. Berret, Univ. Paris 7-Denis Diderot (France)

The possibility to use inorganic nanoparticles as building blocks for the fabrication of supracolloidal assemblies has attracted much attention during the last years. It is thought that these constructs could be made of different shapes and functionalities and could constitute the components of future nanodevices such as sensors, actuators or nanocircuits. In a first part, I report a protocol that allows us to fabricate supracolloidal assemblies in a controlled manner. The building blocks of the constructs are sub-10 nm iron oxide nanocrystals and polymers. I show that a fine control of the electrostatic interactions between the building blocks result in the formation of spherical or anisotropic aggregates at the micrometer length scale [1,2].

With lengths comprised between 1 and 100 μm, the anisotropic aggregates, also called nanorods were found to be very rigid (persistence length 10 cm) and to reorient themselves with an externally applied magnetic field. In a second part, I will review recent results on the toxicity and uptake of the nanomaterials and in particular those of the nanorods by murine fibroblasts and human lymphoblasts. Our studies revealed that the physico-chemical characteristics of engineered nanomaterials play an important role in the interactions with living cells. I will also show that the rods can be utilized for passive and active micro rheology experiments of complex fluids inconfined environments. The mechanical responses of the intracellular medium will be presented and compared to that of model viscoelastic liquids.


Gold nanoparticles in biomedical applications

A. G. Kanaras, D. Bartczak, O. L. Muskens, T. M. Millar, T. Sanchez-Elsner, Univ. of Southampton (United Kingdom)

Realizing the interactions of complex biological systems with chemically produced colloidal nanocrystals is of great importance for the development of new diagnostic and therapy methods, drug delivery, and imaging. A key strategy towards this aim is to understand how different functionalities and types of colloidal nanoparticles affect specific cells and their functions.

In this presentation we demonstrate a new strategy to manipulate cell operations, which is based on the membrane-receptor specific interactions between colloidal peptide-capped gold nanoparticles and human umbilical vein endothelial cells. Colloidal gold nanoparticles of similar charge and size but capped with different sequences of peptides can deliberately trigger specific cell functions. On the other hand, we demonstrate the development of a mild type of hyperthermia, the nondestructive nanoparticle hyperthermia, which can be used to manipulate the viability and cellular functions of non-cancerous cells. Different types of colloids such as hollow gold, gold nanorods and silica-gold core-shell particles are employed as a toolbox for the nondestructive hyperthermia, in order to further tune the cellular manipulation.

The nanoparticles that we use in our experiments are coated with polyethylene glycol-based ligands (OEG), in order to retain biological stability, and they are functionalized with peptides which target receptors allocated on the cellular membrane. Specificity and efficiency of the binding of gold/peptide conjugates are investigated and compared to the non-specific internalization of plain PEG-coated nanoparticles through the endocytosis pathway. Our findings open up new avenues towards the deliberate control of cellular functions using strategically designed nanoparticle and laser hyperthermia.
Alloy metal nanoparticles for multicolor cancer diagnostics

P.V. Baptista, G. Doria, J. Conde, Univ. Nova de Lisboa (Portugal)

Cancer is a multigenic complex disease where multiple gene loci contribute to the phenotype. The ability to simultaneously monitor differential expression originating from each locus results in a more accurate indicator of degree of cancerous activity than either locus alone. Metal nanoparticles have been thoroughly used as labels for in vitro identification and quantification of target sequences. We have synthesized nanoparticles with assorted noble metal compositions in an alloy format and functionalized them with thiol-modified ssDNA (nanoprobes). These nanoprobes were then used for the simultaneous specific identification of several mRNA targets involved in cancer development - one pot multicolor detection of cancer expression. The different metal composition in the alloy yield different "colors" that can be used as tags for identification of a given target. Following a non-cross-linking hybridization procedure previously developed in our group for gold nanoprobes, these multicolor nanoprobes were used for the molecular recognition of several different targets involved in chronic myeloid leukemia (e.g. BCR, ABL, BCR-ABL fusion product) as well as alternatively spliced variants of other relevant genes (e.g. p53, c-myc, BCRA1). Based on the spectral signature of mixtures, before and after induced aggregation of metal nanoparticles, the correct identification could be made. Further application to differentially quantify expression of each locus in relation to another will be presented. The differences in nanoparticle stability and labeling efficiency for each metal combination composing the colloids, as well as detection capability for each nanoprobe will be presented. Additional studies will be conducted towards allele specific expression studies.

Locally increased mortality of gamma-irradiated cells in presence of lanthanide-halide nanoparticles

N. J. Withers, J. B. Plumley, A. McBride, B. A. Akins, A. C. Rivera, N. C. Cook, G. A. Smolyakov, G. S. Timmins, M. Osinski, The Univ. of New Mexico (United States)

Cerium-doped lanthanum fluoride colloidal nanocrystals offer a way to improve radiation therapy through the enhanced absorption of high energy photons. 10% cerium-doped polyethylene-glycol-capped lanthanum fluoride nanocrystals were synthesized in water as platelets 2-4 nm in diameter and 1-3 nm thick and suspended in phosphate buffered saline. The nanocrystals were characterized by transmission electron microscopy, muffle furnace ashing, absorbance spectroscopy, dynamic light scattering, and photoluminescence spectroscopy. The lanthanum fluoride nanocrystals were used in radiation dose enhancement experiments that involved an incoming gamma flux from a Cs-137 source. Finally, increased cell mortality of radiation sensitive S. Cerevisiae, ATCC#208466, under gamma irradiation at varying concentrations of the PEG capped nanocrystals was explored using flow cytometry.

Multifunctional fluorescent nanoparticles for biomedical applications

S. T. Selvan, A*STAR Institute of Materials Research and Engineering (Singapore)

Hybrid multifunctional nanoparticles (NPs) are emerging as useful probes for magnetic based targeting, delivery, cell separation, magnetic resonance imaging (MRI), and fluorescence-based bio-labeling applications. Assessing from the literature, the development of multifunctional NPs for multimodality imaging is still in its infancy stage. This talk will focus on our recent work on quantum dots (QDs), magnetic NPs and bi-functional NPs (composed of either QDs or rare-earth NPs, and magnetic NPs - iron oxide or gadolinium oxide) for multimodality imaging based biomedical applications. The combination of MRI and fluorescence would ally each other in improving the sensitivity and resolution, resulting in improved and early diagnosis of the disease. The challenges in this area will be discussed.

Imageing heterostructured quantum dots in cultured cells with epifluorescence and transmission electron microscopy

E. M. Rivera, C. Trujillo Provencio, New Mexico State Univ. (United States); A. Steinbrueck, P. Rastogi, A. M. Dennis, J. A. Hollingsworth, Los Alamos National Lab. (United States) and Center for Integrated Nanotechnologies (United States); E. Serrano, New Mexico State Univ. (United States) and Center for Integrated Nanotechnologies (United States)

Quantum dots (QDs) are semiconductor nanocrystals with extensive imaging and diagnostic capabilities, including the potential for single molecule tracking. Commercially available QDs offer distinct advantages over organic fluorophores, such as improved photostability and tunable emission spectra, but their cadmium selenide (CdSe) core raises toxicity concerns. For this reason, replacements for CdSe-based QDs have been sought that can offer equivalent optical properties. The spectral range, brightness and stability of InP QDs may comprise such a solution. To this end, LANL/CINT personnel fabricated moderately thick-shell novel InP QDs that retain brightness and emission over time in an aqueous environment. We are interested in evaluating how the composition and surface properties of these novel QDs affect their entry and sequestration within the cell. Here we use epifluorescence and transmission electron microscopy (TEM) to evaluate the structural properties of cultured Xenopus kidney cells (A6; ATCC) that were exposed either to commercially available CdSe QDs (Qtracker® 565, Invitrogen) or to heterostructured InP QDs (LANL). Epifluorescence imaging permitted assessment of the general morphology of cells labeled with fluorescent molecular probes (Alexa Fluor® phalloidin; Hoechst 33342), and the prevalence of QD association with cells. In contrast, TEM offered unique advantages for viewing electron dense QDs at higher resolution with regard to subcellular sequestration and compartmentalization. Preliminary results show that in the absence of targeting moieties InP QDs can passively enter cells and sequester nonspecifically in cytosolic regions whereas, commercially available targeted QDs principally associate with membranous structures within the cell. Supported by: NIH R01GM084702.
Quantum dots (QDs) have the potential for bioimaging contrast agents by bright luminescence, resistance against photobleaching, and tunable emission wavelengths. Lanthanide-doped nanoparticles (LNs) can have advantages in bioimaging applications due to their narrow emission bandwidth, nonblinking, and relatively low toxicity. Both QD and LN can emit at near-infrared (NIR) wavelengths which can provide maximal tissue penetrations from the minimal interferences by water and biomolecules and from the reduced auto-fluorescence. Multiplexed imaging techniques provide the opportunity to investigate the complex biological phenomena governed by multiple biomolecules. QDs with different emission wavelengths can be multiplexed by single excitation light. Multiplexing between QDs and LNs can be obtained by switching the excitation sources while they emit at same wavelength domain. NIR emitting QDs and LNs were synthesized with judicious emission wavelength control by pyrolysis method to expand the multiplexing capability in 700 to 900 nm. Surface chemistry of QDs and LNs will be addressed including the biocompatibility. Using small animal models, in vivo real-time multiplexed imaging will be demonstrated with QDs and LNs exploited simultaneously and complimentarily for the contrast agents. Following issues will be also discussed such as the penetration depth, signal-to-noise ratio, and the limit of multiplexing.

Quantum dots (QDs) can have advantages in bioimaging applications due to their narrow emission bandwidth, nonblinking, and relatively low toxicity. Both QD and LN can emit at near-infrared (NIR) wavelengths which can provide maximal tissue penetrations from the minimal interferences by water and biomolecules and from the reduced auto-fluorescence. Multiplexed imaging techniques provide the opportunity to investigate the complex biological phenomena governed by multiple biomolecules. QDs with different emission wavelengths can be multiplexed by single excitation light. Multiplexing between QDs and LNs can be obtained by switching the excitation sources while they emit at same wavelength domain. NIR emitting QDs and LNs were synthesized with judicious emission wavelength control by pyrolysis method to expand the multiplexing capability in 700 to 900 nm. Surface chemistry of QDs and LNs will be addressed including the biocompatibility. Using small animal models, in vivo real-time multiplexed imaging will be demonstrated with QDs and LNs exploited simultaneously and complimentarily for the contrast agents. Following issues will be also discussed such as the penetration depth, signal-to-noise ratio, and the limit of multiplexing.

The use of semiconductor quantum dots (QDs) as optimized Förster resonance energy transfer (FRET) donors in a variety of sensing and imaging configurations with fluorescent dye acceptors has become well-established. Here we examine the ability of QDs to transfer energy through a DNA photonic wire. In these types of assemblies, the QD acts as both a central nanoscaffold and FRET donor to a series of dyes sequentially placed along an attached DNA sequence. The DNA, attached to the QD via a hexahistidine peptide linker, provides a rigid and controllable template for the dye attachment with complementary labeled DNA sequences. The dyes are arranged in a manner that allows consecutive FRET interactions along the photonic wire. The effects on overall energy transfer of modifying dye placement on the DNA sequence, homo-FRET interactions among identical dyes and the use of a DNA intercalating dye in these hybrid assemblies were also examined. Results suggest that energy transfer is limited by dye re-emission properties and by energy loss through non-FRET pathways. Optimization in choice of acceptor dyes used and their placement along the DNA strand are expected to improve energy flow and allow for efficient photonic wire assemblies with widespread potential in nanotechnology.
Quantum dots as FRET acceptors: multiplexing biosensors for in-vitro diagnostics and molecular ruler applications

D. Geißler, Univ. Potsdam (Germany); F. Morgner, Fraunhofer-Institut für Angewandte Polymerforschung (Germany); N. G. Butlin, Lumiphore Inc. (United States); H. Löhmannsröben, Univ. Potsdam (Germany); N. Hildebrandt, Fraunhofer-Institut für Angewandte Polymerforschung (Germany)

FRET applications play an important role for the determination of concentrations and distances within nanometer-scale systems in vitro and in vivo in many fields of biotechnology. Semiconductor quantum dots (QDs) possess ideal properties for their application as FRET acceptors when the donors have long excited state lifetimes and when direct excitation of QDs can be efficiently suppressed. Therefore, luminescent terbium complexes (LTCs) with excited state lifetimes up to milliseconds are ideal FRET-donor candidates for QD-acceptors. Here we present the application of LTC-QD FRET pairs for multiplexed ultra-sensitive diagnostics and nanometer-resolution molecular distance measurements by time-resolved luminescence analysis. A time- and spectrally-resolved simultaneous measurement of five FRET-sensitized InNitrogen QdotsTM using one commercial LTC (www.lumiphore.com) as FRET-donor within a biotin-streptavidin bioassay is demonstrated. Our color-coded, nearly background-free multiplexed homogeneous assay yields sub-picomolar detection limits for all five QDs in one single sample, making the quantum dot-based FRET probes ideal candidates for highly specific and sensitive companion diagnostics. Different donor-acceptor distances are determined by decay-time analysis, demonstrating the application as multiplexed nanometer scale spectroscopic ruler over very large molecular distances (> 10 nm). Further investigation of the pre-exponentials of the multi-exponential donor and acceptor luminescence decay functions show that the FRET-nanoprobes are suitable for size and shape determination of QDs under physiological conditions and potentially suited for high-resolution multiplexed conformational and functional studies in cell imaging. The results also provide insight into the energy transfer mechanism from LTCs to QDs showing a FRET type (r^-6) distance dependence.

Nanoprobes of fluorescent gold nanoclusters for cells labeling

W. H. Chang, W. Yu, C. Lin, W. Chan, J. Shen, Chung Yuan Christian Univ. (Taiwan); H. Yeh, H. Wang, Mackay Memorial Hospital (Taiwan)

Semiconductor quantum dots are attracted by its stability and tunable wavelengths, but containing toxic ions such as Cd2+, Pb2+ is a concerned issue for broad clinical application. To face this problem, researchers have focused on developing new biocompatible materials with fluorescent properties. Fluorescent gold nanoclusters are becoming the alternative nanomaterials for nontoxic cellular labeling. Gold nanoclusters, consisting of several atoms, exhibit discrete electronic states and fluorescent properties. As a biocompatible materials, gold nanoclusters show a good candidate of novel fluorophore with many advantages, such as chemical stability, and general surface chemistry. In this study, synthesis of ultrafine fluorescent gold nanoclusters is included in this report. We focus on the issue how to efficiently label cells using specific carrier. We study the cell labeling efficiency of fluorescent gold nanoclusters using different forces (electric, magnetic). The nanoprobes design of fluorescent gold nanoclusters are also included. Specific staining of cells and nonspecific uptake by living cells are studied.

Quantum dot-based time-resolved adhesion assay for cell co-cultures

P. Rivera Gil, W. J. Parak, F. Yang, Philipps-Univ. Marburg (Germany); H. Thomas, A. Terfort, Johann Wolfgang Goethe-Univ. Frankfurt am Main (Germany)

Colloidal inorganic semi-conductor nanocrystals - commonly known as Quantum dots (QDs) - are prepared as fluorescent probes in biological staining. Compared with conventional fluorophores, QDs have a narrow, tunable (depending on the size), symmetric emission spectrum and are photochemically stable [1]. The bright fluorescence allows for sensitive detection, the reduced photo-bleaching [2] permit measurements over long periods of time, and enable live cell imaging. Due to their narrow emission peaks they are suitable for multiplexing, in which multiple colors can be obtained in parallel from single excitation sources. Furthermore, QDs are spontaneously ingested by living cells [3], are confined in the cell and are only transferred to daughter cells upon cellular division. According to the advantageous characteristic of QDs, one of the main applications in cell biology is the use of QDs as marker for cell lineage. In this work, a QD label-based time resolved adhesion assay for co-cultures is presented. This is a novel technique, which allows for quantifying the adhesion properties of cell co-cultures on one substrate. Two different cell lineages were labeled with fluorescent QDs of two different colors and were grown within a co-culture onto different substrates. Due to the high contrast and the low brightness variations of the background, a software was developed to count the cells automatically. The adhesion of one against the other cell type was quantified by the ratio of the different colors. With this technique, the effect of different nano- and micro-structured surfaces on the adhesion behavior within co-cultures can be quantified.

Getting control in the antibody-nanoparticle stoichiometry

M. V. Grazú Bonavia, E. Polo, P. Pina, J. Santamaría, J. M. de la Fuente, Univ. de Zaragoza (Spain)

Gold nanoparticles (GNPs) have attracted huge attention recently for both biosensor and biomedical applications. This interest is based mostly owing to their unique optical and catalytic properties, excellent colloidal stability, and relative ease of preparation. Up to now, a large number of different approaches to conjugate different biomolecules to GNPs had been reported. However, controlling the number of functional ligands and biomolecules conjugated to NPs still remains as a significant challenge. Controlled nanoparticle valency would open new perspectives in bottom up nanotechnology for controlling nanomaterial orientation and new properties generation. Besides, it will be also advantageous for biological applications such us single-molecule imaging in cells, as a tool for imaging protein dynamics at the single-molecule level, to improve nanoparticle amplification methods for biodetection (mass enhancers), etc.

In this sense, only a few limited approaches have been developed for making NPs with a discrete number of chemical functional groups: by solid phase synthesis methodology, using polymers with single functional groups at their ends, taking advantage of higher reactivity of surface gold atoms at certain areas of NPs, etc. Besides, the papers that report the union of only one molecule of antibody per NP are even less. Here, we present an easy strategy that allows both to control the binding orientation and the number of molecules of antibodies attached on the GNPs surface. Different techniques were used to demonstrate this monovalent antibody functionalization (TEM, ELISA assays, UV-VIS adsorption spectra, etc).
7909-32, Session 9

**Immobilized quantum dot bioprobes: microfluidics for the development of nucleic acid assays and bioconjugate assemblies**

U. J. Krull, A. J. Tavares, L. Chen, W. R. Algar, Univ. of Toronto Mississauga (Canada)

Quantum Dots (QDs) have been used as donors in fluorescence resonance energy transfer (FRET) for the multiplexed detection of fluorescence from DNA targets in a single assay. This presentation will explore the use of QD-bioprobes that are immobilized within microfluidic channels as a multiplexed assay platform, and the use of microfluidics to bioconjugate probe oligonucleotides and peptides for the assembly of QD-bioprobes.

The typical challenges associated with assembly of unique QD-bioprobe systems on a surface include: non-specific adsorption, slow kinetics of hybridization, and sample manipulation. Our work has considered immobilization of mixtures of different QD-bioprobes onto glass-PDMS microfluidic chips using various chemistries. The ability to dynamically control stringency by adjustment of the potential in an electroosmotic-based microfluidics experiment is advantageous. QD-bioprobes can be covalently anchored, or can be immobilized using a labile tethering system for removal and replacement. As a specific example of the latter, bi-conjugated QDs can be used where one oligonucleotide sequence on the QD is available for immobilization by hybridization with complementary oligonucleotide on a glass surface, and a different oligonucleotide sequence on the QD serves as a probe to transduce hybridization with target in a sample solution.

We are also exploring microfluidic-based solid phase synthesis for QD-bioprobe assembly, purification, and recovery. The process involves multi-step assembly on a layer of QDs that are immobilized in a microfluidic channel. Initial work has considered click chemistry for activation, and then further conjugation with the desired biomolecules for QD-bioprobe assembly.

7909-33, Session 10

**Plasmonic Ag/SiO2 composite nanoparticles doped with a europium chelate and their metal enhanced fluorescence**

W. Deng, K. Drozdowicz-Tomsia, D. Jin, E. M. Goldys, Macquarie Univ. (Australia); J. Yuan, Dalian Univ. of Technology (China)

We report silver nanostructure-enhanced fluorescence of a europium (Eu) chelate, BHHC-Eu-DPBT, which was covalently bound in Ag/SiO2 nanocomposites. The fluorescence enhancement was examined as a function of core and shell size and optimum thicknesses of 52 ± 10 nm and 25 ± 2 nm were found, in agreement with theoretical predictions. An increase in the fluorescence intensity by a factor of ~10.4 and decrease in the lifetime by a factor of ~3.5 were observed. Single nanocomposite particles were bright enough to be observed in fluorescence microscopy under 365 nm LED excitation. The increased brightness and reduced lifetime of such fluorescent core-shell nanocomposites will enhance their applicability for ultrasensitive bioassays and bioimaging, especially with time-gating.

7909-34, Session 10

**Diagnosis and imaging with SERS encoded particles**

R. A. Alvarez-Puebla, Univ. de Vigo (Spain)

SERS encoded particles have been established as a solid and reliable analytical technique for the detection in extremely low amounts of a wide variety of bioanalytes. SERS encoded particles for indirect detection and labeling can be implemented on chip or even inside living cells, tissues or a variety of microorganisms.

However, there are still open challenges, mainly related to the reproducibility of the methods for substrate fabrication, in particular when dealing with the formation of hot spots, which are responsible for the highest enhancement factors, but their efficiency is extremely sensitive toward small geometrical details within the nanostructure. Additionally, although portable Raman spectrometers are available, most of the published reports are based on very sophisticated instruments that will not find a place in routine analysis labs or hospitals. Thus, the field of SERS codification, in particular toward biomedical applications has a great potential, as demonstrated by many examples, but is open to new developments that will undoubtedly continue amazing us in the near future.

7909-35, Session 10

**Ion sensing with colloidal nanoparticles**

W. J. Parak, Philipps-Univ. Marburg (Germany)

Colloidal nanoparticles composed out of an inorganic core and a polymer shell have been synthesized. Both, the core and the polymer shell are either fluorescence, magnetic, or radioactive, so that they can be imaged with fluorescence, magnetic resonance, or radioactivity, respectively. By combining different cores with different polymer shells nine different types of particles for dual imaging have been obtained, as for example fluorescent cores with radioactive polymer shells. In this way a toolkit of nine types of nanoparticles has been created out of which each can be imaged with two different modes. Due to the topography of the polymer shell all nine different types of particles possess very similar surface chemistry and thus have virtually the same interface for consecutive conjugation with ligands.

7909-36, Session 10

**Plasmonic nanostructures: new design methodologies and high-resolution mode imaging for applications in nanobiophotonics**

S. A. Maier, Imperial College London (United Kingdom)

We present new design principles for plasmonic nanostructures based on transformation optics and plasmon hybridization theory. This allows us to create structures optimized for electromagnetic field enhancement, a strong or suppressed scattering response, and indeed broadband light absorption. We further present correlative mode imaging of such nanostructures using electron and optical spectroscopies. We show that particularly electron energy loss spectroscopy is well suited for the investigation of electromagnetic hot spots, and present results obtained both for colloidal systems as well for cavities fabricated using electron beam lithography on ultrathin silicon nitride membranes. Applications in the nanobiosciences will be outlined. We will further discuss a new methodology for bioassays exploiting metal nanoclusters with Raman-active reporter molecules as linking units.
Quantum dots and metal nanoparticle agents for manipulating cellular trafficking

G. F. Strouse, The Florida State Univ. (United States)

Nanomaterials and Quantum Dots are finding wide ranging applications as phosphors, energy transfer agents, and cargo delivery vehicles in cellular applications. Whether the end goal is a drug delivery agent, a phosphor, or a molecular beacon understanding the potential of these materials in biological applications is crucial. In this presentation we will explore the transport of nanomaterials across skin (in-vivo), the fate and transport of QDs modified by cell penetrating peptides for delivery of genetic information in-vitro, and simple molecular beacon applications to measure cellular metabolite levels.

Distribution of quantum dots after intraperitoneal administration, with reference to area-specific distribution in the brain

S. Fushiki, K. Itoh, S. Kato, T. Yaoi, M. Umekage, T. Tozawa, Kyoto Prefectural Univ. of Medicine (Japan); Y. Yoshikawa, H. Yasui, Kyoto Pharmaceutical Univ. (Japan); A. Hoshino, N. Manabe, K. Yamamoto, International Medical Ctr. of Japan (Japan)

Quantum dots (QDs) are well-known for their potential application in biosensing, ex vivo live-cell imaging and in vivo animal targeting. The brain is a challenging organ for drug delivery, because the blood brain barrier (BBB) functions as a gatekeeper guarding the body from exogenous substances. Here, we evaluated the distribution of bioconjugated QDs, i.e., captopril-conjugated QDs (QDs-cap) following intraperitoneal injection into male ICR mice as a model system for determining the tissue localization of QDs, employing ICP-MS and confocal microscopy coupled with spectrometric analysis. We have demonstrated that intraperitoneally administered QDs-cap were delivered via systemic blood circulation into liver, spleen, kidney and brain at 6 hours after injection. Although QDs-cap were located predominantly inside the blood vessels in liver, kidney and brain, but a few were distributed in the parenchyma, especially noteworthy in the brain. In addition, we have studied the effects of chronic exposure to QDs-cap in the brain, and demonstrated a significant increase of the oxidative stress-mediated products in the brain, especially in the hippocampus. Further studies on acute as well as chronic toxicity of QDs in the brain are required prior to clinical application to humans.

Peptide-mediated cellular delivery and endosomal escape of quantum dots

K. Boeneman, J. B. Delehanty III, M. H. Stewart, K. Susumu, U.S. Naval Research Lab. (United States); J. B. Blanco-Canosa, P. E. Dawson, The Scripps Research Institute (United States); I. L. Medintz, U.S. Naval Research Lab. (United States)

Currently there is considerable interest in using bioconjugated nanoparticles for in vivo imaging and sensing applications along with theranostics. Luminescent CdSe/ZnS core shell semiconductor quantum dots (QDs) have unique optical properties and bioconjugation capabilities that make them ideal prototypes for these purposes. We have previously described the metal-affinity association between the imidazole groups of terminal hexahistidine residues of peptides and proteins and the ZnS shell of quantum dots as a useful bioconjugation technique [1]. We have also demonstrated that QDs labeled with an oligohistidine tagged cell penetrating peptide (CPP) derived from the HIV TAT-protein could undergo specific endocytosis-mediated cellular uptake in both HEK293T/17 and COS-1 cells [2]. However, the QDs were predominantly sequestered in the endosomes. This remains a significant hindrance to future potential cellular imaging applications which require the QDs to access other subcellular organelles. Here we describe the design, synthesis and cellular application of a hexahistidine-labeled modular peptide containing various functionalities including a palmitate group that is capable of both cellular uptake and endosomal escape in multiple cell lines without concomitant toxicity [3]. Optimal cellular uptake and endosomal escape of QDs bearing these peptides takes approximately 48 hours. We have also tested various modifications of this peptide to identify the attributes required for both its cellular uptake and its endosomal escape capabilities. A model of how the various functionalities within the peptide contribute to its activity will be presented.

References:
Novel synthesis of gold asymmetric nanocrystals: molecular heaters

P. del Pino, B. Pelaz, J. M. de la Fuente, Univ. de Zaragoza (Spain)

In the last years, gold nanoparticles have found a great deal of interest in the area of bioscience. This is due to the interesting physicochemical properties that these materials bear including biocompatibility, localized surface plasmons or ease of biofunctionalization by means of molecules bearing thiol groups. More recently, asymmetric gold nanoparticles (NPs) such as nanorods, triangular nanoprisms or core-shell dielectric-gold NPs have achieved an increasing popularity; this trend is mainly originated from the absorption band that they present in the NIR range of the electromagnetic spectrum. Upon excitation with NIR radiation, these asymmetric materials can release heat to their most immediate vicinity. Here we describe a novel synthesis route to produce gold triangular nanoprisms. These asymmetric tabular single-crystalline NPs exhibit a characteristic absorption band in the near infrared (NIR) range which can be tuned by varying the aspect ratio (edge to thickness) of the NPs. The aspect ratio is ultimately controlled by the synthesis conditions. Photothermal conversion of NIR radiation can be exploited for nanomedicine applications such as remote drug release or photothermal therapy.

Synthesis of NaYF4:Yb3+/Er3+ upconverting nanoparticles in a capillary based continuous-flow microfluidic reaction system

H. Liu, O. Jakobsson, C. T. Xu, Lund Univ. (Sweden); H. Xie, Lund Univ. Hospital (Sweden); T. Laurell, S. Andersson-Engels, Lund Univ. (Sweden)

Upconverting nanoparticles doped with lanthanide ions have drawn much attention due to their potential as optical imaging probes in biomedical applications[1,2]. Among upconverting nanomaterials, lanthanide-ions-doped NaYF4 nanoparticles have been shown to be the most efficient. Currently, NaYF4 nanoparticles are synthesized in batch-control methods in small volumes[3,4]. Although great achievements have been made, batch syntheses tend to suffer from irreproducibility of nanoparticles quality from batch to batch and difficulty to implement fast screening. Microfluidic reaction systems offer a solution to these challenges and have an increasingly important role in synthesis of nanoparticles as highly controlled thermal and stoichiometric microenvironments can be obtained in the synthesis process.[5]

In this paper, we report continuous flow based synthesis of NaYF4 nanoparticles in a capillary-based system with sequential temperature zones [6]. In a typical synthesis, first lanthanide (0.01 M) and NaF (0.03 M) ethylene glycol (EG) solutions were prepared by dissolving stoichiometric amounts of Ln(NO3)3 6H2O (Ln=Y, Yb, Er) and NaF in EG, respectively. Two solutions were subsequently aspirated into two syringes respectively and injected into a coaxial mixing system by syringe pumps. The coaxial mixture was injected in a polytetrafluoroethylene microcapillary with an inner diameter of 800 µm and heated in a 180 oil bath for 5 s, and subsequently heated in a 110 oil bath for 60 s. Finally, the samples were collected from the outlet of the capillary. The nanoparticles were separated by diluting the obtained suspension using acetone followed by centrifugation for several times.

The nanoparticles show good water-dispersibility and emit visible light when excited at 980 nm. In depth characterizations of the obtained nanoparticles are ongoing and will be included in future manuscript.

References

From inorganic nanocrystals towards their assembly in polymeric mesoscale structures designed for biological applications

T. Pellegrino, National Nanotechnology Lab. (Italy)

In the fast advancing scenario of nanotechnology, the development of multifunctional nano-tools, able to carry out at the same time different tasks, is a subject under exploration and it will provide opportunities for the diagnosis and the cure of diseases. The bricks of such nanostructures are inorganic nanocrystals of different materials (such as metals, semiconductors, magnetic oxides) that at the nanoscale present new and unexpected properties.

Assembling them together, in a controlled manner into still nano/meso structures, characterizing their performances and exploiting them in biomedical applications is the subject of this talk.

As an example, the preparation, the characterization and the application of nanostructures which display at the same time fluorescence and magnetic properties will be discussed. Such nanostructures are interesting biomedical platforms and can find applications as in vivo dual modal imaging probes based on optical and magnetic resonance imaging (MRI), or in vitro bio-separation, as for example the simultaneous magnetic separation and multiplexing optical detection of tumour cells from a pool of different cell populations. A proof of concept for such application will be shown in this work.1

Additionally, surface functionalization of such magnetic or magnetic/fluorescent mesoscale structures with intelligent coatings able to sense stimuli deriving from the internal cellular environment (as for instance, the pH of different cellular compartments, the reducing environment of tumor cells) or from the external surroundings (such as for instance a physical stimulus like a magnetic field applied) will be also discussed. These nanostructures might find application as carriers for the controlled delivery of drugs under defined stimuli.


Indium phosphide-based core-shell quantum dots optimized for biological applications

A. M. Dennis, A. Steinbrueck, J. A. Hollingsworth, Los Alamos National Lab. (United States)

Diverse arrays of biological sensors and imaging tools have been developed using semiconductor nanocrystal quantum dots (QDs) (1).
Concerns linger, however, regarding the toxicity of traditional cadmium-containing nanomaterials (2). Indium phosphide (InP) offers a “green” alternative to the traditional cadmium-based NQDs, but suffers from an extreme susceptibility to oxidation. Coating InP cores with more stable shell materials has been shown to significantly improve nanocrystal resistance to oxidation and photostability (3), although higher quantum yields of InP core-shell NQDs in an aqueous milieu are needed to rival the more common CdSe/ZnS preparations.

We have developed synthetic methods for producing bright and tunable InP-based core-shell QDs suitable for transfer into water. Our one-pot synthesis technique minimizes oxidation of the InP cores while maintaining control of the shell process to produce well-defined core-shell structures. Thicker shells enhance photostability while affording potential for suppressing blinking, improving on current InP core-shell preparations. NQDs made water-soluble using suitable ligands (e.g. mercaptoundecanoic acid) were analyzed for their quantum yield and stability. Thicker shells were shown to enable better retention of the quantum yield in aqueous milieu. Several shell materials, including zinc sulfide and zinc selenide have been utilized, and the different photophysical effects elicited on the InP by the various shell materials have been investigated. Using shell materials of various bandgaps can yield type I or quasi-type II semiconductor materials, allowing the InP core-shell material properties to be tuned not only in emission wavelength, but also in electronic properties, to best suit the biological application.


7909-44, Session 13

Compact and highly stable quantum dots through optimized aqueous phase transfer

P. Reiss, S. Tamang, G. Beaune, I. F. Texier-Nogues, Commissariat à l’Énergie Atomique (France)

In recent years a large number of different approaches for the aqueous phase transfer of quantum dots, synthesized in organic solvent, has been proposed. Among those, surface ligand exchange with small hydrophilic thiols has been shown to yield the lowest hydrodynamic diameter, on the order of 5-15 nm. Compact quantum dots are required for specific targeting are given.

We will demonstrate that the precise control of the pH value during aqueous phase transfer of quantum dots, synthesized in organic solvent, has been proposed. Among those, surface ligand exchange with small hydrophilic thiols has been shown to yield the lowest hydrodynamic diameter, on the order of 5-15 nm. Compact quantum dots are required for specific targeting are given.

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Interactions between iron oxide nanoparticles and human lymphoblastoid cells studied by flow cytometry

M. Safi, Univ. Paris 7-Denis Diderot (France); V. Garnier-Thibaud, Univ. Pierre et Marie Curie (France); A. Galimard, M. Seigneuret, H. Conjeaud, J. Berret, Univ. Paris 7-Denis Diderot (France)

We report here the effect of the coating and aggregation state of engineered nanoparticles on their interactions with human lymphoblastoid cells. The particles put under scrutiny were magnetic iron oxide sub-10 nm nanocrystals, which are used in several biomedical applications (MRI, hyperthermia). Coating strategies comprise low-molecular weight ligands such as citric acid and polymers such as poly(acrylic acid). Electrostatically adsorbed on the surfaces, the organic moieties form an adlayer around the particles and provide a negatively charged coating in physiological conditions. Flow cytometry performed in side- and forward-scattering configurations at 4°C and 37°C reveals that cell/nanoparticle interactions depend on the coating, on the dose ([Fe] = 0.01 - 30 mM) and on the incubation time (0 - 24 h). One important result is the strong increase of the side-scattered intensity with increasing dose of citrate-coated particles. These later particles are found to precipitate in the cell culture medium [1], resulting in submicronic aggregates which adsorb at the surface of the cells in large amount, around 100 pg of iron per cell. This adsorption causes the increase of the flow cytometry signal. In contrast, the polymer-coated particles are taken up at much lower levels, 10 pg of iron per cell [2]. These results were confirmed by transmission electron microscopy (TEM). TEM reveals the existence of 200 - 500 nm layers of densely packed citrate-coated particles at the cell surface, as well as large clusters inside endosome-like structures. In contrast, polymer-coated particles are barely detectable at the cellular membranes and accumulate only in endosomes. The kinetics of adsorption and uptake for both particles are also presented. In this study, we demonstrate that the uptake of nanomaterials by living cells depends on the coating of the particles and on the ability of the coating to preserve the colloidal nature of the dispersions.

References:
Imaging enabled platforms for development of therapeutics

T. Hasan, B. Q. Spring, P. R. Rai, A. O. Abu-Yousif, Z. Mai, S. Mallidi, Massachusetts General Hospital (United States); K. S. Samkoe, B. W. Pogue, Dartmouth College (United States)

Advances in imaging and spectroscopic technologies have enabled the optimization of many therapeutic modalities in cancer and non-cancer pathologies either by earlier disease detection or by allowing therapy monitoring. Amongst the therapeutic options benefiting from developments in imaging technologies, photodynamic therapy (PDT) is exceptional. PDT is a photochemistry-based therapeutic approach where a light-sensitive molecule (photonsensitizer) is activated with light of appropriate energy (wavelength) to produce reactive molecular species such as free radicals and singlet oxygen. These molecular entities then react with biological targets such as DNA, membranes and other cellular components to impair their function and lead to eventual cell and tissue death. The somewhat unique feature of PDT is that the same molecules that are used for therapy often have finite fluorescence quantum yield so that they can be used in imaging also. This clinical-enhanced treatment outcome due to therapy monitoring, optimal dosimetry and potentially detection followed by treatment in a “seek and destroy” approach. Development of PDT-based imaging also provides a platform for rapid screening of new therapeutics in novel in vitro models prior to expensive and labor-intensive animal studies. Results, both in 3-dimensional in vitro models of cancer and orthotopic in vivo models, using small molecules and nanoparticle based PDT agents will be presented.

Molecular imaging of cancer with activatable fluorescence probes: rational design, synthesis, and in-vivo applications

H. Kobayashi, National Institutes of Health (United States)

Conventional imaging methods rely on contrast agents (iodine, gadolinium, radioisotopes) that are “always on. Therefore, these agents are not sufficiently sensitive because of the inadequate target to background ratio. A unique aspect of optical imaging is that fluorescence probes can be designed to be activatable, i.e. only “turned on” under certain conditions. These probes can be designed to emit signal only after binding a target tissue leading to increased sensitivity and specificity. There are two basic types of activatable fluorescence probes; 1) enzymatically activatable probes, which exist in the quenched state until activated by enzymatic cleavage, and 2) target-cell specific activatable probes, which are quenched until activated in targeted cells by lysosomal processing that follows cell binding and subsequent internalization. Designing probes based on their photo-chemical (e.g. activation strategy), pharmacological (e.g. biodistribution), and biological (e.g. target specificity) properties has recently allowed the rational design and synthesis of target-cell specific activatable fluorescence imaging probes which can be conjugated to a wide variety of targeting molecules. Several different photo-chemical mechanisms have been utilized including self quenching, homo- and hetero-fluorescence resonance energy transfer (FRET), H-dimer formation and photon-induced electron transfer (PeT). A further consideration in the design of a new probe is whether the activation should be irreversible or reversible. Given the wide range of photochemical mechanisms and properties, target-cell specific activatable probes possess considerable flexibility and can be adapted to specific diagnostic needs. Herein, we summarize the chemical, pharmacological, and biological basis of activatable imaging probes for in vivo cancer imaging.

Magnetomotive molecular probes for targeted contrast enhancement and therapy

S. A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

The diagnostic, interrogational, and therapeutic potential of molecular probes is rapidly being investigated and exploited across virtually every biomedical imaging modality. While many types of probes enhance contrast or delivery therapy by static localization to targeted sites, significant potential exists for utilizing dynamic molecular probes. Recent examples include molecular beacons, photoactivatable probes, or controlled switchable drug-releasing particles, to name a few. We have developed a novel class of dynamic molecular probes that rely on the application and control of localized external magnetic fields. These magnetomotive molecular probes can provide optical image contrast through a modulated scattering signal, can interrogate the biomechanical properties of their viscoelastic microenvironment by tracking their underdamped oscillatory step-response to applied fields, and can potentially delivery therapy through nanometer-to-micrometer mechanical displacement or local hyperthermia. This class of magnetomotive agents includes not only magnetic iron-oxide nanoparticles, but also new magnetomotive microspheres or nanostructures with embedded iron-oxide agents. In vitro three-dimensional cell assays and in vivo targeting studies in animal tumor models have demonstrated the potential for multimodal detection and imaging, using magnetic resonance imaging for whole-body localization, and magnetomotive optical coherence tomography for high-resolution localization and imaging.

Targeted multifunctional multimodal multishell microspheres for cancer imaging and drug delivery

R. John, A. Ahmad, F. T. Nguyen, E. J. Chaney, K. S. Suslick, S. A. Boppart, Univ. of Illinois at Urbana-Champaign (United States)

Recently, there has been increasing interest in the development of molecular imaging techniques by the intelligent design and application of contrast agents. A large number of agents have been developed, including engineered microspheres, plasmon-resonant nanoparticles, near-infrared absorbing dyes, and magnetomotive agents. In this study, we demonstrate the fabrication and use of protein-shell microspheres of size from 2 to 5 µm filled with oil suspensions of iron oxide nanoparticles, a near-IR dye (DiR), and an anti-cancer drug (paclitaxel). These microspheres are used as multi-modal contrast agents in NIR fluorescence imaging, magnetomotive optical coherence tomography (MM-OCT), ultrasound imaging, and MRI. We describe the development, characterization, and use of multifunctional multi-modal microspheres as targeted contrast and therapeutic agents. These engineered protein microspheres are made through the use of high-frequency ultrasound. The sonication process creates a fluid mixing state allowing the protein to form a shell encapsulating the oil-dye-drug suspension and the hydrophobic nanoparticles. Traditionally, similar microspheres have been filled with perfluorcarbon and used for ultrasound imaging. We have functionalized our microspheres with RGD peptide ligand, which is targeted to Alpha(v)Beta (3) integrin receptors that are overexpressed in tumors and atherosclerotic lesions. These microspheres, containing iron oxide nanoparticles in their cores, can be modulated externally using a magnetic field to create dynamic contrast in MM-OCT. With the presence of iron oxide nanoparticles, these agents also show significant negative T2* contrast in MRI. These protein
microspheres have previously been demonstrated as contrast agents in ultrasound imaging. In this paper, we demonstrate the capability of these protein microspheres as drug delivery vehicles with high load capacity, as well as contrast agents for multimodal imaging. Preliminary results demonstrate tracking in vivo dynamics of these functionalized microspheres in real-time using NIR fluorescence imaging in a dark box followed by ex vivo MM-OCT. We expect these microspheres to have a great potential for targeted delivery of lipophilic drugs under ultrasound or MR image-guidance, and for high-resolution monitoring using MM-OCT. Further studies are being carried out to explore the possibility of localized delivery of drugs through rupture of these microspheres.

7910-05, Session 1

New materials for imaging bacteria

N. Murthy, Georgia Tech Research Institute (United States)

Bacterial infections cause millions of deaths each year and new strategies for imaging them are greatly needed. In this presentation we will present a new strategy for imaging bacterial infections. This strategy is based on using small molecules that are designed to target bacteria specific transport pathways. We have completed in vitro studies demonstrating that we can specifically target bacteria and are in the process of performing in vivo imaging studies.

7910-06, Session 1

Optimizing tumor imaging by bioconjugated QDs via Kupffer cell inactivation

S. Krishnan, P. Diagaradjane, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States)

Clinical translation of tumor-targeted quantum dot (QD) imaging probes is hindered by (i) significant background noise arising from QDs entrapment within the liver and spleen (the reticuloendothelial system, RES) and (ii) concerns about the biocompatibility of heavy metal-laden particles that are retained for long durations within the body. A potential solution to the sequestration of QDs in the liver, a determinant of both long-term retention (and toxicity) and nonspecific background signal in imaging applications, is to minimize the detection and capture of nanoparticles by the RES. Earlier attempts have focused on (i) surface modification of nanoparticles to evade RES capture or (ii) inhibition of RES uptake by saturating RES capacity. Unfortunately, neither strategy has yielded reliable and reproducible amplification of signal-to-noise ratios in nanoparticle-based imaging. In contrast to these approaches, we chose to inhibit RES macrophage activity to serve as a class solution to the challenge of nonspecific accumulation of all classes of nanoparticles in the liver. Pretreatment with gadolinium chloride (GdCl3) to inactivate Kupffer cells resulted in increased circulatory time and amplified tumor-specific signal of conjugated nanoparticles in vivo. The improvement in tumor-specific imaging following intravenous administration of receptor-targeted QDs was established in a xenograft tumor model, analyzed by compartmental modeling of nanoprobe pharmacokinetics, confirmed by biodistribution analyses at the organ/tissue/cellular levels, and validated as secondary to Kupffer cell inactivation using immunohistochemical and ultrastructural analyses. This pretreatment strategy could have broad applicability across biomedical applications utilizing all classes of theranostic nanoparticles that are sequestered by the RES.

7910-07, Session 2

Target cell specific antibody based photosensitizers for photodynamic therapy

L. T. Rosenblum, M. Mitsunaga, P. L. Choyke, H. Kobayashi, National Institutes of Health (United States)

In photodynamic therapy (PDT), localized monochromatic light is used to activate targeted photosensitizers (PS) to induce cellular damage through the generation of cytotoxic species such as singlet oxygen. While first-generation PS passively targeted malignancies, a variety of targeting mechanisms have since been studied, including specifically activatable agents. Antibody internalization has previously been employed as a fluorescence activation system and could potentially enable similar activation of PS. TAMRA, Rhodamine-B and Rhodamine-6G were conjugated to trastuzumab (brand name Herceptin), a humanized monoclonal antibody with specificity for the human epidermal growth factor receptor 2 (HER2), to create quenched PS (Tra-TAM, Tra-RhoB, and Tra-Rho6G). Specific PDT with Tra-TAM and Tra-Rho6G was demonstrated in HER2+ cells: Minimal cell death (<6%) was observed in all treatments of the HER2- cell line (BALB/3T3) and in treatments the HER2- cell line (3T3/HER2) with light or trastuzumab only. There was significant light-induced cell death in HER2 expressing cells using Tra-TAM (3% dead without light, 20% at 50 J/cm2, 46% at 100 J/cm2) and Tra-Rho6G (5% dead without light, 22% at 50 J/cm2, 46% at 100 J/cm2). No efficacy was observed in treatment with Tra-RhoB, which was also non-specifically taken up by BALB/3T3 cells and which had weaker PS-antibody interactions (as demonstrated by visualization of protein and fluorescence on SDS-PAGE).

7910-08, Session 2

Gold nanorods in photodynamic therapy as hyperthermia agents, and in near-infrared optical imaging

W. Kuo, C. Chang, Y. Chang, National Cheng Kung Univ. (Taiwan); M. Yang, National Chung Hsing Univ. (Taiwan); Y. Chien, S. Chen, C. Yeh, National Cheng Kung Univ. (Taiwan)

The development of multifunctional nanomaterials is currently a topic of interest in the field of nanotechnology. Integrated systems that incorporate therapeutics, molecular targeting, and diagnostic imaging capabilities are considered to be the next generation of multifunctional nanomedicine. In this work, we present the first example of using Au nanorods simultaneously serving not only as photodynamic and photothermal agents to destroy A549 malignant cells but also as optical contrast agents simultaneously to monitor cellular image. Au nanorods were successfully conjugated with hydrophilic photosensitizer, indocyanine green (ICG), to achieve photodynamic therapy (PDT) and hyperthermia. With the combination of PDT and hyperthermia proved to be efficiently killing cancer cells as compared to PDT or hyperthermia treatment alone and enhanced the effectiveness of photodestruction. Moreover, Au nanorods conjugated with ICG displayed high chemical stability and simultaneously acted as a promising cellular image probe. As a result, the preparation of Au nanorods conjugated with photosensitizers as well as their use in biomedical applications is valuable developments in multifunctional nanomaterials.

7910-09, Session 2

Halogenated porphyrins as PDT sensitizers, something more than the heavy atom effect?

A. A. Rocha Gonsalves, A. C. Serra, M. Pineiro, M. Laranjo, A. M. Abrantes, C. Gonçalves, B. Oliveira, A. B. Sarmento, M. F. Botelho, Univ. de Coimbra (Portugal)

In our approach to the development of PDT sensitizers the incorporation of halogen atoms in the structure of tetraarylporphyrins was exploited in the rationale that the heavy atom effect would allow achieving a much better PDT sensitizer. First biological activity studies led to partly deceptive results (2). Elaborating
on our previous observations, we addressed our attention to halogenated diarylporphyrins supported by the concept that having unsubstituted meso-bridges these were structurally more similar to the natural ones. Experimental results proved that our halogenated diarylporphyrins present superior activity relatively to the tetraaryl counterparts (3). However these porphyrins showed a PDT activity similar to the corresponding non-halogenated ones despite being better singlet oxygen generators. In addition, the halogenated porphyrins showed much lower affinity for cancer cells, meaning that they present a much higher intrinsic photoactivity (ISP) (3).

In this work we compare the photodynamic activity of tetraryl and diarylporphyrins on urinary bladder carcinoma cells (CRL1472). In particular we attempt to explain this higher activity of halogenated porphyrins, comparing the activity of a brominated (I) and a non-brominated (II) porphyrin derivatives. Cellular uptake measurements and cytotoxicity assays were carried out. Flow cytometry studies showed marked differences between the two kinds of porphyrins. Intracellular localization is also different. Together these facts can explain the different ISP of the porphyrins pointing to a multiple role of the presence of halogens in modeling the PDT activity. 

I - 5,15-bis(3-hydroxyphenyl)porphyrin  
II - 5,15-bis(2-bromo-5-hydroxyphenyl)porphyrin  

7910-10, Session 2  
A minimally invasive multifunctional nanoscale system for selective targeting, imaging, and NIR photothermal therapy of malignant tumors  
H. Green, D. V. Martyshkin, E. L. Rosenthal, S. B. Mirov, The Univ. of Alabama at Birmingham (United States)  
The anti-EGFR antibody, cetuximab, was labeled with Cy5.5 fluorescent dye and conjugated to gold nanorods (GNR). GNR with aspect ratio of ~ 4 and plasmon resonance peak at ~785 nm were fabricated for use in experiments. The Cy5.5:ctuximab:nanorod conjugate treatment with NIR light selectively heated the GNR and was sufficient to treat cancers. Excitation induced fluorescence of the Cy5.5-dye enabling real-time imaging. We characterized and optimized the parameters for the conjugation of the GNR to cetuximab. This combination of selective targeting, imaging, and photothermal treating of malignant cells is a viable approach for a variety of squamous cell carcinomas.

7910-11, Session 3  
Modeling structure and spectra of the kindling fluorescent protein asFP595  
J. Collins, I. Topol, SAIC-Frederick, Inc. (United States); A. P. Savitsky, A.N. Bach Institute of Biochemistry (Russian Federation); A. V. Nemukhin, Lomonosov Moscow State Univ. (Russian Federation)  
Modern computational approaches based on quantum mechanical methods to characterize structures and optical spectra of biological chromophores in proteins are intensively used to gain knowledge of events occurring upon of their photo-excitation. Primary attention is paid to the chromophores from the family of the green fluorescent protein widely used as a biomarker in living cells. We apply modern quantum chemical approaches for accurate calculations of the structures of the chromophore binding pockets and to estimate spectral bands corresponding to the 50-51 optical transitions of the intriguing kindling protein asFP595. A special attention is paid to evaluate effects of point mutations in the vicinity of the chromophore group. Theoretical data provide important information on the chromophore properties aiming to interpret the results of experimental studies and applications of this fluorescent protein.

7910-12, Session 3  
Developing targeted fluorescent contrast agents for in-vivo micropathology guided resection of medulloblastoma  
D. Wang, Stony Brook Univ. (United States); F. V. Cochran, H. Haeberle, C. H. Contag, Stanford Univ. School of Medicine (United States); J. T. C. Liu, Stanford Univ. (United States)  
The outcomes of brain tumor patients correlate with the degree of surgical resection. However, cautious resection is necessary to avoid neurological damage, especially in pediatric patients. Real-time image guidance will allow for improved resection in a larger proportion of patients, while reducing the debilitating effects of over-aggressive resections. Confocal microscopy, if modified for deep tissue imaging, enables visualization of sub-surface cells that are in their natural undisturbed tissue microenvironment, where cell-surface proteins may accurately be labeled with exogenous contrast agents. Furthermore, cellular and glandular morphologies may be resolved in real time, providing additional diagnostic information.

We have developed a surgical dual-axis confocal (DAC) microscope with a 2-mm diameter GRIN relay lens at the distal tip for in vivo micropathological guidance during resection of medulloblastoma, the most common form of pediatric cancers. In vivo imaging studies have been performed with tissues from a transgenic mouse (Ptc+/−/p53−/−; Math1-GFP) that spontaneously develops medulloblastoma with co-localized GFP expression. We are using this mouse model, and our DAC microscopes, to develop and validate topically-applied fluorescent contrast agents for delineating tumor margins with molecular specificity. The first probe being developed is a fluorescent monoclonal antibody that targets the vascular endothelial growth factor receptor 1 (VEGFR1), which has been reported to be over-expressed in human medulloblastomas. For the second group of probes, an M13 phage-display random peptide library (New England Biolabs), in which individual phage are engineered to express up to 10^9 unique 12-amino-acid-long peptide sequences, was screened to identify sequences that bind with high affinity to fresh tumor tissues obtained from our mouse model. These techniques will allow surgeons to unambiguously distinguish between normal and cancerous tissues for chemically-specific and spatially-precise tumor debulking.

7910-13, Session 3  
Quadratic Stark effect tunes spectrum of fluorescent proteins  
M. Drobitshev, N. S. Makarov, B. H. Davis, A. K. Rebane, T. E. Hughes, P. R. Callis, S. E. Tillo, Montana State Univ. (United States)  
Intrinsically fluorescent proteins (FPs) exhibit a wide breadth of excitation and emission spectra across the visible wavelength range. We studied two families of red and green FPs; both with a respective chromophore. We found that the excitation energy of a pure electronic transition...
between the ground and first excited states varies quadradically with the internal electric field among FPs sharing a common chromophore, in agreement with the theory of Stark effect. The internal field at the chromophore was calculated using Coulomb’s Law and a number of approximation schemes. A first approximation was to consider only contributions to the field by four charged amino acids (K+ R+, D-, E-), approximated as point charges. The approximation was refined by considering the protonation of histidine, surrounding water molecules, and the distribution of charge on both charged and uncharged residues. These calculations are compared to experimental data obtained by two-photon spectroscopy. As both models possess their own approximations and assumptions, the two can be compared so that the method can be refined. For example, the experimental method assumes a uniform field. The calculations ignore effects from hydrogen bonds, salt bridges, etc. Our results show that these calculated values correlate with the experimental data with varying degrees according to the approximation scheme, but always within an order of magnitude. Recent access to FPs with high structural resolution has allowed for more accurate calculations that correlate more strongly with experiment. Matching experiment to a theoretical model could provide such insight as the protonation states of histidine within FPs.

7910-66, Session 3
Compact intraoperative fluorescence imaging device for imaging tumor margins and mapping sentinel lymph nodes
Y. Liu, A. Q. Bauer, W. J. Akers, G. Sudlow, K. Liang, D. Shen, M. Berezin, J. P. Culver, S. Achilefu, Washington Univ. in St. Louis (United States)

We have developed a real-time intraoperative fluorescence imaging device that can identify tumor margins, guide surgical resections, map sentinel lymph nodes (SLNs) and transfer pathologival information wirelessly. Unlike conventional imaging instruments which are generally bulky and expensive, our device is compact, wearable, portable, battery-operated and allows hand-free operation by surgeons. Using this device, NIR fluorescence from molecular probes is converted to visible light with adjustable amplification and subsequently displayed on its goggle eyepiece. Consequently, by wearing this special goggle device, tumors and SLNs targeted by NIR molecular probes can be tracked in real-time during surgery. Adjustable NIR light sources for dye excitation are also integrated in the device, which allows the device to operate in a similar way as head mirrors. It is further equipped with wireless communication capability to transfer pathological information in real-time. The detection sensitivity of our device is down to 1 nM, and we have successfully demonstrated its capacity in identifying breast tumor margins, guiding surgical resections, mapping SLNs and transferring pathological information wirelessly in a murine model. Moreover, the total cost of the proposed device is much lower as compared to other imaging instruments, with total device cost under $1000. These results suggest that our device hold great promise as a clinical tool for ensuring real-time during surgery. Adjustable NIR light sources for dye excitation are also integrated in the device, which allows the device to operate in a similar way as head mirrors. It is further equipped with wireless communication capability to transfer pathological information in real-time. The detection sensitivity of our device is down to 1 nM, and we have successfully demonstrated its capacity in identifying breast tumor margins, guiding surgical resections, mapping SLNs and transferring pathological information wirelessly in a murine model. Moreover, the total cost of the proposed device is much lower as compared to other imaging instruments, with total device cost under $1000. These results suggest that our device hold great promise as a clinical tool for ensuring point-of-care medical interventions.

7910-14, Session 4
Development of ultra-sensitive Ca2+ indicators, yellow cameleon nano
T. Nagai, K. Horikawa, Hokkaido Univ. (Japan)

There are so many Ca2+ indicators such as Oregon green 488 BAPTA-1 and cameleon, all of which have moderate Ca2+ affinities (Kd > 150 nM). These indicators successfully report changing in Ca2+ concentration in most of cells. However, it is known that some cells are estimated to have a very low resting [Ca2+] and display very small Δ[Ca2+], which is below the detection limit of the indicators. Although a possible way to detect subtle [Ca2+] changes at low concentrations (< 100 nM) is to utilize a high-affinity indicator, only a small number of high-affinity indicators (Kd < 100 nM; Quin2, D2cpV) are available. Thus, we sought to develop high-affinity indicators by modifying a FRET-based Ca2+ indicator, yellow cameleon (YC) 2.60 (Kd = 100 nM) because of the brightness and the large dynamic range (> 6 fold). By engineering the Ca2+ sensing domain, we obtained a series of ultra-sensitive Ca2+ indicators. Their high Ca2+ affinities (Kd = 15, 30, and 50 nM) enabled detection of subtle Ca2+ transients associated with spontaneous network activity in mice brain and zebrafish. Our measurements revealed that both the resting Ca2+ level and the amplitude of Ca2+ transients significantly differed by cell type and stimulation, indicating that the selection of indicators with the appropriate Kd is essential for successful in vivo Ca2+ imaging. A lineup of such indicators with finely tuned Kd values optimized to detect [Ca2+] from 10 nM to 100 nM would enable precise and reliable Ca2+ imaging.

7910-16, Session 4
Synthesis of gold nanoparticle-based beacon for measurement of STAT5b protein expression
J. Xue, China Pharmaceutical Univ. (China); Z. Qian, Nanjing Univ. of Aeronautics and Astronautics (China); Y. Gu, China Pharmaceutical Univ. (China)

STAT5b is an important protein in JAK-STAT signal pathway and is responsible for the metastasis and proliferation of tumor cells. The determination of the STAT5b expression provides a way to study the mechanism of tumor progress. In this study, gold nanoparticles with different diameters were conjugated to the fluorescein modified STAT5b specific DNA sequence to form the beacon. The procedures for the beacon with better fluorescence properties were optimized. The fluorescence quenching and the recovery properties after hybridizing with mRNA of STAT5b were intensively investigated. Results indicate the gold nanoparticle based beacon is an effective probe for the determination of STAT5b protein expression in JAK-STAT signal pathway and has great potential in the study of drug screening and discovery.

7910-17, Session 4
Optical heating and sensing with plasmonic gold shell and phosphorescent core nanoparticle
S. Lakshmana, I. M. Kennedy, Univ. of California, Davis (United States)

Nanoparticles with a rare-earth doped, up-converting phosphorescent core and plasmonic gold shell were synthesized by a modified sol-gel method and were employed to demonstrate optical heating and temperature sensing. The up-converting phosphor consisted of an Er3+ emitter with Yb3+ sensitizer in a NaYF4 matrix. The typical sizes of the nanoparticle were 20-50 nm and the thickness of the gold shell was 5-8 nm [1,2]. Optical heating and sensing from this novel core-shell architecture was achieved by employing two lasers. The heating of the shell was accomplished by 10 ns pulsed laser with an average energy ranging from 1-10 mJ per pulse. The wavelength was either a visible light that excited the surface plasmon resonance of the gold shell centered around 532 nm or a near infrared (NIR) 1064 nm that directly heated the shell. The temperature was measured by a low power picosecond pulsed laser or a continuous laser operating at 980 nm. The nanoparticles were immobilized on a solid matrix and the emission spectrum from Er3+ at 520 nm and 540 nm were analyzed. The ratio of the two green lines gives a quantitative estimate of temperature within one degree of accuracy. 1. L. Sudheendra, Jin-Hee Han and I. M. Kennedy Proc. of SPIE Vol. 7576, 75760Y (2010). 2. L. Sudheendra, Volkant Orltin, Sanchita Dey, Nigel Browning, I.M. Kennedy, Manuscript under review.
Molecular specific silica-coated gold nanorods for enhanced photoacoustic imaging and image-guided photothermal therapy

Y. Chen, C. L. Bayer, K. A. Homan, S. Y. Emelianov, The Univ. of Texas at Austin (United States)

We designed and fabricated silica-coated gold nanorods - a multifunctional agent for molecularly sensitive photoacoustic imaging and image-guided photothermal therapy. The nanorods are characterized by their strong optical absorption, superior photothermal stability and efficient light-to-sound conversion. Silica-coated gold nanorods were prepared by a modified Stöber methods using PEG-thiol as a gold-to-silica coupling agent. Transmission electron microscopy imaging revealed fairly uniform and conformal silica coating of the gold nanorods. The controllable deposition of silica allowed rod-to-sphere variation of the nanoparticle shape as the thickness of the silica shell increased. The silica surface of the nanoparticle was functionalized with amine groups and directionally bio-conjugated to the antibody for epidermal growth factor receptor (anti-EGFR), a receptor known to be upregulated by a variety of cancer cells. Using human epithelial cancer cells (the A431 cell line), low toxicity of silica-coated nanorods was confirmed using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell viability assay. The targeting efficacy of the silica-coated nanorods was assessed using dark field optical microscopy imaging of A431 cells incubated with the nanoparticles. The effect of the shape and the surface charge of the anti-EGFR conjugated silica-coated gold nanorods on cellular uptake was also investigated by studying cells incubated with gold nanorods having various thickness of silica coating. The effectiveness of the silica-coated gold nanorods as a multifunctional agent was measured using a combined ultrasound and photoacoustic imaging system. The result confirmed that the silica coated gold nanorods can be an effective and superior molecular specific agent for noninvasive photoacoustic imaging and image-guided photothermal therapy.

Micro- to nanosized spherical aggregates through self-assembly approach for biosensing applications

M. Khorasaniejad, S. Saini, Univ. of Waterloo (Canada)

Nanoparticles are potential building blocks to realize nanoscale devices. Various methods for manipulation and dispersion of metallic and non-metallic nanoparticles into solid form shapes have been proposed in the recent years. Arranging nanoparticles into spherical aggregates has been of interest because of their large specific area, a property important for sensing applications and also their opportunity to use as photonic crystals, nanosphere lithography, nano-capacitors etc. In this paper, we demonstrate a simple method to create self-assembled alumina spherical structures using 3-Aminopropylmethyoxysilane (APTES). This structure can be used as a promising platform for biosensing applications. Efforts toward immobilizing fluorescence sensing chemistry in a biocompatible polymeric matrix have been pursued to decrease concerns over cytotoxicity of the assay components, protein binding, and diffusion of dye from the area of interest, all of which affect the surrounding tissues as well as the experimental results. However, an optical approach based on transdermally implanted, intradermally implanted fluorescent glucose transducers with significant advantages over previous methods has been proposed: the “smart tattoo”. These transducers typically comprise microsphere carriers containing an appropriate fluorescent glucose assay, which are implanted in the skin and may be queried across the skin using light. Nanoparticles arranged in spherical aggregates can allow for larger surface area and discretized surface to attach multiple assays. Our proposed method has an added advantage that the surface of the spherical aggregates is already amine-terminated allowing for simple attachment of biological tags like DNA using the well known glutaraldehyde chemistry. Further, our method should be easily extended to other dielectric nanoparticles including silica and ZnO.

Silver nanoplates for enhanced photoacoustic imaging and therapy of pancreatic cancer


Approaches to cancer treatment are becoming increasingly multipronged where several methods are employed simultaneously to eradicate the cancerous tissue. Chemotherapy and radiation are commonly used together, and new methods such as photothermal therapy with nanoparticles are now in clinical trials. We developed silver nanoplates that can aid in this multiplexed approach by carrying chemotherapeutic drugs, targeting cancer cells, and acting as photothermal contrast agents for pancreatic cancer. Specifically, silver nanoplates were synthesized using wet chemistry techniques in an aqueous environment, employing biocompatible agents such as sodium citrate and ascorbic acid. The nanoplates were then functionalized with three molecules: a thiolated, prodrug version of the chemotherapeutic drug gemcitabine used in pancreatic cancer treatment, the antibody to epidermal growth factor receptor (a-EGFR), and polyethylene glycol. Cytotoxicity studies revealed that the prodrug version of gemcitabine is just as toxic to the pancreatic cancer cell line L3.6pl as the native drug and that silver nanoplates without drugs are non-toxic to cells up to a concentration of 2 mg/mL. Furthermore, darkfield microscopy of cells mixed with silver nanoplates showed selective endocytosis of nanoplates with a-EGFR functionalization. Additionally, the large absorption cross section of the nanoplates made them excellent photoacoustic contrast agents. Their ability to enhance photoacoustic imaging in vivo in a mouse model of pancreatic cancer following tail vein injection was demonstrated. Overall, silver nanoplates represent a largely unexplored nanosystem for cancer imaging and treatment. Here we present ways to chemically functionalize nanoplates and show their potential for multifunctional imaging and treatment of pancreatic cancer.

Multiphoton luminescence of gold nanorods upon excitation with wavelengths away from their absorption maxima

N. K. Balla, C. J. R. Sheppard, National Univ. of Singapore (Singapore); P. T. C. So, Massachusetts Institute of Technology (United States)

Gold nanoparticles are quite popular as contrast agents for optical microscopy. Their strong linear and nonlinear interaction with light, coupled with their biocompatibility and resistance to photobleaching make them suitable contrasts agents for bioimaging applications. Gold nanorods have been used for in vivo two photon microscopy in small animals [PNAS 102, 15752 (2005)]. Conventional two photon microscopy with gold nanorods involves exciting these particles with femtosecond laser at wavelengths close to their longitudinal plasmon resonance (LPR). Most of the reported works used Ti:Sapphire laser with excitation wavelengths ranging from 780 nm to 850 nm. The rational was to maximize absorption of excitation wavelengths, a fraction of which gives rise to two photon luminescence. This however causes intense heating of the nanorods and unless the excitation powers are kept low, gold nanorods tend to melt [Phys Rev Lett 95, 267405 (2005)]. Another less explored way of getting multiphoton emission from gold nanorods is to excite them at long wavelengths far away from their LPR wavelength [Jour Amer Chem Soc 131, 14186 (2009)]. We are interested femtosecond lasers operating around 1200 nm wavelengths because of their lower scattering and absorption by tissue and water. Here we
compare multiphoton photon luminescence properties of gold nanorods when excited at wavelengths around 800 nm and 1200 nm. Excitation with wavelengths around 1200 nm has certain advantages like lower heating of the particles and hence prolonged durations of imaging. Other advantage is the ability to collect emission in the near infrared regions (NIR) up to 800 nm which is not possible when using excitation wavelengths around 800 nm. These features are good for deep tissue imaging. One disadvantage of this approach is lower luminescence intensity.

7910-58, Poster Session

**A compression program for chemical, biological, and nanotechnologies**

B. S. Tice, Advanced Human Design (United States)

The paper will use various radix based number systems for compression values that will be used in theoreetical models in the fields of chemistry, biology and nano technologies.

7910-59, Poster Session

**Development and application of fluorescent, green-light activatable caged compounds**

N. Umeda, Y. Urano, T. Nagano, The Univ. of Tokyo (Japan)

Caged compound is one of the most powerful tools for spatiotemporal control of biomolecules in cells, which can be activated by irradiation of light. However, ultra violet light, which is required for activation of caged compounds, can damage cells and has poor permeability into tissues. In addition, invisibility of caged compounds makes it difficult to tell distribution of released small molecules.

At the conference, we will describe the development of novel caging group and new caged compounds which are fluorescently visible and efficiently activatable with green light. We have found that boron dipyrromethene (BODIPY), known as a widely used fluorophore, is a potential caging group for phenol, carboxyl acid and amine, which can be photolized with irradiation of green light at around 500 nm wavelength. Based on the result, we have developed caged compounds including caged histamine and caged glutamate which can be efficiently photolized with green visible light. Since the light of that range of wavelength is much harmlesss and far more permeable into biological tissues, developed caged compounds can be applied to UV-susceptible samples such as developing embryo, or slices of organs and cellular tissues. Moreover, fluorescence of the BODIPY caging group makes the caged compounds visible and supports to see or control the distribution of them. These characteristics of the new caged compounds will expand the way to control small molecular activity in a spatially and temporally precise manner.

7910-60, Poster Session

**Gold nanocages as contrast agents for two-photon luminescence endomicroscopy**

Y. Chen, Y. Zhang, The Johns Hopkins Univ. (United States); K. L. Davis, North Carolina State Univ. (United States); X. Li, The Johns Hopkins Univ. (United States)

Plasmonic nanomaterials have received considerable attention for image-guided therapies. Gold nanocages are porous nanoparticles with hollow interiors, have easily tunable surface plasmon resonance (SPR) peaks in the near-infrared region, and produce a broad two-photon photoluminescence band when excited by femtosecond laser irradiation. The bright luminescence signal indicates a possible use of Au nanocages as a new class of optical contrast agents for two-photon imaging. In this study, we demonstrated the use of nonlinear optical endomicroscopy as a novel tool to directly examine the uptake of antibody-conjugated gold nanocages with average size of ~50 nm into A431 cancer cells. The cells overexpress anti-epidermal growth factor receptors (EGFR) on the cell membrane and an anti-EGFR monoclonal antibody was used to tag the nanocages. We examined the two-photon luminescence imaging of Au nanocages in tissues (such as liver, spleen etc.) after injection of PEGylated Au nanocages. The results show gold nanocages exhibit strong two-photon luminescence signals from cells and tissues. Our future research will be focused on targeting bioconjugated gold nanocages to tumor cells for in vivo two-photon imaging of cancers. Overall, Au nanocages are promising as contrast agents for two-photon luminescence imaging with a strong signal, resistance to photobleaching, chemical stability, ease of synthesis, simplicity of conjugation chemistry, and biocompatibility. Combined with optical endomicroscopes capable of nonlinear optical imaging, Au nanocages offer potential for optical imaging in vivo.

7910-22, Session 5

**Single-cell imaging detection of nanobarcoded nanoparticle biodistributions in tissues for nanomedicine**

T. Eustaquio, J. F. Leary, Purdue Univ. (United States)

In nanomedicine, biodistribution studies are critical to evaluate the safety and efficacy of any given nanoparticle formulation. However, detection of small numbers of nanoparticles in whole tissues using standard imaging techniques, such as brightfield or fluorescent microscopy, is feasible only when they are agglomerated and subsequently able to generate a sufficient detection signal. Moreover, such imaging techniques do not allow the direct association of nanoparticle uptake with a specific cell type. In contrast, single-cell imaging techniques, such as electron microscopy (EM) and atomic force microscopy (AFM), are able to detect small numbers of nanoparticles, but these techniques are not practical for extensive ex vivo biodistribution studies concerning large clearance organs. To ameliorate these limitations, we have developed a novel method for single nanoparticle detection that incorporates a non-endogenous oligonucleotide on the nanoparticle surface for use as a unique “nano-barcode” for detection. After these nanoparticles are internalized by cells, the nano-barcode can then be amplified by in situ PCR and the resulting amplicons can be detected by fluorescence or colorimetric systems at the optical level. Thus, the nano-barcoding strategy magnifies the detection signal from single nanoparticles, facilitating rapid evaluation of nanoparticle uptake by specific cell type over larger areas. Preliminary data demonstrates proof-of-concept with nano-barcoded magnetic iron oxide nanoparticles in a model HeLa cell culture system. Work in progress includes nano-barcoding other nanoparticle types and detection of these nanoparticles in mixed cell cultures and pertinent clearance organ tissues. Additionally, associated cytotoxicity of nano-barcoded nanoparticles will be evaluated at the single-cell level.
In our study we used bifunctional gold nanoparticles which are optimal for optical coherence tomography (OCT) diagnostics and laser heating. Solution of gold nanoparticles was injected intravenously in dose 108 particles per animal. The study was performed on female mice of CBA line bearing cervical carcinoma.

Noninvasive control of nanoparticle accumulation in tumor was carried out in vivo by the OCT device designed at the Institute of Applied Physics of the Russian Academy of Sciences.

Laser treatment was performed in 5 hours after nanoparticle injection. The tumor temperature mean was controlled to be between 44-45°C. The surface temperature during treatment was measured with a cooled NIR-thermograph Histopathology and transmission electron microscopy (TEM) studies were conducted to observe apoptotic and necrotic damages in tumor tissues.

An optimal regime of laser therapy was found that ensures pronounced therapeutic effect on a tumor marked by plasmon resonant nanoparticles. No visible tumor damage (burns, hyperemia) was observed during treatment. The anti-tumor impact of hyperthermia is confirmed by inhibition of tumor growth and induced apoptotic death of tumor cells.

7910-24, Session 5

Dual-modality Chitosan-coated magnetic nanoparticles as carrier of cisplatin and MRI contrast agent: an in-vitro study

Y. Arum, Pukyong National Univ. (Korea, Republic of); J. Kim, Kyungpook National Univ. (Korea, Republic of); Y. Song, J. Oh, Pukyong National Univ. (Korea, Republic of)

The chitosan-coated magnetic nanoparticles (CS MNPs) were prepared as carriers of cisplatin through a reverse micro-emulsion method. The characteristics of CS-cisplatin MNPs were analyzed by using FT-IR spectroscopy, transmission electron microscopy (TEM), and superconducting quantum interference device (SQUID). It was found that the synthesized CS-cisplatin MNPs were spherical in shape with an average size of 80nm, with low aggregation and high magnetization property. Meanwhile, the anticancer drug, cisplatin content and encapsulation rate of the nanoparticles was 15-30% and 50-94%, respectively. These CS-cisplatin MNPs also demonstrated sustained release of cisplatin at 37°C in buffer solutions. The extent of CS-cisplatin MNPs in the cells was evaluated in vitro to Hela cancer cells by labeling cells with Prussian blue stain. The result showed that CS-cisplatin MNPs were uptake by cells after 48 h of incubation at 37°C. The cytotoxicity of CS-cisplatin MNPs was investigated using MTT assay. The result showed that CS-cisplatin MNPs retained significant antitumor activities.

The chitosan-coated magnetic nanoparticles were synthesized and safe, and they are potentially useful for a number of applications such as therapy, imaging, and therapeutic system such as magnetic hyperthermia.

7910-26, Session 5

Synthesis and characterization of CdHgTe/SiO2 nanoparticles for in-vivo study of their dynamic distribution in mouse model

H. Chen, S. Cui, Y. Gu, China Pharmaceutical Univ. (China)

Quantum dots (QDs) have been increasingly used in biomedical field recently due to their advantages over organic fluorophores. Meanwhile, the silica shell can prevent the leakage of toxic metal ions. In this study, CdHgTe/SiO2 core-shell nanoparticles were synthesized by coating of silylating reagent on the surface of CdHgTe QDs. The size change after coating a silica shell had been characterized by laser size analyzer. The average size of the nanoparticles is 30±8.7 nm and the dispersion coefficient is low to 0.13. Photoluminescence studies showed that the silica shell resulted in a minor decline of fluorescence intensity and greatly increased photostability in phosphate-buffered saline buffers. Acute toxicity study indicated the obvious toxicity reduction of CdHgTe QDs after coating with silica shell. The dynamic bio-distribution of CdHgTe/SiO2 nanoparticles in living mouse was in vivo monitored by a NIR imaging system. Results indicated the liver-intestine metabolic pathway of these Nanoparticles. The silica-capped QDs are easily synthesized and safe, and they are potentially useful for a number of applications in biolabeling and imaging.

7910-27, Session 5

Effect of nano-encapsulation on photophysical properties of ICG

S. Gupta, K. Thenkondar, H. Mehta, B. Bahmani, V. Vullev, B. Anvari, Univ. of California, Riverside (United States)

Indocyanine green (ICG) is an FDA-approved infrared fluorescent dye used for various biomedical applications such as cardiac and hepatic function evaluation, and ophthalmic angiography. Despite its clinical applications, freely dissolved ICG binds non-specifically to various plasma proteins resulting in changes in its near infrared (NIR) emission properties and rapid elimination from the vasculature. To overcome these shortcomings, we have encapsulated ICG within polymeric nano-constructs composed of poly allylamine hydrochloride cross-linked with di-sodium hydrogen phosphate (Na2HPO4). To optimize the photophysical properties of nano-encapsulated ICG (NE-ICG) for clinical imaging applications, we report measurements of fluorescent quantum yield of NE-ICG. Our preliminary results indicate that NE-ICG shows crossing improvements compared to freely dissolved ICG.
higher quantum yield compared to freely-dissolved ICG, and provide
evidence for the effectiveness of nano-encapsulation as an effective
methodology for improved fluorescence properties of ICG.

7910-48, Session 5
Near-IR triggered release from polymeric nanoparticles
A. Almutairi, Univ. of California, San Diego (United States)
A new light-sensitive polymer containing multiple lightsensitive triggering
groups along the backbone and incorporating a quinone-methide self-
immoliative moiety was developd and formulated into nanoparticles
encapsulating a model pharmaceutical Nile Red. Triggered burst release of
the payload upon irradiation and subsequent degradation of the
nanoparticles were observed. This system is designed to be versatile
where the triggering group can be sensitive to a number of wavelengths.

7910-28, Session 6
Fluorescent molecular probes based on excited state prototropism
A. K. Mishra, M. Mohapatra, Indian Institute of Technology
Madrass (India)
Excited state prototropism (ESPT) is observed in molecules having
one or more ionizable protons, whose proton transfer efficiency
is different in ground and excited states. The interaction of various ESPT
molecules like naphthols and intramolecular ESPT (ESIPT) molecules
like hydroxyflavones etc. with different microheterogeneous media
have been studied in detail and excited state prototropism as a probe
to concept has been gaining ground [1]. The fluorescence of different
prototropic forms of such molecules, on partitioning to an organized
medium like lipid bilayer membrane, often show sensitive response to
the local environment with respect to the local structure, physical
properties and dynamics. Our recent work using 1-naphthol as an
ESPT fluorescent molecular probe has shown that the incorparation
of monomer bile salt molecules into lipid bilayer membranes composed
from dipalmitoylphosphatidylcholine (DPPC, a lung surfactant) and
dimyristoylphosphatidylcholine (DMPC), in solid gel and liquid crystalline
phases, induce appreciable wetting of the bilayer up to the hydrocarbon
core region, even at very low (< 1 mM) concentrations of the bile
salts. The interaction of fisetin, an ESIPT molecule having antioxidant
properties, with lipid bilayer membranes could be sensitively monitored
by its intrinsic fluorescence. The molecule appears to fulfill many essential
criteria of a good fluorescence molecular probe with regard to the
fluorescence parameters of its phototautomer form.
Processes, Eds V. Ramamurthy and K. S. Schanze, Marcel Dekker, Inc.,

7910-29, Session 6
FRET as a tool for the investigation of the fate
of Lipidots® contrast agents in vivo
J. J. Gravier, Commissariat à l’Énergie Atomique (France); L.
Sancey, J. Coll, Institut Albert Bonniot (France); F. Vinet, I. F.
Texier-Nogues, Commissariat à l’Énergie Atomique (France)
The recent years have seen the development of different approaches for the
in vivo delivery and targeting of poorly soluble contrast agents and
active ingredients in diseased tissues. In this context, we developed new
lipid nanoparticles (Lipidots®) with size being easily varied from 25 to
120 nm. Lipidots® display numerous advantages: they are composed of
low-cost and biocompatible lipids; they can be stored in injection-
ready formulations for long duration; their manufacturing process is
versatile and up-scalable. Several indocyanines have been efficiently
encapsulated in the particles while retaining their spectroscopic
properties, with emission wavelengths ranging from the 500 to 820 nm.
Thus, dye loaded-Lipidots® have been proved suitable for both in vitro
and in vivo applications.
To better understand Lipidots® behavior in biological systems,
formulations based on Förster Resonance Energy Transfer (FRET) have
been studied. Different pairs of the selected indocyanines were co-
encapsulated and calculations proved that transfer efficiency within
nanoparticles (i) behaves as in a continuous medium; (ii) depends on local
acceptor concentration. Only one FRET-pair, using quencher QSY21,
shows a different behavior upon encapsulation, suggesting specific
localization or orientation of the dark dye within Lipidots®. Nevertheless,
a >75% quenching of DiD fluorescence (670 nm) is achieved with
reasonable QSY21 concentrations. Thanks to the local dye concentration
dependence of FRET, these formulations are used to understand where
and when the particles are delivered in biological systems. They are
also promising tools to enhance imaging contrast in vivo by making
a clear difference between circulating and uptaked particles.

7910-30, Session 6
Quantum dot-fluorescent protein FRET pair
for intracellular imaging of pH
A. M. Dennis, D. Sotto, G. Bao, Georgia Institute of Technology
(United States)
Fluorescence resonance energy transfer (FRET)-based biosensors have
been designed to fluorometrically detect everything from proteolytic
activity to receptor-ligand interactions and structural changes in proteins.
While a wide variety of fluorophores have demonstrated effectiveness
in FRET probes, several potential sensor components are particularly
notable. Semiconductor quantum dots (QDs) are attractive FRET
donors because they are bright, exhibit high quantum yields, and their
nanoparticulate structure enables the attachment of multiple acceptor
molecules. Fluorescent proteins (FPs) are also of particular interest for
fluorescent biosensors because design elements necessary for signal
transduction, probe assembly, and device delivery and localization for
intracellular applications can all be genetically incorporated into the FP
polypeptide.
We developed a ratiometric pH sensor based on the QD-FP FRET
probe platform. The sensor, which uses the intrinsic pH-sensitivity of the
FP mOrange to modulate the FP/QD emission ratio, exhibits a
20-fold change in its ratiometric measurement over a physiologically
interesting pH range, is extraordinarily photostable under typical
fluorescence imaging conditions, and has been demonstrated in sub-
cellular fluorescence microscopy. The pH sensor was used to image the
acidification of the endosomes following polyarginine-mediated uptake
of the probe into the endosomes. In addition to exhibiting improved
sensitivity and photostability over commercially available pH-sensitive
fluorophores, the modular nature of the probe offers the opportunity to
customize the sensor for a variety of applications through the genetic
engineering of the FP. This was demonstrated with a probe using a single
point mutation of mOrange that shifted the pH range of the probe.

7910-31, Session 6
Spatiotemporal transport of fluorescent
markers in live cells
R. Wang, S. Sridharan, L. Lei, Y. Wang, Univ. of Illinois at Urbana-
Champaign (United States); A. Levine, Univ. of California, Los
Angeles (United States); G. Popescu, Univ. of Illinois at Urbana-
Champaign (United States)
Fluorescence is the most widely applied microscopy technique to study
the dynamics and function in both medical and biological sciences due
to its sensitivity and specificity. Fluorescence correlation spectroscopy
(FCS) is an attractive experimental tool to monitor the dynamic molecular activities which result in the fluctuations of fluorescence emission of the molecules. Inspired by the spirit of spatial FCS, we proposed here a new method to study the transport dynamics over a broad spatial and temporal scale owing to the access to the wide range of both time and space. The molecules of interest are labeled with a fluorophore whose motion gives rise to spontaneous fluorescence intensity fluctuations that can be further analyzed to quantify the governing molecular mass transport dynamics. These data are characterized by the effective dispersion relation in the form of a power law, which describe the relaxation rate of fluorescence intensity fluctuations (is the frequency bandwidth whose inverse describes the characteristic time of moving particles to travel an 1/q mean distance). In particular we expect to observe, respectively, for directed and diffusive mass transport. We performed experiments in epifluorescence, where the fluctuations were captured by an electron multiplier charged coupled device (EMCCD).

Our results indicate that diffusion of particles can be quantified without the need for particle tracking. Furthermore, we show that in combination with GFP, our method reports on the spatiotemporal dynamics of cytoskeleton.

7910-32, Session 7
Near-infrared dipyrrin-based fluorogenic chelators for metal ions
S. A. Vinogradov, S. Thyagarajan, Univ. of Pennsylvania (United States); B. Ghosh, A. V. Moore, Harvard Medical School (United States); A. V. Cheprakov, Lomonosov Moscow State Univ. (Russian Federation)

A simple structural modification of the dipyrrin molecule results in a dramatic enhancement of its ability to form fluorescent complexes with metal ions. The spectral properties of the new family of dipyrrins are tunable over the visible/near infrared range by way of annealing of the pyrrolic residues with external aromatic fragments. The developed method of synthesis allows convenient introduction of various peripheral functions into the dipyrrin molecule. Complexes of pi-extended dipyrrins with many metal ions were found to be brightly fluorescent, and their fluorescence could be switched on and off upon changing the mode of metal coordination. Structural and spectroscopic data are consolidated on the basis of the exciton coupling theory, suggesting rational pathways to practically useful fluorogenic dipyrrin-based ligands. Water-soluble dendritic pi-extended dipyrrins were prepared and evaluated as turn-on fluorescent sensors for Zn2+ showing micromolar binding affinity and bright fluorescence.

7910-33, Session 7
Development of fluorescent tracers for the real-time monitoring of renal function

The measurement of glomerular filtration rate (GFR) is widely accepted by the medical community as the best indicator of kidney function, and is a key component in the diagnosis and management of renal impairment. Accurate measurement of GFR at the bedside is highly desirable in order to assess renal function in real-time, which is currently unmet clinical need. In our pursuit to develop exogenous fluorescent tracers as GFR markers, four classes of hydrophilic derivatives of 3,6-diaminopyrazine-2,5-dicarboxylic acid were synthesized. These include polyhydroxyalkyl based small molecules with high volume of distribution, and polyethylene glycol derived moderate molecular weight compounds with low volume of distribution. Further classification was made on the basis of photophysical properties and includes analogs having blue excitation with green emission, and longer wavelength analogs having green excitation with orange/red emission. Lead compounds were identified in each of the four classes on the basis of structure-activity relationship studies, which included plasma protein binding, urine clearance, and in vivo optical monitoring. The in vivo optical monitoring experiments with lead candidates have been correlated with plasma pharmacokinetic data for measurement of clearance and hence GFR. Renal clearance of all these advanced candidates was superior to that of the iothalamate, which is currently an accepted standard for the measurement of GFR. Clinical evaluation of one of these analogs, a small molecule short wavelength compound, for human studies is being planned.

7910-34, Session 7
Synthesis and characterization of indocyanine green embedded biodegradable-biodegradable-compatible polymeric (PLGA) nanoparticles for prostate cancer imaging
N. L. Patel, A. Wadajkar, R. Patel, K. T. Nguyen, H. Liu, The Univ. of Texas at Arlington (United States)

The aim of this study is to develop and characterize biodegradable-biodegradable compatible polymeric (di-lactide-co-glycolic acid) (PLGA) nanoparticles loaded with indocyanine green (ICG) for enhanced prostate cancer imaging. ICG encapsulated PLGA nanoparticles were synthesized using a double emulsion technique with the particle size of ~250 nm and low polydispersity index (0.07±0.01, n=3). To see the surface morphology of the nanoparticles, scanning electron microscope (SEM) was employed. Absorption, excitation and emission spectra of the particles were measured for the optical characterization of particles. ICG encapsulation in polymeric PLGA did not affect the peak excitation (770 nm) and peak emission (810 nm) wavelengths of ICG. Moreover, the particles showed high absorption in the Near Infrared region. The fluorescence lifetime of prepared nanoparticles in aqueous solution was measured using an intensified CCD camera and found to be 0.65 nm at the peak excitation and emission wavelengths. Wide field fluorescence microscopy images showed successful cell uptake of the nanoparticles in vitro. Then, ICG embedded nanoparticles were made cancer specific by conjugating them with Folic acid which binds to over expressed Folate group on cancer. Our results of in-vitro flow study suggested successful targeting of prostate cancer cells (PC3) by folic acid conjugated nanoparticles. Feasibility of the synthesized nanoparticles as a contrast agent for diffuse optical tomography will be tested using laboratory phantoms. Scope of the project will be expanded to in-vivo prostate cancer targeting by conjugating RGD peptide, instead of folic acid, which binds to Integrin αvβ3 of tumor vasculature.

7910-35, Session 8
Application of near-infrared fluorescence imaging to monitor changes in HER2 expression after therapeutic intervention
V. V. Chernomordik, M. Hassan, R. Zielinski, A. H. Gandjikhche, J. Capala, National Institutes of Health (United States)

The goal of this study is to find specific molecular probes that do not interact with therapeutic agents and analyze the kinetic of the probe binding to human epidermal growth factor receptor type 2 (HER2) in the tumor. It enables quantification of HER2 expression in vivo. This well-known biomarker is overexpressed in many breast carcinomas. Level of HER2 overexpression is an important factor to optimize the therapeutic strategy and monitor the treatment. We used albumin-binding domain-
fused HER2-specific Affibody molecules, labeled with AlexaFluor750 dye, as targeting agent. Quantitative in vivo NIR optical imaging studies were carried out using xenografts mice with subcutaneous HER2-positive tumors. Fluorescence images were obtained at several time points after intravenous injection of the dye to investigate binding kinetics. Mice were divided into groups of 5: no treatment, 12 hours and one week after the treatment of the tumors with Hsp90 inhibitor 17-DMAG. We have shown that compartmental ligands-receptor model can be used to estimate HER2 expression from data obtained by NIR optical imaging of Affibody-ABD-based, HER2-specific probes. Initial slope, characterizing the temporal dependence of the fluorescence intensity detected in the tumor, linearly depends on the HER2 expression, as measured ex vivo by an ELISA assay for the same tumor. We observed strong correlation between downregulation of HER2 expression in human tumor xenografts, treated with 17-DMAG, and temporal characteristics of fluorescence images. Our results indicate that optical imaging using Affibody-ABD-based probes, combined with mathematical modeling, allows non-invasive monitoring effects of therapeutic intervention on receptor expression and tumor vasculature.

7910-36, Session 8

Storable near-infrared chemiluminescent probes for in-vivo optical imaging

B. D. Smith, Univ. of Notre Dame (United States)

We report a new set of near-infrared dyes that are both chemiluminescent and fluorescent probes for dual modality optical imaging. The chemiluminescence is thermally-activated (that is, no chemical or electrical stimulus is needed) which means that the probes can be stored at low temperature and they only become chemiluminescent when warmed to body temperature. Self-illuminating, chemiluminescent systems are especially attractive since they have inherently high signal contrast due to the lack of background emission. The near-infrared wavelength leads to maximal penetration of the light through skin and tissue. Planar imaging results in mice show that near-infrared chemiluminescence imaging permits identification of target sites that are more than two centimeters below the animal surface, which is about five times deeper than currently achieved using planar fluorescence imaging. The results highlight an attractive feature with these probes as dual modality molecular imaging agents. They can be used first in high contrast chemiluminescence mode to locate relatively deep anatomical locations in vivo and subsequently employed in fluorescent mode to identify the microscopic targets within thin histopathology sections taken from the same specimen. The ability to optically image relatively deep animal organs will greatly expand the experimental capabilities of commercial small-animal planar optical imaging stations and facilitate efforts to develop in vivo optical tomography.

7910-37, Session 8

Dye-biomolecule conjugates and NIR-fluorescent particles for targeting of disease-related biomarkers

J. Pauli, T. Behnke, R. Brehm, M. Grabolle, K. Hoffmann, C. Würth, Bundesanstalt für Materialforschung und -prüfung (Germany); J. Mathejczyk, F. Alves, Max-Planck-Institut für experimentelle Medizin (Germany); F. Hamann, I. Hilger, Univ. Hospital Jena (Germany); U. Resch-Genger, Bundesanstalt für Materialforschung und -prüfung (Germany)

The sensitive, reliable, and fast detection of tumors is of utmost importance for the interrogation of the molecular basis of cancer pathogenesis, preventing the onset of complications, enabling the timely detection of metastases, and providing a tool for therapy monitoring [1]. The high sensitivity, and comparatively inexpensive equipment of optical imaging techniques, that use light to visualize the optical characteristics of tissue via measurement of its absorption, scattering or fluorescence, have turned these techniques into important tools in biomedical research [2]. Indispensable for especially attractive fluorescence imaging are highly specific and sensitive molecular probes, that absorb and emit in the near infrared (NIR) spectral region between ca. 650 and 950 nm and respond to or target molecular species or processes. Such probes typically consist of a biomarker-specific ligand like an antibody or antibody fragment covalently bound to a fluorescent reporter (e.g. organic dye molecule, nanocrystalline label or fluorophore-doped particle) [3,4]. Here, we present different approaches to targeted fluorescent probes for in vivo imaging in the intensity and lifetime domain exploiting NIR dyes and NIR-fluorescent polymeric particles. In addition, strategies for the introduction of a sensor function, e.g. for pH, are developed, that yield targeted and analyte-responsive probes. With this respect, screening schemes for the fast identification of suitable fluorophores are derived based upon the spectroscopic properties of the fluorescent reporters, their aggregation behavior, interaction with serum proteins, stability, and cytotoxicity and, in a second step, design criteria for highly emissive optical probes.


7910-38, Session 8

Novel design of multimodal NIR optical/MR agents for in vivo imaging

K. Guo, M. Berezin, J. Zheng, W. J. Akers, F. Lin, S. Achilefu, Washington Univ. in St. Louis (United States); B. Teng, A. H. Gandjbakhche, G. L. Griffis, National Institutes of Health (United States)

Integration of optical and magnetic resonance imaging (MRI) methods in one setting is appealing due to the complementary nature of MRI's high spatial and temporal resolution and the high molecular sensitivity of optical imaging. A major challenge in constructing dual MRI/optical probes is matching the relatively low contrast agent's detection sensitivity (micromolar) by MRI with the single molecule detection capability of fluorescence imaging. To bridge this gap, we have developed a multimodal contrast agent that is composed of a near-infrared cyanine dye covalently linked to a chelated gadolinium. Upon administration, the cyanine dyes bind strongly to hydrophobic pockets of albumin, significantly reducing the molecular rotation of gadolinium and consequently resulting in a dramatic increase in T1 relaxivity. The increase of T1 was demonstrated in vitro using NMR and after intravenous administration in vivo on small animal models. Strong fluorescence originated by the developed probe was also utilized immapped in vivo by steady-state and fluorescence lifetime optical imaging.

7910-39, Session 8

Functional imaging of tumor-associated lymphatics

S. Kwon, E. M. Sevick-Muraca, The Univ. of Texas Health Science Ctr. at Houston (United States)

The lymphatic system provides a major route of cancer cell dissemination from the primary lesion site to regional draining lymph nodes (LNs). Yet to date there has been no in vivo imaging technique available to demonstrate functional and structural changes of overall lymphatic system in response to tumor growth and metastasis. Herein, we employed a dynamic near-infrared (NIR) fluorescence imaging technique...
Functional blood vessels can be identified by intravital labeling with a GM, which can be utilized in one-photon fluorescence bioimaging, are not absorbing chromophores. Most of the known fluorescent compounds, features of microvasculature. This application relies on the development imaging, for the study of dynamic processes in living cells, and molecular Research Institute (United States); M. Komatsu, Sanford-Burnham Medical States); T. Urakami, J. Sawada, Sanford-Burnham Medical K. D. Belfield, A. R. Morales, Univ. of Central Florida (United Two-photon fluorescence vascular imaging

7910-40, Session 9

A novel fluorescence lifetime bioassay for screening receptor internalization

N. Cade, G. O. Fruhwirth, King’s College London (United Kingdom); S. J. Archibald, The Univ. of Hull (United Kingdom); T. C. Ng, D. R. Richards, King’s College London (United Kingdom)

We have investigated the use of gold substrates in a novel bioassay to screen for cell receptor internalization.

The distance-dependence of the fluorescence lifetime above a gold film was quantified using a FITC labelled sphere; this shows a significant reduction in lifetime occurs within 100nm of the Au surface.

Similar effects are seen in cells on Au films which express eGFP in the membrane receptor CXCR4, with a strong reduction in lifetime for those fluorophores in the bottom membrane. The addition of the ligand CXCL12 induces internalization of the receptor, thus moving the eGFP away from the Au film and increasing its lifetime.

By measuring the spatially integrated lifetime from large regions of cells, we have been able to distinguish the degree of internalization with a high degree of sensitivity, without the need for any image acquisition or analysis. Furthermore, using this technique we have been able to quantify the efficacies of different internalization inhibitors.

7910-41, Session 9

Two-photon fluorescence vascular imaging with a new fluoresce-RGD peptide conjugate

K. D. Belfield, A. R. Morales, Univ. of Central Florida (United States); T. Urakami, J. Sawada, Sanford-Burnham Medical Research Institute (United States); C. O. Yanez, Univ. of Central Florida (United States); M. Komatsu, Sanford-Burnham Medical Research Institute (United States)

Multiphoton fluorescence microscopy is a powerful tool in biological imaging, for the study of dynamic processes in living cells, and molecular features of microvasculature. This application relies on the development of highly fluorescent, water-soluble, photochemically stable, multiphoton absorbing chromophores. Most of the known fluorescent compounds, which can be utilized in one-photon fluorescence bioimaging, are not optimized for two-photon excitation, and, as a rule, are characterized by relatively small two-photon absorption (2PA) cross sections (~10–100 GM).

Functional blood vessels can be identified by intravitral labeling with a tracer that can reach vascular targets only through the circulation. For example, a fluorescent marker injected intravenously before tissue fixation labels functional vessels but not lumens endothelial sprouts. This approach can be used to monitor blood vessel regression after treatment or determine whether blood flows through channels lined by tumor cells.

In the present study, a two-photon fluorescence microscopy (2FPM) interactive image-analysis method was utilized to evaluate the efficiency of a new 2PA conjugate which was designed to target αvβ3 integrin. The linear and nonlinear photophysical properties of this RGD peptide fluorescent conjugate were carefully measured. This conjugate was injected into the tail vein of a male C5BL/6 mouse that had been implanted subcutaneously with Lewis Lung Carcinoma cells. The excised tumors consisting of ~1 cm3 in volume were whole-mounted and imaged by 2FPM. Ex vivo 2FPM revealed the structure of functional vessels deep within the tumor mass.

7910-42, Session 9

Fluorescence lifetime imaging to quantify subcellular oxygen measurements in live macrophage during bacterial invasion

J. Dragavon, M. Amiri, S. Shorte, P. Sansonetti, Institut Pasteur (France)

Fluorophore concentration, the surrounding microenvironment, and photobleaching greatly influence the fluorescence intensity of a fluorophore, increasing the difficulty to directly observe micro-environmental factors such as pH and oxygen. However, the fluorescence lifetime of a fluorophore is essentially independent of both the fluorophore concentration and photobleaching, providing a viable alternative to intensity measurements. The development of fluorescence lifetime imaging (FLI) allows for the direct measurement of the microenvironment surrounding a fluorophore. Pt-porphyrin is a fluorophore whose optical properties include a very stable triplet excited state. This energy level overlaps strongly with the ground triplet state of oxygen, making the phosphorescent lifetime directly proportional to the surrounding oxygen concentration. Initial experiments using this fluorophore involved the use of individual micro-wells coated with the Pt-porphyrin. Cells were allowed to enter the micro-wells before being sealed to create a diffusionally isolated volume. The decrease in the extracellular oxygen concentration was observed using FLI. However, this isolation technique provides only the consumption rate but cannot indicate the sub-cellular oxygen distribution. To improve upon this, live macrophages are loaded with the Pt-porphyrin and the fluorescence lifetime determined using a Lambert Instruments Lifa-X FLI system. Initial results indicate that an increase in sub-cellular oxygen is observed upon initial exposure to invasive bacteria. A substantial decrease in oxygen is observed after about 1 hour of exposure. The cells remain in this deoxygenated state until the bacteria are removed or cell death occurs.
and utilize in vivo optical imaging, such as photoacoustics, capable of imaging nanoparticles at sufficient depth. In the current study, we investigated nanoparticle uptake in MSCs and the effect on cell function using various gold nanoparticle formulations. MSCs could be loaded with citrate-stabilized and poly-L-lysine coated gold nanospheres of various sizes as observed with darkfield microscopy. Cell viability and proliferation were maintained over a two-week period for all nanoparticle formulations as assessed using a live/dead stain and MTT assay, respectively. MSC differentiation was not affected by nanoparticle uptake. Also, nanoparticle uptake and retention overnight was analyzed using mass spectrometry. Lastly, MSCs loaded with 20 nm citrate-stabilized gold nanospheres were imaged using ultrasound and photoacoustic imaging. Our results demonstrate MSCs can be loaded with gold nanoparticles and imaged, thus demonstrating the potential for MSCs to be tracked in vivo and provide a better understanding of the mechanisms of neovascularization.

7910-44, Session 9

Quantifying enzyme activity with MOMIA in combined DOT-PET imaging

R. E. Nothdurft, M. Solomon, H. Lee, Y. Tai, S. Achilefu, J. P. Culver, Washington Univ. in St. Louis (United States)

The goal of our study was to quantify the presence of an enzyme by combining DOT and PET measurements. A monomolecular multimodality imaging agent (MOMIA) is envisioned with two components: one a radiolabel, the other an activating fluorophore sensitive to an enzyme of interest. DOT provides a three-dimensional map of resulting fluorescence yield, a measure dependent on both the amount of agent and the amount of activating enzyme present. PET meanwhile provides a map based on the radiolabel, hence only dependent on the amount agent.

We performed a simple phantom experiment that illustrates the scenario where varying probe and enzyme concentrations confound fluorescence imaging. Inclusions containing probe and enzyme are placed within a tissue simulating phantom. We then imaged with DOT to obtain three-dimensional maps of yield. The resulting fluorescence images did not correspond to either the probe or enzyme concentrations individually. An equivalent phantom with radio-labeled dyes was then subsequently imaged in a commercial microPET scanner.

Spatial co-registration was accomplished via fiducials visible in both modalities. Since the PET data has finer resolution we examined two methods to resolve the miss-matched point spread functions. In the simplest solution we convolved PET data with a Gaussian kernel to increase the point-spread function. In the second we introduced the DOT data as a prior into the DOT reconstruction. In both cases by normalizing the DOT data by the PET data we recovered a quantitative measurement of the fluorescence activation (enzyme activity).

7910-45, Session 10

Uptake of PEGylated indocyanine green loaded nanocapsules by cells of reticuloendothelial system

B. Bahmani, S. Gupta, V. Vullev, B. Anvari, Univ. of California, Riverside (United States)

Optically active nanoparticles are widely pursued as exogenous chromophores in diagnostic and phototherapeutic applications. However, the blood circulation time of nanoparticles remains limited due to the rapid clearance of the nanoparticles by reticuloendothelial system (RES). Coating with Polyethylene glycol (PEG) is used as a strategy to modify surface properties, biocompatibility, water solubility of nanoparticles, and extend their circulation time. Here, we report synthesis and cellular studies of polymeric-based nanocapsules loaded with Indocyanine green (ICG), an FDA approved near-infrared dye, and coated with PEG molecules of various molecular weights through reductive amination.

We quantify the effect of PEG's molecular weight on uptake of these nanocapsules by human spleen macrophages and hepatocytes using fluorescent microscopy. Our results indicate reduced uptake of PEGylated nanocapsules by human spleen macrophages as compared to uncoated nanocapsules. Among PEGylated nanocapsules, low molecular weight (5000 Da) PEG-coated nanocapsules displayed lower intracellular uptake by spleen macrophages than high molecular weight (30,000 Da) PEG-coated nanocapsules. Our results suggest that reduced uptake of nanocapsules by RES cells can result in prolonged blood circulation time of these nanoconstructs.

7910-46, Session 10

Biodegradable NIR gold nanoclusters: photoacoustic imaging and in-vivo clearance

J. O. Tam, A. Murthy, S. J. Yoon, S. Emelianov, K. P. Johnston, The Univ. of Texas at Austin (United States); K. V. Sokolov, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States)

Gold nanoparticles have shown strong potential as molecular-specific NIR imaging and therapeutic agents. Current plasmonic nanoparticles under investigation for biomedical applications are 20-150 nm. Due to their size and non-biodegradability, they could potentially exhibit long-term systemic toxicity in vivo. Previous reports have shown that nanoparticles < 5.5 nm can undergo efficient clearance in vivo. We previously reported the synthesis of plasmonic nanoclusters, which are assemblies of ca. 5 nm gold spheres into sub-100 nm clusters surrounded by a biodegradable polymer. These nanoclusters provided high NIR absorbance and degraded into primary sub-5nm components within cells. Here, we studied the clearance of the nanoclusters over 1 month in Balb/c mice. Nanoclusters were injected via tail vein, and their biodistribution and clearance were analyzed using neutron activation analysis (NAA) and dark-field reflectance (DR) imaging at 1 day, 1 week, and 1 month post injection. PEGylated gold spheres (60 nm) were used as control. NAA showed significantly higher excretion rate of nanoclusters in both urine and feces as compared to solid gold nanoparticles. These results were confirmed by DR images of liver slices where no gold was detected in the case of biodegradable nanoclusters after 1 month while gold nanoparticles were clearly visible in the liver of the mice treated with solid particles. These results provide evidence of drastically improved clearance kinetics of the biodegradable assemblies of gold nanoclusters versus solid particles. Future work will include a combination of clearance studies with photoacoustic imaging for cancer detection in mouse xenograft tumour models.

7910-47, Session 10

Fabrication, bioconjugation and optical stability of silanized gold nanorods as multifunctional transducers of near-infrared light

F. Ratto, P. Matteini, Istituto di Fisica Applicata Nello Carrara (Italy); S. Centi, Univ. degli Studi di Firenze (Italy); F. Rossi, Istituto di Fisica Applicata Nello Carrara (Italy); F. Fusi, Univ. degli Studi di Firenze (Italy); R. Pini, Istituto di Fisica Applicata Nello Carrara (Italy)

We give new insight into multifunctional nanoparticles with light extinction in the therapeutic window, optical stability even on aggregation, and possibility of bio-conjugation. Over recent years, intense interest for innovative near infrared (NIR) dyes for mini invasive biomedical applications has driven the advent of new noble metal nanoparticles. For instance gold nanorods may serve as excellent contrast agents for dark field, two photon luminescence, and photoacoustic microscopy, and transducers for photothermal microsurgeries and drug delivery. Moreover gold nanoparticles are suitable for bio-conjugation to gain biomolecular
specificity to phenotypical anomalies. Gold nanorods respond to NIR light via plasmon oscillations, which is a genuine boundary effect and depends on all physiochemical conditions at the interface with their environment. Therefore their feasibility for biomedical applications is challenged by a poor definition of their dispersion medium, aggregation (e.g. inside endocytic vesicles) and morphological transformations, which are likely to occur in the biological sample and under excitation.

Here silanization of the gold nanorods is proposed as one effective solution to overcome these issues. A shell of porous silica confers isolation from the environment and additional stability, and also proves suitable for PEGylation and bio-conjugation with proteins, which is discussed in detail. In particular we engineer models of aggregation of gold nanoparticles, in order to investigate its principal effect on their optical response. While in the absence of silica gold nanorods undergo substantial degradation of their plasmon oscillations, silanization proves excellent to maintain pristine optical properties even after critical flocculation.

7910-49, Session 10
Fluorescence imaging reveals expedited tumor accumulation, intracellular delivery, and enhanced potency of bevacizumab encapsulated in a multifunctional nanoconstruct for combined cytotoxic and anti-VEGF therapy of pancreatic cancer
B. Q. Spring, P. R. Rai, Z. Mai, S. K. Chang, T. Hasan, Massachusetts General Hospital (United States)

We present quantitative fluorescence imaging of orthotopic murine pancreatic tumors following simultaneous delivery of multiple therapeutic agents for a mechanism-based combination therapy. A nanoconstruct co-encapsulates (1) a polymer formulation of densely packed BPD (benzoporphyrin derivative, a photosensitizing agent for photodynamic therapy) molecules, and (2) bevacizumab (Avastin), a cell impermeant anti-vascular endothelial growth factor (VEGF) monoclonal antibody. Photodynamic therapy (PDT) is a photochemistry-based modality in which a photosensitizing agent is activated by light irradiation to produce cytotoxic reactive oxygen species. Tumor cells that survive cytotoxic therapy often increase their production and secretion of cytokine growth factors (such as VEGF) as part of a complex network of pro-survival molecular signaling cascades. The nanoconstruct represents a new paradigm for neutralizing the intracellular pool of VEGF following subcurative cytotoxic therapy before these molecules are mobilized systemically. Small animal hyperspectral fluorescence imaging reveals rapid delivery of bevacizumab mediated by nanoconstruct delivery in comparison to the conventional administration. Ex vivo microscopy of tumor tissue sections confirms that the nanoconstruct facilitates intracellular delivery of bevacizumab. Finally, objective quantification of microvessel density using automated, batch processing routines demonstrates an increased antiangiogenic potency resulting from targeting the intracellular VEGF pool.

7910-50, Session 11
Protein nanospheres: synergistic nanoplatform-based probes for multimodality imaging
M. A. McDonald, National Institute of Standards and Technology (United States)

No single clinical imaging modality has the ability to provide both high resolution and high sensitivity at the anatomical, functional and molecular level. MRI and CT have excellent resolution but low sensitivity for probe detection. PET, SPECT and optical imaging provide high sensitivity but poor resolution. Synergistically integrated detection techniques overcome these barriers by combining the advantages of different imaging modalities while reducing their disadvantages. However the integration is not seamless. Image misalignment occurs in 2-50% of clinical PET/CT scans, which is the standard bearer for combined high resolution and molecular imaging. Co-registration is hampered by the absence of co-localized CT contrast, yet simply adding two different classes of imaging probe together does not solve the problem due to differences in pharmacodynamic properties, detection sensitivity and toxicity. We report the development of protein nanospheres optimized for enhancing MRI, CT and US contrast while also providing high sensitivity optical detection. Protein nanosphere’s advantages include the potential to sonochemically convert any protein into monodisperse < 90 nm spheres while keeping the proteins’ native function intact. In addition, nanoparticles, drugs and genes have been encapsulated so as to extend diagnostic and therapeutic functionality. Protein nanospheres in solution or targeted/internalized in cancer cells were prepared in chamber slides, artificial vessels or tissue-mimicking phantoms. Measurements utilizing multiple imaging modalities were performed sequentially at the microscopic and/or tomographic level. These studies reveal protein nanospheres have the potential to couple optical probe detection sensitivity with non-depth-dependent high resolution imaging at the cellular- to whole animal level.

7910-51, Session 11
Gold nanorods for applications in biological imaging
Y. Chen, Y. Zhang, D. J. S. Birch, Univ. of Strathclyde (United Kingdom)

Two-Photon luminescence (TPL) from gold nanorods shows considerable potential in biological imaging for its high resolution, low photodamage, tunable near infra-red (NIR) longitudinal band, polarization dependence, ability to conjugate to bio-molecules and low toxicity. In this work, luminescent properties of gold nanorods were studied using both confocal microscopy and fluorescence lifetime imaging microscopy (FLIM). Luminescent intensity images from single nanorod were obtained and the relationship between the luminescence intensity and the excitation power confirms the two-photon origin. Through a spectroscopy study, TPL from gold nanorods is found to be strong and stable, and mainly governed by the longitudinal surface plasmon band. The application of gold nanorods as TPL imaging agents is demonstrated by studying the gold nanorods taken up by Madin-Darby canine kidney (MDCK) cells. TPL intensity from gold nanorods is found to be several times stronger than that from DAPI. The characteristic fluorescence lifetime of gold nanorods is found to be less than 100ps, which can be used to distinguish gold nanorods from other fluorescent labels and endogenous fluorophores in lifetime imaging. Compared with intensity image, the FLIM image provides better contrast and more details that are lost when using traditional imaging due to the low luminescence intensity. Moreover, luminescence lifetime analysis confirmed the uptake of gold nanorods by MDCK cells, indicating that FLIM can also provide more information than intensity imaging.

7910-52, Session 11
Design of graphene nanoparticle undergoing axial compression: quantum study
O. E. Glukhova, A. S. Kolesnikova, M. M. Slepenchenkov, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

We report the results of quantum investigations of the atomic structure and deformations of graphene nanoparticle undergoing axial compression. We applied the tight-binding (TB) method. Our transferable TB model represents a set of carbon atoms in the graphene nanoparticle. The calculations required to describe the molecular structure and the atomic movements of the nanospheres were performed using the Gaussian 09 package and the 6-31G(d) basis set. The graphene nanoparticle was considered to be a two-dimensional sheet of graphite, with each carbon atom connected to three other carbon atoms by covalent bonds. The carbon-carbon bond length was set to 1.42 Å. The energy of the system was minimized using the steepest descent method, and then the system was allowed to relax using the molecular dynamics method. The relaxation was performed using the NVT ensemble with a temperature of 300 K and a time step of 1 fs. The pressure was maintained at 1 atm using the Berendsen thermostat. The relaxation time was set to 1 ps. The total relaxation time was 10 ps. The total number of carbon atoms in the graphene nanoparticle was 100. The graphene nanoparticle was simulated on a water bath, which was modeled as a dielectric continuum with a dielectric constant of 78.54. The water molecules were modeled using the TIP3P model. The interactions between the graphene nanoparticle and the water molecules were modeled using the Lennard-Jones and Coulomb potentials. The energy and the pressure of the system were calculated using the virial theorem. The energy and the pressure were plotted against time, and the dynamics of the graphene nanoparticle was analyzed.
own program was used. Our own program provides the calculation of the total energy of nanostructures, which consists 10-5000 atoms. We have adapted our TB method to be able to run the algorithm on a parallel computing machine (computer cluster).

To simulate axial compression of graphene nanoparticles atoms on the ends were fixed on the plates. The plates were moved towards each other to decrease the length for some percent. Plane atomic network undergoing axial compression becomes wave-like. Amplitude of wave and its period are not constant and changes along axis. This is, so called, a phase transition. The density of states calculation demonstrates absence of changes in electronic structure. However, the topology has nonzero pyramidalization angles. The strain energy collapse occurs at the strain of axial compression 0.03-0.04.

We can come to the conclusion that elasticity of graphene nanoparticles is more than elasticity of nanotubes the same width and length. Curvature of the atomic network because of compression will decrease the reactivity of graphene nanoparticles.

7910-53, Session 12

Targeting split-enzyme reporter fragments to achieve chemical resolution for molecular imaging

A. Broome, G. Ramamurthy, K. Lavik, L. A. Liggett, J. P. Basilion, Case Western Reserve Univ. (United States)

Determining the status of cell surface receptors has become routine in the care of patients with cancer and has proven to be helpful in guiding treatment. Mutational events that drive a normal cell to become a cancer cell require the coordinated overexpression of not just one receptor at a time, but rather multiple biomarkers. A growing body of evidence from genomic and proteomic research asserts that several receptors contribute to tumor behavior and these expression patterns are referred to as the cancer signature. Many cancers are characterized by an abnormal increase in the activity of epidermal growth factor (EGFR) and transferrin (TfR) receptors. Our data of representative human cancer cell lines demonstrate unique, observable expression patterns for the two receptors. Targeted-reporter imaging agent platforms have real application for noninvasive imaging of the multi-step progression of cancer growth, creating the next frontier in in vivo imaging and allowing chemical resolution. To develop imaging tools that take advantage of the diagnostic molecular signature, new technologies must employ a contrast generating agent whose signal is dependent on the presence of multiple markers. To achieve this we have divided beta-gal into unique sets of complementing subunit pairs that individually have no enzymatic activity. However, when brought into close proximity, complementing pairs associate, resulting in detectable enzymatic activity. To drive complementation at the site of target expression, we have constructed a targeting complex composed of reporter fragment, linker, and targeting moiety. By targeting each subunit to a different cell surface marker, we can drive enzyme complementation and beta-gal activity only on cells that express both markers. Using this strategy, we were able to generate imageable enzyme activity when two cell surface receptors implicated in cancer development, EGFR and TfR, are both expressed simultaneously. Cells expressing only one (or none) of the two receptors do not generate activity or signal. Time course studies indicate that knowledge about receptor cycling is critical for complementation to occur. Further studies to translate this technology to an in vivo setting are under way. [Broome, A-M. et al. (2010) Expanding the functionality of the classic beta-galactosidase complementation assay: piece by piece. Mol Pharmaceuticals 7:60.]
Because the two-photon tensor of the S0 - S1 transition is proportional to \((\text{deltamu})^2\), we suggest an interesting physical effect implying strong Herzberg-Teller coupling of \((\text{deltamu})\) with the \(\text{BLA}\) coordinate. Our model quantitatively explains the vibronic enhancement in 2PA spectrum and also provides upper limit estimation for the 2PA peak cross section of any FP with red anionic chromophore.

7910-56, Session 12

**Novel harmonophores for third-harmonic generation microscopy**

D. B. Tokarz, A. E. Tuer, R. Cisek, V. Barzda, Univ. of Toronto Mississauga (Canada)

Third harmonic generation (THG) microscopy is a valuable technique used for imaging biological structures. Although highly valued for noninvasive structural visualization without labeling, THG microscopy is not highly specific to particular biomolecules or cellular organelles. Structural specificity can be achieved by designing molecular labels that demonstrate large third-order optical nonlinearities. Several molecules have been shown to label and enhance THG signal intensity of stained intracellular structures. Such compounds have been designated the term, harmonophores. Recently, we have found that cancerous tissue when stained with hematoxylin solution, a label used by pathologists for better contrast under white light microscopy, also demonstrated intense THG due to the aggregation of hemalum particles, inorganic aluminum oxide oligomers formed in tissue under specific pH conditions. Since this discovery, we have been able to synthesize various sizes of hemalum nanoparticles and we have tested the size dependency of the hemalum nanoparticles on their ability to generate third harmonic signal using THG microscopy. The THG ratio method was used to determine the third-order nonlinear susceptibility, \(\chi(3)\), of nanoparticle hemalums in aqueous solution. These findings revealed the optimal size of the hemalum nanoparticles for maximal ability to generate THG. In parallel, we have also studied the ability of hemalum nanoparticles to label cells via endocytosis. The discovery of hemalum nanoparticles opened new perspectives for developing high brightness bleach-free harmonophore labels for the study of cellular structures with nonlinear microscopy.

7910-57, Session 12

**STED superresolution microscopy in drosophila tissue**

L. Lau, M. Matis, J. Axelrod, W. E. Moerner, Stanford Univ. (United States)

Farfield superresolution imaging is a rapidly emerging technique that opens up new opportunities into studying biology and medicine. Most superresolution microscopy is currently limited to imaging in fixed or live single cells. However, many biological functions critically depend upon fine details of molecular architecture in tissue, especially cell-cell communication and signaling processes. To address this, we have constructed a Stimulated Emission Depletion (STED) microscope for fluorescence studies of intact tissue samples. Images with red-emitting dyes can be obtained with 50-80nm resolution in tens of seconds and with optical sectioning to reject background from thick samples. First, we compared the STED performance of single molecules of various dyes, including the common commercial ATTO and Alexa dyes, in different mounting media. For the dyes studied, the photostability and performance in STED microscopy differed significantly. We suggest several dye and embedding conditions which are useful for STED microscopy in the red. Second, we apply STED to image signaling proteins and protein complexes in intact Drosophila wing tissue for the first time. Distinct protein complexes near the intercellular junction are imaged at high signal-to-background ratio. This work shows that STED microscopy is a viable superresolution method for biological studies in tissue.
Manipulation of silver nanoparticles in a droplet for label-free detection of biological molecules using surface-enhanced Raman scattering

M. Culha, Yeditepe Univ. (Turkey); M. Altunbek, Yeditepe University (Turkey); S. Keskin, A. D. Saatci, Yeditepe Univ. (Turkey)

Detection and identification of biomacromolecules is of critical importance in many fields ranging from biotechnology to medicine. Surface-enhanced Raman scattering (SERS) is an emerging technique for the label-free detection and identification of biological molecules and structures with its fingerprinting properties and high sensitivity. However, there are a number of obstacles for its applications for biological macromolecules due to their complexity. In this report, manipulation of microscopic processes in play during the drying of a sessile droplet as a tool to influence the nanoparticle-macromolecule packing, which has a dramatic effect on SERS performance, before the SERS acquisition is demonstrated. A process known as the coffee ring phenomenon jams all particles and molecular species to the edges of the droplet during drying. This uncontrolled process has dramatic effects on a SERS experiment, using colloidal metal nanoparticles as substrates, by sweeping everything to the edges and influencing the packing of nanoparticles in the droplet area. A plastic tip was dipped into a drying sample droplet to influence the uncontrolled piling up. A negatively-charged protein, BSA, a positively-charged protein, cytochrom c, and a 20-base long oligonucleotide, were used as model biomacromolecules in this study. While a minimum of one order of magnitude lower concentration improvement in detection limit was observed with negatively-charged biomacromolecules, no significant improvement was observed with positively-charged ones compared to a sample droplet left on the surface without any interference. With the demonstrated approach, picomolar-level biomolecular detection using SERS is possible.

Numerical optimization of periodic hole arrays for plasmonic Raman sensor

K. Yamaguchi, Toyohashi Univ. of Technology (Japan); M. Fuji, Toba National College of Maritime Technology (Japan); D. K. Gramotnev, Nanophotonics Pty Ltd. (Australia); M. Fukuda, Toyohashi Univ. of Technology (Japan)

Recently, biosensors exploring the surface plasmon resonance have received a lot of attention. Using this approach, binding reactions of molecules can be detected in real time with high sensitivity. However, to achieve this, the electromagnetic field and Raman radiation must be greatly enhanced. Nanofocusing structures with surface plasmon could present a break-through in achieving both nano-scale confinement of the electromagnetic energy (for achieving nano-scale resolution) and large, highly controlled local field enhancement (i.e., hot spots) required for efficient surface-enhanced Raman spectroscopy. Previously, we had fabricated nanofocusing hole array in a thin metal film on a dielectric substrate using focused ion beam lithography and succeed in the observation of a SP resonance. The experimental result agreed very well with the simulation results obtained using the finite-difference time-domain method and the finite element analysis. Moreover, we archived large electric filed enhancement with focusing structure more than cylindrical hole. Furthermore, we had determined the optimization in structural design, which uses the focusing hole diameter, periodic hole distance and metal film thickness for a nanofocusing hole array. However we still could not demonstrate the effects of substrate and number of hole.

In this paper, we investigate theoretically substrate and number of hole dependencies for optical characteristics. Finally, we fabricate the nanofocusing hole array with optimized conditions and detect the Raman signal.

Antibiotic sensitivity testing of bacteria using gold nanoparticles and SERS

E. Kastanos, Univ. of Nicosia (Cyprus); K. Hadjigeorgiou, A. Kyriakides, C. Pitriss, Univ. of Cyprus (Cyprus)

Urinary tract infection (UTI) diagnosis and antibiotic sensitivity testing require a minimum of 48 hours using conventional clinical tests. A rise in ineffective treatments, chronic infections, health care costs and antibiotic resistance are some of the consequences of this prolonged waiting period. In this work, Surface Enhanced Raman Spectroscopy (SERS) is used to classify bacteria and determine their sensitivity or resistance to a variety of antibiotics. SERS spectra of six species of gram negative and two species of gram positive bacteria, isolated from urine cultures, were classified after mixing bacterial samples with concentrated gold nanoparticles. The classification algorithm used for the analysis involved a novel feature set based on ratios between different bands of the SERS spectra as well as discriminant analysis and resulted in ~90% accuracy of classification. For antibiotic sensitivity testing, SERS spectra were collected just two hours after exposure to eight different antibiotics (ciprofloxacin, amoxicillin, amoxicillin/clavulanate, norfloxacin, penicillin, cefuroxime, cefixime, or cefaclor). Analysis of the spectra revealed clear separation between bacterial samples exposed to various antibiotics to which they were sensitive and samples exposed to antibiotics to which they were resistant. With the enhancement provided by SERS, the technique can be applied directly to urine samples, bypassing the need for urine cultures. This technology can become the basis for the development of a new, rapid method for UTI diagnosis and antibiogram avoiding all undesirable consequences of current practice.
Portable surface-enhanced Raman spectroscopy for insecticide detection using silver nanorod film fabricated by magnetron sputtering

K. Wong-ek, P. Eiamchai, M. Horprathum, P. Limnonthakul, V. Patthanasettakul, P. Chindaudom, N. Nuntawong, National Electronics and Computer Technology Ctr. (Thailand)

In order to increase agricultural productivity, most Asian countries heavily rely on deadly insecticides, known to be toxic to most living organisms and thus significantly affect the food chain. The most obvious impact is to human beings who come into contact, or even consume, pesticide-exposed crops. This work hence focused on an alternative method for insecticide detection at trace concentration under field tests. We proposed a compact Raman spectroscopy system, which consisted of a portable Raman spectroscope, and a SERS substrate, developed for the purpose of such application, on a chip. For the selected portable Raman spectroscope, a laser diode of 785 nm for excitation and a thermoelectric-cooled CCD spectrometer for detection were used. The affordable SERS substrates, with a structure of uniformly distributed silver nanorods, were however fabricated by a low-energy magnetron sputtering system. Based on an oblique-angle deposition technique, several deposition parameters, which include a deposition angle, a rotation speed, an operating pressure, and a sputtering power, were studied for their immediate effects on shapes and size distributions of the nanorods. The effects of the nanorod topology, as confirmed by a field-emission scanning electron microscope, on the SERS activities from probe organic molecules would be discussed. Several trace concentrations of organophosphorous chemical agents, including methyl parathion, malathion, dichlorvos, and chlorpyrifos, adsorbed on the silver nanorod film fabricated by magnetron sputtering system, were exposed crops. This work hence focused on an alternative method for insecticide detection at trace concentration under field tests. We proposed a compact Raman spectroscopy system, which consisted of a portable Raman spectroscope, and a SERS substrate, developed for the purpose of such application, on a chip. For the selected portable Raman spectroscope, a laser diode of 785 nm for excitation and a thermoelectric-cooled CCD spectrometer for detection were used. The affordable SERS substrates, with a structure of uniformly distributed silver nanorods, were however fabricated by a low-energy magnetron sputtering system. Based on an oblique-angle deposition technique, several deposition parameters, which include a deposition angle, a rotation speed, an operating pressure, and a sputtering power, were studied for their immediate effects on shapes and size distributions of the nanorods. The effects of the nanorod topology, as confirmed by a field-emission scanning electron microscope, on the SERS activities from probe organic molecules would be discussed. Several trace concentrations of organophosphorous chemical agents, including methyl parathion, malathion, dichlorvos, and chlorpyrifos, adsorbed on the silver nanorod film fabricated by magnetron sputtering system, were analyzed. The obtained results indicated, to a great degree, a highly sensitive detection for the trace organic analyses of the toxic chemical agents from the purposed portable SERS system.

Tip-enhanced Raman spectroscopy: nanoscale resolution combined with single-molecule sensitivity

V. Deckert, Institut für Photonische Technologien e.V. (Germany)

Tip enhanced Raman spectroscopy (TERS) enables the label-free investigation of interfaces and surfaces with a resolution down to a few nanometers. This is achieved by using the field enhancing effect of a single plasmonic feature, in most cases a single metallic particle. Conceptually this is very similar to surface enhanced Raman spectroscopy (SERS), just only one particle is used in contrast to many or even aggregates.

Due to the use of single plasmonic particles the electromagnetic field enhancement is confined to areas directly related to the diameter of the particles usually down to a few nanometers. This enhancement region can then be arbitrarily positioned on the surface thus for instance creating a Raman map.

Similarly to SERS also in TERS very low limits of detection can be achieved and single molecule sensitivity has been demonstrated. Here examples from life science related problems, ranging from single molecule detection to nanoscale protein/lipid distinction on cell membranes will be addressed. The high sensitivity (without labels) in combination with the high lateral resolution provides a unique tool to address molecule specific problems.

Selective analyte adsorption on mixed-metal SERS substrates

P. A. Munoz, P. Peng, R. Olivares-Amaya, A. Aspuru-Guzik, Harvard Univ. (United States)

We present the effect of mixed metals on the preferential binding of analyte molecules to femtosecond-laser structured surface enhanced Raman scattering (SERS) substrates. While we have demonstrated previously the robustness, reproducibility, and high surface enhancement (> 10^7) of such substrates on increasing the Raman cross-section of benzenethiol, there is a need for sensitive detection of a broader class of molecules. By coating a native gold- or silver-based SERS substrate with an additional thin layer of transition metal (nickel, platinum, or palladium) atoms, we show that preferential binding to molecules with primary amine groups may be achieved. Consequently, there is an improvement to the Raman enhancement factor in a mixed-metal substrate over a native SERS substrate. Our results are supported by time-dependent density functional theoretical calculations that predict a contribution of chemical enhancement after the inclusion of transition metal adatoms, in addition to the electromagnetic enhancement afforded by the underlying noble metal. We examine in detail the impact of type and thickness of transition metal on analyte adsorption, as well as the applicability of mixed-metal SERS substrates to sensitive detection of biological molecules.

Stragegies to maximize the performance of refractometric nanoplasmonic biosensors

B. Sepúlveda, M. A. Otte, M. C. Estévez, L. García Carrascosa, L. M. Lechuga, Ctr. d’Investigacions en Nanociència i Nanotecnologia (Spain)

Refactometric sensing with plasmonic nanostructures is acquiring a growing interest in the last years due to its high sensitivity and multiplexing capabilities, allowing real-time and label-free detection schemes. In this work we theoretically and experimentally present several routes to improve the refractometric limit of detection of nanoplasmonic sensors.

The first important step consists of tuning the localized surface plasmon resonance (LSPR) wavelength within the region where the quotient between the real and imaginary parts of the dielectric constant of the plasmonic metal is maximized, thus optimizing the ratio between sensitivity and LSPR peak width. In the case of gold, this region is comprised between 700 and 900 nm. Related to this feature, we demonstrate that the shape of the nanostructure plays a secondary role, since nanoparticles with different shapes exhibit similar sensing performance if the resonance position and the volume of the particle are fixed, while a further improvement of the sensing features can be achieved by minimizing the volume of the nanoparticle.

On the other hand, we will discuss the adverse effects of the supporting substrate and the commonly employed adhesion layers (Ti, Cr). To avoid these effects, we propose the reduction of the effective refractive index of the substrate by means of an isotropic chemical etch, and the elimination of the adhesion layers by vapor phase silanization processes, thus providing sensitivities similar to those of free nanoparticles in homogenous media.

Finally, we will show that the excitation of surface modes via total internal reflection leads to a great improvement of the signal-to-noise ratio of the biosensing measurements (larger than one order of magnitude), as we experimentally demonstrate with the detection of triplex DNA structures associated to the iap gene of Listeria innocua. The combination of all these features can boost the development of highly competitive and multiplexed nanoplasmonic biosensors.
Enhanced SPR sensing based on micropatterned thin films
L. S. Live, J. Masson, J. Breault-Turcot, K. Nguyen, Univ. de Montréal (Canada)

Micro-patterned thin films interrogated in the Kretschmann configuration of SPR extend the detection range to lower concentrations and small biomolecules. This was achieved with the same instrumentation and analysis methodologies developed for SPR with continuous films. The plasmonic properties of micro-patterned thin films were investigated to find an optimal structure for biosensing application. The analytical parameters and biosensing performances were also evaluated for analysis of crude biological samples. Au microhole arrays of various periodicities and hole diameters were prepared using a modified nanospheres lithography (NSL) technique to map the optical properties of micro-patterned thin films. Au microhole arrays with a 3.2 µm periodicity and hole arrays of 1.6 µm diameter showed optimal plasmonic properties for biosensing applications as they exhibit a 50% increase in sensitivity to refractive index changes and a shorter penetration depth compared to continuous thin films of the same thickness. Moreover, microhole arrays presented a faster response time to refractive index changes while analytical parameters such as the resolution and the noise in biosensing measurement were comparable to continuous films. When combined to the appropriate surface chemistry, a greater SPR response was measured using microhole arrays with small protein molecules in complex biological matrix. Although microhole arrays required an additional preparation step, a cleaning step using oxygen plasma allowed multiple measurements with the same sensing surface with great repeatability. Further investigations were performed using similar microhole arrays with Au and Ag multilayers to evaluate their plasmonic properties. Hence, microhole arrays proved to be a simple and easy way to improve the current SPR biosensing technique.

Surface plasmon resonance biosensing via differential spectral phase interferometry
S. P. Ng, L. Wu, City Univ. of Hong Kong (Hong Kong, China); S. Wu, H. A. Ho, S. Kong, The Chinese Univ. of Hong Kong (Hong Kong, China)

A novel surface plasmon resonance (SPR) sensor based on differential spectral phase interferometry is introduced. Our scheme incorporates a broadband white-light emitting diode (WLED) with double-pass Michelson interferometer for highly sensitive Kretschmann SPR phase detection over the visible spectrum. This scheme addresses two important limitations of conventional approaches: (i) Laser based SPR interferometers are vulnerable to nonlinear phase saturation. (ii) Spectroscopic SPR sensors only offer limited resolution performance because of weak variations in spectral intensity. The proposed spectral phase interferometer directly acquires the optimal SPR phase response of every spectral component which is equivalent to having infinitely many SPR laser interferometers operating simultaneously at fixed angle of incidence. Therefore the inherent phase saturation problem due to monochromatic laser source can be readily addressed. Experimental results have demonstrated the expected merits by (i) achieving a comparable detection limit as high as 10-7 RIU and (ii) extending the phase measurement range as far as 10-2 RIU, (iii) simplifying the phase modulation scheme through direct acquisition of the spectral oscillation instead of adding a temporal carrier. Biosensing experiments based on the BSA-anti-BSA binding interaction indicate that our system is capable of achieving an ultimate sensitivity of 0.5ng ml-1 (3.3pM), which is among the best reported in literature. Yet such sensitivity is maintained over a wide range of measurement as each wavelength specific SPR phase jump is monitored over the entire visible spectrum. Further biosensing application such as detection of Cytochrome C with aptamer immobilized on the SPR sensing surface is currently under investigation. We believe that the performance merits of high sensitivity, wide dynamic range and simplicity of operation offered by our system will lead to truly practical label-free biosensing applications.

Design, manufacture, and testing of Bragg grating embedded trapezoidal SPP waveguide sensor
M. Xu, S. Aitchison, Univ. of Toronto (Canada)

Abstract: We are presenting a patent-pending plasmonic-sensor enabled microfluidic channel design. Unlike conventional SPP-waveguide enabled sensors, the trapezoidal channel Surface Plasmon Polariton (SPP) waveguide provides the dual-function of guiding SPPs and channeling analytes both in one structure. We demonstrate experimentally a Bragg-grating-embedded trapezoidal SPP waveguide, an index sensing structure ideal for integration with microfluidic channel platforms which has not been previously demonstrated. Our SPP waveguide structure uses silver for the active metal surface, and Electron Beam Lithography (EBL) patterned PMMA stripes as Bragg gratings. We used a sample having grating period \( \lambda_{Bragg} = 467 \text{ nm} \), and index-matching oils with indices \( n_d = 1.3442, 1.3538, \) and 1.3634 at 1550 nm wavelength. We characterize the Bragg resonance of the samples by illuminating the waveguide with a tunable laser, and using a fiber circulator to capture the reflection. The Bragg resonance shift can be caused by changing the waveguide dielectric filler index, \( n_d \), and/or changing the grating periodicity. In both cases, we observe a distinct ~ 11 nm wavelength shift for each 0.01 change in refractive index, which corresponds to ~ 1100 nm/RIU. This result agrees with theoretical calculations.

In addition to the inherent properties of SPP sensors, such as high sensitivity, our trapezoidal design allows single mode operation at micron-size channel cross-section, which is comparable to the dimensions of the biological cell. Also, the waveguide fabrication procedures require readily available micro-fabrication tools. Therefore, this large Bragg-grating-embedded trapezoidal waveguide has good potential to be used in fluidic chemical sensing and adapted by biological lab-on-a-chip devices.

Aptamer-based localized surface plasmon resonance sensor for monitoring glycated proteins
R. Zheng, B. D. Cameron, The Univ. of Toledo (United States)

The peak extinction wavelength of the nano-size noble metal localized surface plasmon resonance (LSPR) spectrum is unexpectedly sensitive to nanoparticle size, shape, and local external dielectric environment. This sensitivity to the nanovenvironment has enabled the development of a new class of nanoscale affinity biosensors. An ssDNA aptamer and fiber optics based LSPR sensor has been developed for the rapid, small volume and label-free detection of glycated proteins for the assistance in the diagnosis and treatment of diabetes. The sensor was fabricated by nanosphere lithography with excellent reproducibility to achieve sensitive and selective nanoscale sensing. Thrombin was used initially as the first target protein for demonstration purposes. A thiol-modified ssDNA aptamer was immobilized onto the gold or silver sensing surface as the specific receptor. Different density and thickness of the self assembly monolayers (SAM) were coated to control the distance between the biosensing and sensing surface. Nonspecific and specific binding effects were studied. The performance of the sensor was evaluated by a custom designed flow cell with competitive samples loaded. Results are compared to the simulation of the discrete dipole approximation (DDA). New aptamers for other target proteins were also produced by Magnetic beads based Systematic Evolution of Ligands by Exponential Enrichment (MB-SELEX).
Development of a molecularly imprinted polymer-based surface plasmon resonance sensor for theophylline monitoring

R. Zheng, B. D. Cameron, The Univ. of Toledo (United States)

Molecularly imprinted polymer (MIP) hydrogel and both planar and localized surface plasmon resonance (SPR) sensing technologies were combined together to develop a novel sensing platform to monitor real-time theophylline concentration, which is a compound of interest in environmental monitoring and a probe molecule for phenotyping certain cytotoxic enzymes. The MIP hydrogel is easy to synthesize and provides shape-selective recognition with high affinity to specific target molecules. Different polymerization formulas were tested and optimized in this study. The influence of the monomer concentration, monomer ratio, and polymerization condition in the formula to the final selective and sensitive factors were addressed by SPR. SPR is an evanescent wave optics based sensing technique that is suitable for real-time and label free sensing purposes. In this study, the planar SPR (PSPR) was based on a commercially available sensor with modified sensing surface to achieve reliable sensing. The localized SPR (LSPR) nano-noble metal sensing surface was fabricated by nanosphere lithography with excellent reproducibility to achieve sensitive and selective nanoscale sensing. The interaction between the nano-structure and the external sample was assessed by a fiber optics coupled spectrometer. Both sensing platforms were investigated and a direct comparison will be provided to show the advantages and disadvantages of each other. This technique can be also applied to assess the activities of other small organic molecules by adjusting the polymerization formula, thus the approach has many potential applications.

Optical-fiber based localized surface plasmon resonance biochemical sensor

Y. Lin, Y. Zou, Y. Mo, J. Namkung, J. Guo, R. G. Lindquist, The Univ. of Alabama in Huntsville (United States)

Planar nanofabrication technology was applied to the facet of optic fiber tip to realize periodic arrays of gold (Au) nanodots with subwavelength periodicity on the tips of silica fibers using electron beam lithography (EBL) and reactive ion etching (RIE). There are two remarkable advantages of this method. One is that it results in an excellent adhesion of metal nano-structures to the fiber end facet. The optical fiber based LSPR sensor can be cleaned in piranha solution and reused for more than 5 times. The other one is that the resulted sensor device can be reproduced and mass fabricated by conventional planar nanofabrication technology.

To demonstrate the effectiveness of the fiber tip LSPR sensor in the affinity-based optical and chemical sensing, the system biotin/ streptavidin was chosen as model receptor and the model analyte, respectively. Using the fiber tip LSPR sensor, we were able to enhance the sensitivity in monitoring the binding of streptavidin to the gold nanodots surfaces functionalized with a monolayer of biotin, which was deposited onto the gold nanodots surface via a covalent bond between biotinylated thiol group and the Au. These results represents new applications in biochemical research and medical diagnostics.

Surface plasmon-induced photothermal effect and image contrast enhancement of Au nanorings

H. Tseng, C. Lee, S. Wu, T. Chi, K. Yang, J. Wang, National Taiwan Univ. (Taiwan); M. Tsai, Chang Gung Univ. (Taiwan); Y. Kiang, C. Yang, National Taiwan Univ. (Taiwan)

Preparation of a high-concentration Au nanoring (NR) water solution and its applications to the enhancement of image contrast in optical coherence tomography (OCT) and the generation of photothermal effect in a bio-sample through localized surface plasmon (LSP) resonance are demonstrated. Au NRs are first fabricated on a sapphire substrate with colloidal lithography and secondary sputtering of Au, and then transferred into water solution through a lift-off process. By controlling the NR geometry, the LSP dipole resonance wavelength in tissue can cover the spectral range of 1300 nm for OCT scanning of deep tissue penetration. The extinction cross sections of the fabricated Au NRs in water are estimated to give quite high levels near their LSP resonance wavelengths. The optical measurement results are quite consistent with those from numerical simulations based on the finite element method. In numerical simulation, the factor of random orientation distribution of Au NRs in tissue is considered. The fabricated Au NRs are then delivered into pig adipose samples for OCT scanning. It is observed that when resonant Au NRs are delivered into such a sample, LSP resonance-induced Au NR absorption results in a photothermal effect, making the opaque pig adipose cells transparent. Also, the delivered Au NRs in the intercellular substance enhance the image contrast of OCT scanning through LSP resonance-enhanced scattering. By continuously OCT scanning a sample, both photothermal and image contrast enhancement effects are observed. However, by continually scanning a sample with a low scan frequency, only the image contrast enhancement effect is observed.

Plasmon-enhanced ultrafast laser cell transfection

E. D. Diebold, Harvard Univ. (United States); A. Koh, Stanford Univ. School of Medicine (United States); P. Peng, V. Nuzzo, Harvard Univ. (United States); A. Heisterkamp, Laser Zentrum Hannover e.V. (Germany); E. Mazur, Harvard Univ. (United States)

The lack of a high throughput, high efficiency cell transfection technique is an outstanding problem in molecular cell biology and gene therapeutics. We present a method for transfecting biological cells using ultrafast plasmons excited on large areas (> 5 cm^2) of bio-compatible, nano-pyramid substrates. This technique does not employ any potentially toxic chemical transfection reagents or metallic nanoparticles. Leveraging the field enhancement supported by these pyramidal plasmonic nanostructures, we generate localized, transient pores in the membranes of large numbers (~10^6) of biological cells at a rate of approximately 10^4 per second. Diffusion through these pores enables the delivery of functional short interfering RNA (siRNA) molecules into the cells. We present results on the fabrication and optical characterization of these plasmonic substrates, and demonstrate both cellular uptake of biomolecules and cell transfection after plasmon-enhanced laser cell perforation. This new technique represents a viable method to genetically modify cells difficult to transfect using conventional protocols.
7911-18, Session 4
Study of 3D rotational diffusion of plasmon-resonant gold nanorods using optical coherence tomography
R. K. Chhetri, The Univ. of North Carolina at Chapel Hill (United States); K. A. Kozeck, J. B. Tracy, North Carolina State Univ. (United States); A. L. Oldenburg, The Univ. of North Carolina at Chapel Hill (United States)

Optical scattering from gold nanorods at the plasmon resonant frequency is strongly polarized along the orientation of their long axes. This property of gold nanorods is exploited to investigate the rotational diffusion of an ensemble of freely rotating gold nanorods in viscous media using an Optical Coherence Tomography (OCT) system. OCT is a micron-resolution, depth-ranging optical imaging modality that detects coherent backscattering using interferometry. Gold nanorods, plasmon resonant at 800nm, the central wavelength of a custom Polarization Sensitive OCT (PS-OCT) system, were synthesized using a seed-mediated growth method. The PS-OCT setup utilizes a polarization-sensitive interferometer and a single-camera spectrometer to simultaneously detect backscattered light into two orthogonal polarization states. In this study, the rotational diffusion coefficients of the gold nanorods in media of varying viscosity were determined from the autocorrelations of the intensity fluctuations due to the polarization dependent backscattering from the gold nanorods. The experimental rotational diffusion coefficients were observed to decrease as the viscosity of the solution increased, suggesting a longer rotation time due to an increased viscous drag. This is consistent with several theoretical models of the Brownian rotation of rods. Gold nanorods have previously been used as contrast agents for OCT in biological tissues, and the results from this study suggest that plasmon resonant gold nanorods investigated using OCT can also be used as viscosity sensors. Thus, plasmon resonant gold nanorods in conjunction with PS-OCT can find application toward real-time viscosity sensing for various biological tissues.

7911-19, Session 4
Plasmonic manipulations of biomolecular targets using single-femtosecond pulses
G. Bisker, L. Minai, D. Yelin, Technion-Israel Institute of Technology (Israel)

Gold nanoparticles play an important role in biomedical research due to their unique optical properties and high biocompatibility. When illuminated with intense short laser pulses at their plasmonic resonance, gold nanoparticles may affect their nearby environment through a variety of mechanisms, including near field enhancement, local heating, generation of acoustic shock waves, and the formation of cavitation bubbles. In this work, we utilize these effects to alter the structure and functionality of various types of biomolecules. Our experimental method involves illuminating samples of the target molecules, in the presence of small concentrations of gold nanoparticles, with single femtosecond pulses tuned to the particles’ plasmonic resonance. A sample of a solution containing green fluorescence protein (GFP) and 20 nm gold nanoparticles coated with Polyethylene glycol was resonantly irradiated by 50 fs long pulses at 545 nm wavelength from a tunable optical parametric amplifier. Results show a decrease of up to 75% in GFP fluorescence emission following a single pulse with a peak power of approximately 10^11 W/cm^2. Gel electrophoresis of the irradiated GFP-nanoparticles solution showed the dissociation of the protein into small fragments, which correlated well with the observed decrease in fluorescence. Experiments with other proteins indicated that different experimental parameters are required for their loss of function and their fragmentation. The talk will present our experimental observations, and discuss possible interaction mechanisms between the pulses, the particles and the biomolecules.

7911-20, Session 4
Damaging cancer cells using gold nanoparticles and femtosecond pulses
L. Minai, L. Golan, G. Bisker, D. Yelin, Technion-Israel Institute of Technology (Israel)

Illuminated by intense laser light at their resonance wavelength, gold nanoparticles significantly absorb and enhance the optical fields at their near vicinity. This feature could be recruited for the manipulation of cells and tissues via physical processes which include photo-thermal effects, photo-ionization, and formation of cavitation bubbles. Previous experimental demonstrations of this approach used mainly nano-shells and nanorods, which allow resonance excitation at the near infrared part of the spectrum.

In this work we demonstrate damage to cancer cells with a few high intensity femtosecond pulses, using 20 nm gold nanospheres coated with polyethylene glycol that were irradiated at their plasmon resonance (550 nm). Using gold nanospheres and resonance pulse illumination in the visible part of the spectrum has several advantages which include the wide availability of gold nanospheres and their morphological stability under high laser energies. Cell viability was assessed using time lapse microscopy of the irradiated cells, and various parameters were evaluated including nuclei condensation, appearance of apoptotic bodies, and cell motility. We have found that up to ten pulses at resonance wavelength could initiate apoptosis in human fibroblast cells which were co-cultured with gold nanospheres. Cancer cells (A431), on the other hand, were less sensitive to the intense pulses, demonstrating the requirement of larger numbers of pulses to initiate their apoptosis. The talk will outline the new approach, present our results with and without specific targeting, and discuss its advantages and limitations.

7911-21, Session 4
Coupled gold nanorod structures: polarization dependent behavior and their use as contrast agents
K. B. Mehta, N. Chen, National Univ. of Singapore (Singapore)

Unique optical response of metallic nanoparticles due to collective oscillation of free electrons has been widely used to enhance contrast in various coherent imaging modalities. Due to the resonance in collective oscillation of electrons, nanoparticles provide enhancement in scattering and absorption cross-sections. By changing the particle size, shape and composition it is possible to tune the resonance frequency. Various forms of nanoparticles have been designed and used for enhancing the contrast in various imaging modalities. With the advancement in nanofabrication, now it is possible to fabricate nanostructures with unique optical properties which can be used for enhanced contrast. For example stellated shaped gold particles have been designed and it has been shown that enhance depolarization in light due to such particles can be useful in providing contrast in imaging.

In our work we have discussed about a bi-layer structure consists of gold nanorods. It has been demonstrated that this type of structure has different response to left and right circularly polarized incident light. We have used numerical simulations based on the Finite Difference Time Domain method to characterize such particles. We have discussed the possibility of using such particles, which provides enhanced scattering as well polarization dependent behavior, as contrast agent for coherent imaging modalities provides
7911-42, Poster Session

Angle-resolved surface-enhanced Raman scattering (SERS) for rationally designing two-dimensional hole arrays as high-performing SERS substrates

C. Chan, J. Xu, M. Waye, H. Ong, The Chinese Univ. of Hong Kong (Hong Kong, China)

Because of its high specificity, surface-enhanced Raman scattering (SERS) is a promising technique for molecular identification. Unfortunately, this technique so far has only been proven to be of limited use due to a major drawback: lack of reliable SERS substrates that produce stable Raman signals. To obtain strong and stable SERS, one must controls the size, shape, and position of the metallic nanostructures with high precision. However, knowing that the plasmonic effect is so geometry dependent that slight modification of the structure can lead to large variation in the outcome, it is of importance to optimize the geometry (size, shape, depth and period) of periodic arrays in order to produce the strongest possible SERS. In this presentation, we have systematically studied the dependence of SERS on the geometry of two-dimensional circular hole arrays. In particular, we employ angle-resolved reflectivity and Raman spectroscopy to map out the dispersion relations of all resonance modes arising from the arrays and to study their contributions to the Raman enhancement individually. As a result, both the excitation and emission enhancements induced by Bloch-like propagating and localized waveguide modes can be identified and studied quantitatively. More importantly, we find that, for a given mode, the resulting SERS is strongly associated with its decay lifetime and coupling efficiency; shorter lifetime and stronger coupling efficiency lead to stronger SERS. In the end, we will lay out a general scheme on how one can rationally design high sensitive and stable SERS substrates based on periodic arrays.

7911-43, Poster Session

Fabrication of photonic force device for biomolecule sensor

S. S. Choi, Sun Moon Univ. (Korea, Republic of); M. J. Park, Korea Military Academy (Korea, Republic of); N. K. Park, D. S. Kim, Seoul National Univ. (Korea, Republic of); S. M. Park, L. P. Lee, S. G. Hong, Univ. of California, Berkeley (United States)

The fabricated plasmonic cavity with nanosize waveguide presents the huge transmission ratio possibly due to cavity-waveguide resonance and cavity-cavity resonance. The huge photonic pressure device can be utilized as single molecule dynamics study.

7911-44, Poster Session

High-resolution fluorescence excitation profile generated by surface plasmon standing waves induced via focused optical vortices

P. S. Tan, Nanyang Technological Univ. (Singapore); X. Yuan, Nankai Univ. (China)

Surface plasmon polaritons (SPPs) are electromagnetic surface waves generated through strong interaction between electromagnetic field and free electron oscillations at a metal-dielectric interface. Due to such an excitation scheme, SPPs are highly confined near the interface and intrinsically localized in a small volume, resulting in spatially localized to dimensions smaller than the wavelength of free-propagating radiation. More recently, there is a growing interest in a phenomenon of light beams interacting with SPPs, aims at harnessing the unique characteristics of SPPs for various applications such as high-resolution imaging, generation of non-diffracting evanescent Bessel beam, nanolithography and sub-wavelength waveguides to miniaturize optical components to the nanoscopic dimensions of their electronic counterparts. In this Paper, we propose the propagation of SPPs standing wave on the metal film which is excited by localized fields originating from the optical vortex (OV) beams on a homogeneous silver (Ag) metal film. The near-field two-dimensional intensity distribution near the focal plane can be experimentally examined by using near-field scanning optical microscopy (NSOM) and well explained by use of the angular spectrum representation. The realization of the proposed excitation scheme demonstrates a potential for locally exciting standing surface plasmon polaritons and serve as the sub-wavelength fluorescence excitation profile in enhancing the image resolution.

7911-46, Poster Session

Combined near-infrared photothermolysis and photodynamic therapy by association of gold nanoparticles and an organic dye

F. Ratto, Istituto di Fisica Applicata Nello Carrara (Italy); E. S. Tuchina, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); S. Centi, Univ. degli Studi di Firenze (Italy); B. N. Khlebtsov, Institute of Biochemistry and Physiology of Plants and Microorganisms (Russian Federation); P. Matteini, F. Rossi, Istituto di Fisica Applicata Nello Carrara (Italy); V. V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); F. Fusi, Univ. degli Studi di Firenze (Italy); N. G. Khlebtsov, Institute of Biochemistry and Physiology of Plants and Microorganisms (Russian Federation); R. Pini, Istituto di Fisica Applicata Nello Carrara (Italy)

We investigated the combination of near infrared (NIR) photothermolysis and photodynamic therapy against different models of bacteria (s. aureus, s. epidemidis both methicillin susceptible and resistant), in order to identify possible synergistic pathways in the fight against cancer. Photothermolysis was mediated by NIR light absorption from gold nanorods and nanoshells, which were coated with polyethylene glycol to provide for biocompatibility, and then targeted against the bacteria membranes to pursue local photothermal damage. At the same time photodynamic therapy was delivered by administration of an organic dye such as Indocyanine Green (ICG), whose spectrum of molecular transitions overlaps that of plasmon oscillations of gold nanorods and nanoshells (~ 800 nm). Therefore irradiation with NIR light from a low power diode laser resulted into simultaneous photothermolysis and generation of reactive oxygen species as well as additional cytotoxic byproducts of ICG. We assessed the inhibition of the bacteria colony forming ability upon different treatments. We undertook a systematic investigation on the effect of the gold nanoparticles without ICG, ICG without the gold nanoparticles and both together after various doses of NIR light. Moreover we compared the performance of gold nanorods and nanoshells in this model application, which may exhibit different uptake, near-field enhancement, efficiency of photothermal conversion and stability. Our preliminary results point to the concurrence of synergistic and conflicting interactions, and include enhanced intake of cytotoxic species due to permeabilization of the bacteria membranes, quenching of ICG and modification of the bleaching of ICG due to the noble metal proximity.
Functional nanoscale instrumentation for imaging and monitoring of biological systems

K. Lee, A. Neogi, T. Choi, Univ. of North Texas (United States); B. Kim, KAIST (Korea, Republic of); R. Luchowski, Z. K. Gryczynski, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); N. Calander, Macquarie Univ. (Australia)

In this present work, we developed nanostructure functional instrumentation for kinetically imaging biological systems. Proximately located silver (Ag) nanowire (NW) and nanodots (NDs) was employed in the instrumentation to achieve fairly enhanced surface fluorescence (ESF). These NW and NDs functioned as an ESF-active platform where very high fluorescence signal enhancement is expected. The presence of nanostructures such as metal NW and NDs close to each other, created an enhanced near field optical intensity that resulted in making a biological particle emit higher fluorescence, by increasing the emission quantum yield and decreasing life time. The fluorescence intensity and lifetime measurement for the fluorophores, LDS798 dye (1-Ethyl-4-(4-(p-Dimethylaminophenyl)-1,3-butadienyl)-2-quinoilinium Perchlorate) dissolved in 0.2% poly(vinyl) alcohol (PVA) (Mw=9000-10000, Sigma-Aldrich, St. Louis, MO), shortly LDS-PVA, was accomplished. This was confirmed by a simulation work. The enhanced optical field between closely positioned Ag NW and NDs was clearly found. Fabricating the ESF-active platform was accomplished by the nanolithographical method, which employed a focused ion beam (FIB) and a nanomanipulator, on the Ag thin film (50nm thick) coated on a glass substrate.

This approach would be extended further to monitor exocytotic events and cargo release to the extracellular space in the vicinity of cell membrane. When biological particles were positioned over the ESF-active platform, a strong near field interaction with the enhanced field between NW and NDs can drastically magnify the field near the particle such as vesicles. Thus, their movement near the NW and NDs would be monitored with great sensitivity and low signal-to-noise ratio.

Biological sensing with surface-enhanced Raman spectroscopy (SERS) using a facile and rapid silver colloid synthesis technique

C. Smyth, J. J. Wang, Y. P. Rakovich, E. M. McCabe, Trinity College Dublin (Ireland)

Optical techniques towards the realisation of sensitive and selective biosensing platforms have received a considerable amount of attention in recent times. Techniques based on interferometry, surface plasmon resonance, field-effect transistors and waveguides have all proved popular, and in particular, spectroscopy has a large range of options available. Raman spectroscopy has always been viewed as a highly informative technique, with much to be learned about the structure of a compound from the vibrational bond energies. The issue with Raman spectroscopy has been its rather low cross section, so limits-of-detection have always been somewhat hampered by this. Surface-enhanced Raman Scattering (SERS) has been developed to consider all manners of enhancing substrates. Here we consider a facile and rapid technique for the preparation of colloidal silver suspensions[1], with immediate SERS activity. Pteridine compounds are a family of biochemicals, heterocyclic in structure, and employed in nature as components of colour pigmentation and also as facilitators for many metabolic pathways, particularly those relating to the amino acid hydroxylases. In this work, isoxanthopterin and 7,8-dihydrobiopterin are examined in relation to their response to adsorption to the SERS-active silver colloids. SERS, while far more sensitive than regular Raman spectroscopy, has its own issues relating to the reproducibility of substrates. In order to quantify concentration-based studies of the pteridine compounds mentioned above, a method for implementing an internal standard for normalisation of the signals has been investigated.

References:

Hybrid gold nanorods/polysaccharides composites as new materials for photothermal applications

P. Matteini, F. Ratto, Istituto di Fisica Applicata Nello Carrara (Italy); G. Giambastiani, L. Luconi, Consiglio Nazionale delle Ricerche (Italy); L. Dei, Univ. degli Studi di Firenze (Italy); F. Rossi, R. Pini, Istituto di Fisica Applicata Nello Carrara (Italy)

Gold nanorods (GNRs) are aspherical colloidal nanoparticles with enhanced absorptions in the NIR region, which is the window where light penetration through the body is maximal. Therefore GNRs can be conveniently employed for selective conversion of light into heat within the context of biomedical applications. A remarkable property of GNRs includes an excellent chemical versatility, which ultimately provides for suitable functionalization strategies by means of biomolecules, dyes or drugs. Despite their unique characteristics GNRs cannot be used in biomedical due to contamination with hexadecyltrimethylammonium bromide (CTAB), a strong cytotoxic detergent used as a stabilizing agent during the nanoparticles synthesis. Even after washing by centrifugation, CTAB bilayers usually remain on the surface of the GNRs, while further removal of CTAB irreversibly destabilizes the colloidal suspension. In order to reduce the cytotoxicity and stabilize the nanoparticles, we have succeeded in fabricating hybrid GNRs/polysaccharides composites. Such materials consist in nanostructured gels, films and biocjugated nanoparticles which exhibit both enhanced stabilization and high versatility. The conjugation of GNRs and polysaccharides allowed us to dramatically reduce the CTAB concentration (below 10-6 M), thus resulting in potentially safe systems for applications in humans. The synthesized hybrid composites have been spectroscopically characterized and then tested to assess their...
light-to-heat conversion efficiency under laser irradiation. The results evidenced that these materials are good candidates for applications in laser therapies including tissue repair, active drug delivery and hyperthermia of tumors.

7911-23, Session 5

Tracking of gold nanoparticles exhibiting enhanced Raman scattering in a living cell
J. Ando, K. Fujita, N. I. Smith, S. Kawata, Osaka Univ. (Japan)

Particle tracking has been used for the observation of biological functions, such as organelle transportation or membrane protein diffusion/coupling. Here, we propose the combination of particle tracking technique and enhanced Raman spectroscopy to monitor biological molecules related to intracellular biological events. Gold nanoparticles incubated with macrophages are taken into the cell body by endocytosis and transported for lysosome accumulations and digestion. Therefore, simultaneous tracking of the particle position and detection of enhanced Raman scattering from the particle give information of biomolecules involved in those biological events. We observed motions of nanoparticles by dark-field microscope. 676 nm excitation laser was used for Raman spectroscopy, and the position of laser focus was controlled by feedback system to follow particle motions in real-time. Camera exposures for dark-field imaging were started along with that for Raman spectroscopy to synchronize their operation. Temporal resolution for Raman spectroscopy and particle tracking achieved to be 50 ms. We found out that Raman spectra changed depending on the particle motions. During straightforward motions, Raman bands, which can be assigned to be proteins, is frequently observed. Once their motions stopped and showed confined motions, Raman spectra also changed. Motion dependent spectral change observed here indicates that enhanced Raman scattering detects biomolecules involved in cellular functions, such as organelle transportation or lysosomes accumulations.

7911-24, Session 5

Photothermally activated drug release from temperature-sensitive liposomes coupled to hollow gold nanoshells
N. Forbes, J. A. Zasadzinski, Univ. of California, Santa Barbara (United States)

A drug delivery system designed to rapidly release drug under low energy continuous wave near infrared light will enable precise spatial and temporal control of drug release. The system consists of temperature sensitive liposomes coupled to hollow gold nanoshells. Low energy, continuous wave near infrared light triggers rapid drug release through photothermal heating of the nanoshells, which then locally heat the liposome membrane to the phase transition temperature. The rate of passive drug diffusion across the liposomal membrane is greatest at the transition temperature. Rapid drug release at a phase transition temperature only several degrees above that of physiological is achieved with liposomes composed of primarily dipalmitoylphosphatidylcholine (DPPC) containing a small fraction of lysolipid (phospholipid with a single fatty acid chain). This system could be used for cancer therapy to deliver anticancer drugs directly to a tumor site. Previously, similar lysolipid-containing temperature sensitive liposomes have been shown nearly-complete release of encapsulated doxorubicin within minutes under bulk hyperthermia. In this system, the presence of nanoshells eliminates the need for bulk hyperthermia while improving the spatial control of release. The nanoshell-mediated photothermal trigger thereby acts as both a release and a targeting mechanism, since only those liposomes located directly under the near infrared laser beam will release their contents. Additionally, the laser power can be tuned to control the rate of drug release. The ability to correlate drug release with membrane temperature allows us to empirically determine the local heat generated by the hollow gold nanoshells under continuous wave near infrared laser irradiation.

7911-25, Session 5

Smart gold nanoparticle conjugates for combined photothermal and chemotherapy of cancer cells
J. Nam, S. Jung, S. Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Noble metal nanostructures can be ideal candidates for photothermal cancer therapy by their large light extinctions at surface plasmon resonances and the efficient heat conversions. We developed a new photothermal therapy concept using ‘smart’ gold nanoparticle that exploit coupled surface plasmon modes of nanoparticle aggregates. ‘Smart’ gold nanoparticles are designed to form aggregates under mild acidic conditions such as in intracellular environment. With the relatively small size of 10 nm, ‘smart’ gold nanoparticles can be efficiently internalized into cancerous cells. Triggered by the pH change, the nanoparticles rapidly form aggregates inside cells, and the aggregates accumulate as the exocytosis is blocked by the increased size. The pH-induced formation of aggregates shifts the absorption to far-red and near-infrared. This shift was successively utilized for selective and deep tissue penetrating photothermal therapy. We also developed ‘smart’ gold nanoparticle conjugates where molecular cancer drugs such as doxorubicins were covalently conjugated to the ‘smart’ gold nanoparticles. This ‘smart’ gold nanoparticle conjugate acts as a drug delivery vehicle as well as a sensitizer for photothermal therapy. When trigger by the intracellular environment, ‘smart’ gold nanoparticle conjugates can form gold nanoparticle aggregates and simultaneously release the loaded cancer drugs. This allows localized, region-specific delivery of photo-sensitizer and cancer drugs inside of cancer cells. The gold nanoparticle conjugate and release of cancer drugs are monitored real-time by dark field and fluorescence microscopy. Combination of photothermal and chemo-therapy shows significantly improved therapeutic efficacy of maximally over 6 folds compared to the projected sum of two treatments alone, suggesting huge synergistic effect between two modalities.

7911-26, Session 5

Single-fullerene manipulation inside carbon nanotube
O. E. Glukhova, A. S. Kolesnikova, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

We report the results of the tight-binding molecular modeling of the polymerization process of some fullerenes C28 inside carbon nanotube. The nanotube is located between two electrodes connected with a power source. The positively charged fullerene C60 moves from one end of tube to other. The moving of C60 is controlled by an external electric field. Moving fullerene C60 compresses on the fullerenes C28 located in one of the nanotube ends. Fullerenes C28 undergoing axial compression move toward each other. For example, when the pressure created in the tube provides both the overlap of pi-electrons of the C28 fullerenes (the interatomic distance of about 1.9 Å) and the covalent bonds formation, the intermediate phase of the (C28)2 dimer is synthesized: (C28)2 [6+6]. The pressure becomes equal to ~35 TPa. After returning the fullerene C60 to initial state, the (C28)2dimer is isomerized with the re-orientation in the tube field. So, (C28)2 transfers to stable phase (C28)2 [1+1]. In conclusion, the dimer/polymer synthesis inside carbon nanotube is real, the dimmers and polymers are stable and may be synthesized in the field of the nanotube holding potential, and the fullerene polymerization in the nanotube guarantees the absence of any additives in the final product.

The motions of the atoms are determined by the classical molecular modeling method where Newton’s equations of motion are integrated with a third-order Nordsieck predictor-corrector. Time steps of 0.15-0.25 fs were used in the simulations. The forces on the atoms were calculated using TB method. Our own program was used for researches.
Hyperspectral molecular imaging of multiple receptors using immunolabeled plasmonic nanoparticles

M. J. Crow, K. C. Seekell, A. P. Wax, Duke Univ. (United States)

Plasmonic nanoparticles have many advantages over fluorescent molecules in applications such as molecular imaging. The scattering intensity from one nanoparticle can be 10^{-5} times greater than the fluorescence intensity from a single fluorophore, making single particle detection possible. Nanoparticles also do not photobleach over time and are non-cytotoxic. One of the most significant advantages of using nanoparticles is that their scattering peaks may be modified by altering the size, shape, and composition of the particle. This allows for the particles' scattering peak wavelengths to span the range from 400nm to 2200nm, as opposed to fluorophores which are largely limited to the visible region. Due to this increased range, immunolabeled nanoparticle systems have the potential to tag and monitor a greater number of cell receptors at a time than fluorescence based systems. In this study, the feasibility of a multiplexed nanoparticle labeling system in molecular imaging is analyzed.

Three different types of immunolabeled metal nanoparticles were developed, each targeting a specific cell receptor commonly overexpressed in cancer cells (EGFR, HER2, and IGF1R). A hyperspectral dark-field microscope was developed to image cells throughout the visible wavelength range and to record the scattering spectra of each cell. The same labeling system was used on cells and analyzed by flow cytometry for confirmation. In both applications, distinct scattering signatures were observed for each of the nanoparticles when bound to cells which expressed the targeted receptors. These studies show the potential for multiplexing nanoparticle labels to monitor and image cell receptors.

Ultra-sensitive plasmonic nanosensors for biochemical detection

J. Martin, J. Proust, J. Bijeon, J. Plain, P. Royer, Univ. de Technologie Troyes (France)

The last decade has seen a growing development of very sensitive and selective nano-sensors for the detection of chemical and biological agents. Increasing interest for this research area witnesses the importance of the interface between physics, chemistry, biology and medicine. The principle of those nano-sensors is based upon transduction mechanisms such as chemiluminescence, fluorescence or plasmonics.

In this context, we propose the realization of very sensitive plasmonic based nano-sensors for the detection of very low concentration molecules, typically in the zeptomolar range. Those sensors are based on the sensitivity of Localised Surface Plasmon Resonance (LSPR) of noble metal nanoparticles to their dielectric environment. Our technique relies on the structuration of the glass substrate to optimize the guiding of the light in excitation as in collection. The structuration consists in glass tips functionalized by gold nanoparticles. We showed in a previous study that our nanostructured substrates increase the sensitivity of LSPR detection by two orders of magnitude compared to the sensitivities reported in the literature for a set of nanotips. First, the fabrication of our nano-sensors and their morphological and optical features will be described. Second, we will present our latest results obtained using these nanostructured plasmonic substrate. Sensing recorded on a single nanotip will be presented. Particularly, we will show measurements performed on a single nanotip bringing few metal nanoparticles (from 1 to 10). Moreover, enhanced spectroscopies (SERS or MEF) will be presented.

Biocompatible polymer-coated gold nanorods/ICG for simultaneous active targeting, NIR molecular imaging, and phototherapy

Y. Chen, The Johns Hopkins Univ. (United States); K. L. Davis, North Carolina State Univ. (United States); J. Li, X. Li, The Johns Hopkins Univ. (United States)

Recently there has been considerable interest in combining nanomaterials with different properties, such as fluorescence, magnetization, and near-infrared (NIR) absorption, into one nano-dimensional composite that may lead to the development of multifunctional nanomedical platforms for simultaneous targeting, imaging and therapy. This paper reports a promising and convenient approach to develop a multifunctional nanocomposite made of polymer-coated gold nanorods loaded with an NIR dye and conjugated with an antibody for targeted NIR molecular imaging and phototherapy. Gold nanorods were used as transducers for photothermal therapy due to strong absorption in the NIR region. Indocyanine green (ICG), the only NIR fluorescent dye approved by the FDA for routine clinical use, was chosen for molecular imaging. The nanorod surface was coated with assembly of poly(styrenesulfonate)/poly(allylamine hydrochloride) polyelectrolytes. ICG encapsulation was achieved by the reversible reorganization of polyions in water and water/ethanol mixture leading to changes in permeability. Finally, ICG-loaded gold nanorods were conjugated with anti-EGFR monoclonal antibodies. The nanocomposite was incubated with A431 cancer cells which overexpress EGFR. Microscopy with an ICG filter revealed a strong NIR fluorescence signal from the cancer cells, suggesting successful active molecular targeting by the biocomjugated nanocomposites. Cells with nanocomposites exposed to an 810 nm laser at a power density of 1.7 W/cm^2 for 3 min, exhibited a higher loss of viability compared to similarly irradiated cells without the nanocomposites and non-irradiated cells with the nanocomposites. Preliminary results show efficient and specific targeting, NIR molecular imaging and selective photothermal therapy could be realized simultaneously using the multifunctional nanocomposites.

Label free surface biosensors, exploring novel electrical, mechanical and optical properties at nanoscale dimensions, have recently emerged as promising diagnostic tools. However, the performances of surface sensors operating in fluidic environments are strongly limited by the efficiency of the analyte delivery to the sensing surface instead of the sensors’ intrinsic detection capabilities. Relying only on passive diffusion can severely limits the delivery of the analytes to the sensing surface. At low concentrations, this limitation, commonly known as mass transport problem, causes impractically long detection times extending from days to months.

To overcome this limitation, we proposed and demonstrated a biosensing system merging nanophotonics and nanofluidics in a single platform. So far, one of the main conceptual constraint was that biosensing and microfluidics are always considered as different parts of a biosensing platform completing each other rather than a fully merged single entity. In our system, we merge these two components by employing suspended plasmonic nanohole arrays, resonantly transmitting light as well as actively transporting fluids through them. We developed a multi-inlets/outlets microfluidic scheme to actively...
control the fluidic flow in three-dimensions. Unlike previous approaches where the analytes simply stream pass over the surface, our platform enables perpendicular steering of the analytes to the sensing surface and enables 14-fold improvement in mass transport rate constant appearing in the exponentials. We also introduce a lift-off free plasmonic device fabrication technique based on positive resist electron beam lithography allowing yield/reproducibility and minimal surface roughness while eliminating the need for focused ion beam lithography.

7911-31, Session 6

**Plasmonic and two-photon luminescence of star-like gold nanoparticles used in cervical cancer detection**

S. Ruiz, T. Lopez-Luke, Ctr. de Investigaciones en Óptica, A.C. (Mexico); A. L. Gonzalez, Univ. de Guanajuato (Mexico); R. Curbet, Ctr. de Investigaciones en Óptica, A.C. (Mexico); P. Salas, Univ. Nacional Autónoma de México (Mexico); E. De La Rosa, Ctr. de Investigaciones en Óptica, A.C. (Mexico)

The conventional method to identify malignancy cells obtained as biopsies of uterine cervix is based on the use of different markers such as DAPI and P16INK4. Recently gold nanoparticles has been demonstrated that reflects more light than these fluorescence markers. Another advantage of gold nanoparticles is that the absorption can be tuned changing the size and/or the shape of nanoparticle. The energy absorbed by the nanoparticles from the pumping source, allow destroy the cells by photothermal effect. Therefore gold nanoparticles are very attractive for labelling cells. In this work, the synthesis of stars-like gold nanoparticles with shape and size controlled is reported. Nanoparticles were prepared at room temperature with citric acid with a size ranging from 160 nm to 180 nm, controlled with short time reactions and using seeds nanoparticles. It is presented the method to functionalize the gold nanoparticles to label Ki-67 cervical protein of exfoliated cervical cancer cells. Ki-67 is a nuclear antigen associated to cells proliferation. Different methods to study the capacity of diffusion of nanoparticles were evaluated. Experimental results show a high fluorescence of gold nanoparticles imaging the cervical cells and the destruction by photothermal effect. Results suggest that fluorescence was produced by two-photon absorption after pumping at 720 nm from a femtosecond laser.

7911-32, Session 7

**Surface-wave-enabled dark field aperture: a method for suppressing background during weak signal detection**

G. Zheng, X. Cui, C. Yang, California Institute of Technology (United States)

Sensitive optical signal detection in biomedical and bioscience can often be confounded by the presence of a significant background, and, as such, pre-detection background suppression is substantively important for weak signal detection. In this paper, we present a novel optical structure design, termed surface-wave-enabled darkfield aperture (SWEDA), which can be directly incorporated onto optical sensors to accomplish pre-detection background suppression. This SWEDA structure consists of a central hole and a set of appropriately designed groove pattern that channels incident light to the central hole via surface plasmon wave and surface scattered wave coupling. We show that the surface wave component from the groove pattern can mutually cancel the direct transmission component of the central hole, resulting in near-zero net transmission under uniform illumination. Here, we report the implementation of two SWEDA structures. The first structure, circular-groove based SWEDA, is able to provide polarization independent suppression of uniform illumination with a suppression factor of 1230. The second structure, linear-groove based SWEDA, is able to provide a suppression factor of 5080 for TM wave and can serve as a highly compact (length = 5.5 micron) polarization sensor (the measured TE/TM transmission ratio is 6100). Since the exact destructive interference balance is highly delicate and can be easily disrupted by the non-uniformity of the localized light field, the SWEDA can therefore be used to suppress a bright background and allow for sensitive darkfield sensing and imaging (observed image contrast enhancement of 27dB for the first SWEDA). A detection system that can effectively suppress background contributions (prior to detection) and allow detection of small signals in extremely compact device architectures is potentially useful for a broad range of applications from on-chip bio-sensing to metrology and microscopy.

7911-33, Session 7

**Triply surface-plasmon resonant four-wave mixing imaging of gold nanoparticles**

F. Masia, W. Langbein, P. Watson, P. Borri, Cardiff Univ. (United Kingdom)

Multiphoton microscopy is a powerful tool for imaging subcellular structures with intrinsic three-dimensional spatial resolution. Common labels in multiphoton microscopy include organic fluorophores, which suffer from photobleaching, and semiconductor nanocrystals, which are more photosable but contain cytotoxic elements (such as Cd or In). Gold nanoparticles (GNPs) are ideal optical labels in terms of photo-stability and bio-compatibility, but show weak fluorescence. We have developed a novel multiphoton microscopy technique not relying on (and hence not limited by) fluorescence emission, which exploits the third-order non-linearity called four-wave mixing (FWM) of GNPs in resonance with their surface Plasmon. The coherent, transient and resonant nature of this signal allows its detection free from background limiting other contrast methods with GNPs. We show high-contrast imaging of gold-labeled down to 5nm size in Golgi structures of HepG2 cells with a sub-diffraction lateral (axial) resolution of 140nm (470 nm) at useful imaging speeds (10kHz pixel rate). Since GNPs are well established markers for electron microscopy, our technique offers the prospect of correlative optical and electron imaging of the same gold-labelled structures. We have also demonstrated imaging of single GNPs in the size range of 10nm to 100nm. Furthermore, by detecting the transient nonlinearity of single GNPs using 100fs pulses with adjustable delay we have gained fundamental insights into the physical processes creating FWM.

7911-34, Session 7

**Advances in surface plasmon resonance imaging for parallelized detection of biomarkers**

M. Piliarik, M. Bockova, J. Homola, Institute of Photonics and Electronics of the ASCR, v.v.i. (Czech Republic)

Future medical diagnostic technologies are expected to detect a panel of biomarkers rather than individual biomarkers. Therefore high-throughput biosensors capable of monitoring a large number of different binding events in parallel present a potentially very attractive tool for biomarkers screening and point-of-care diagnostics.

We report a new surface plasmon resonance (SPR) biosensor enabling sensitive and parallelized detection of disease-related biomarkers in blood plasma. The presented approach is based on the advanced SPR imaging sensor platform with polarization contrast and internal referencing. This platform makes it possible to perform measurements simultaneously in more than 100 sensing channels and provides a refractive index resolution as good as 3 x 10−7 RIU. This resolution is superior to other high-throughput SPR systems and comparable with the best available low-throughput SPR systems. Immobilization of antibodies recognizing selected biomarkers on different areas of the sensor surface is accomplished by first microspotting selected single-stranded DNAs in different areas of the sensor surface and then immobilising antibodies.
conjugated with complementary DNA sequences via DNA hybridization. The developed biosensor system is applied to detection of cancer-related protein biomarkers - human chorionic gonadotropin (hCG) and activated leukocyte cell adhesion molecule (ALCAM). Detection is carried out both in buffer and in 10 % blood plasma. It is demonstrated that the biosensor is capable of detecting ALCAM and hCG in blood plasma samples at ng/ml-levels.

7911-35, Session 7

Silver plasmon rulers as probes in polarization-resolved plasmon coupling microscopy

B. M. Reinhard, Boston Univ. (United States)

Individual pairs of polymer-tethered silver nanoparticles, so called silver plasmon rulers, enable distance and orientation measurements on the nanoscale. The reduced linear dichroism and the spectrum of the light scattered from individual plasmon rulers encode information about their orientation and average interparticle separation, respectively. We took advantage of the gain in information silver plasmon rulers offer as probes in optical tracking and analyzed the translational and rotational motions as well as the extension of individual silver plasmon rulers diffusing on the plasma membrane of lysed HeLa cells. Consistent with a compartmentalization of the cell surface on the length scales of the plasmon rulers, most rulers were either immobilized or performed a confined lateral diffusion. Structural details of a plasmon ruler’s confinement region became accessible utilizing the orientation and interparticle separation dependent optical response of the plasmon rulers. This approach, which we refer to as polarization-resolved plasmon coupling microscopy, enabled a detailed structural characterization of individual membrane compartments and provided a quantitative metrics to characterize the structural lateral heterogeneity of cell membranes on submicrometer length scales. In combination with adequate tracking methods, the “dance” performed by membrane confined dimers of flexibly linked noble metal nanoparticles revealed deep insight into the underlying membrane morphology.

7911-36, Session 7

Hyperspectral imaging of surface plasmon resonance effects induced by uncoullimated semiconductor radiation

D. Lepage, J. J. Dubowski, Univ. de Sherbrooke (Canada)

We have recently proposed an innovative SPR microstructure comprising a metal coated dielectric layer deposited on top of a photoluminescence (PL) emitting quantum well (QW) wafer [1-2]. The entire device, thanks to the built-in light source and the application of a SPR imaging technique, has the potential to become a highly compact SPR biosensor for simultaneous detection of numerous biomolecules.

The device takes advantage of uncoullimated and incoherent emission from the QW microstructures, which result in a spectro-angular far-field SPR response. Our results indicated that the injected in-plane wavevectors could increase the SPs coupling efficiency up to 100 times in comparison to indirect SPRs injection [2]. To adequately monitor the emitted spectro-angular far-field, we have presented the general idea of an experimental setup required for the collection the 3D measurements of SPR dispersion relations \( \omega(k_{x,y}) \), enabling a much richer picture of surficial biochemical events. Preliminary results indicated that the proposed methodology would produce simultaneously the equivalent of 10^5 to 10^8 conventional SPR scans achievable with commercial systems [2-3].

In this communication, we present what we believe to be a novel surficial imaging technique, based on the hyperspectral photoluminescence mapping of SPR events (HI-PLM-SPR). A comparison between experimental and analytical dispersion relation maps \( \omega(k_{x,y}) \) are presented, along with initial trials with biochemical agents.

References:

7911-55, Session 7

Development and modeling of surface plasmon resonance imaging (SPRI) biosensor chips based on gold nano- and microstructures

A. Dhawan, Duke Univ. (United States); A. Duval, M. Nakkach, Institut d’Optique Graduate School (France); G. Barbillon, Univ. de Technologie Troyes (France); J. Moreau, Lab. Charles Fabry (France); H. Wang, Duke Univ. (United States); M. T. Canva, Lab. Charles Fabry (France); T. Vo-Dinh, Duke Univ. (United States)

No abstract available

7911-37, Session 8

Novel monolayer and bilayer shell aggregate gold nanostructures

M. Angelidou, C. Pitrîs, Univ. of Cyprus (Cyprus)

The unique optical properties of noble metal, such as gold and silver, nanostructures have led to an increased interest into their potential uses for various biological applications. For intracellular imaging and/or spectroscopy, it would be beneficial to use (i) near infrared (NIR) excitation, for better penetration, and (ii) small gold nanoparticles, for easier distribution into the cytoplasm and cell nucleus and less cytotoxicity. Unfortunately, none of the existing nanostructure shapes can fulfill both requirements simultaneously. This work proposes a novel nanostructure, the “shell aggregate,” which consists of small gold nanospheres aggregated around a core such as an intracellular organelle or vesicle. The extinction efficiency of such monolayer and bilayer shell aggregates is thoroughly investigated with appropriate simulations. Of particular interest is the effect on the extinction efficiency factor of the overall radius of the shell aggregate and the size of and distance between the small nanospheres. While the magnitude of the extinction efficiency is analogous to the overall radius, the shape of extinction spectrum appears to depend heavily on the distance between the small nanospheres. As the distance between the nanospheres decreases, the interaction of their surface electromagnetic fields results in a red-shift of the dipole plasmon resonance and the appearance of the quadrupole interactions and a long NIR tail. The monolayer shell aggregate could be a good candidate for use in various biological, intracellular, applications since it provides a reasonably tunable plasmon resonance wavelength while the small size of its components can be exploited for intracellular distribution.

7911-38, Session 8

A combined T-matrix and polar decomposition study on polarization characteristics of metal nanoparticles in the surface plasmon band

N. Ghosh, A. Banerjee, N. Kaushal, Indian Institute of Science Education and Research (India)

Studies on polarization properties of scattered light from metal nanoparticles possessing surface plasmon resonances is of considerable
current interest both from the point of fundamental understanding and potential applications. Recent experimental studies conducted in this direction have revealed enhanced depolarization of light scattered from such colloidal metal nanoparticles solutions as compared to that scattered from dielectric particles. We have thus investigated the reason for such intriguing depolarization behavior. The polarization characteristics of scattered light from non-spherical metal nanoparticles in their surface plasmon resonance spectral region (we have chosen the optical properties of silver nanoparticles and their corresponding plasmon resonance spectral region, 380 - 600 nm) were investigated using the T-matrix approach for light scattering in combination with a polar matrix decomposition method. The phase matrices (or scattering matrices) for both randomly and preferentially oriented non-spherical (axially symmetric particle shapes, prolate and oblate spheroids) metal nanoparticles having varying sizes, were generated using the T-matrix approach. These were further analyzed through the polar decomposition approach to yield individual polarimetry characteristics of the nanoparticles, namely, retardance, diattenuation and depolarization. The results of the decomposition analysis on the phase matrices from randomly oriented spheroidal metal nanoparticles indeed showed enhanced depolarization (quantified from the decomposition-derived depolarization matrix) in the surface plasmon spectral band as compared to dielectric particles having identical size and shape. On the other hand, the decomposition analysis on the scattering matrices from preferentially oriented nanoparticles revealed the presence of additional linear retardance effects (phase difference between orthogonal linear polarizations, quantified from the decomposition-derived retardance matrix) in the surface plasmon spectral band of the spheroidal metal nanoparticles. This additional phase retardation is identified as being due to interference between two plasmon resonances having a difference in phase between them. When averaged over all possible orientation of the particles (as should be the case for experimental studies on colloidal metal nanoparticles), addition of the retardance matrices having random orientation of retarder axes lead to the observed stronger depolarization effects. The details of these theoretical results will be presented and their implications for polarimetric biomedical imaging and bio-sensing will be discussed.

7911-39, Session 8

Optical properties of silver bowtie nanoantenna arrays fabricated using plasma-assisted nanosphere lithography

S. Wang, Y. Chang, S. Chang, National Cheng Kung Univ. (Taiwan)

Optical nanoantennas have been an important research topic in Nanophotonics in recent years. Problems those were very hard to investigate before, such as fluorescence from single molecule, can now be studied with the help from these nanoantennas. Currently, bowtie nanoantenna, one of the popular nanoantenna designs, has been fabricated using Electron-beam Lithography (EBL) and Focused-Ion-Beam (FIB). However, high fabrication cost has limited these methods for only prototype fabrication. In this study, fabrications of bowtie nanoantenna arrays were demonstrated by plasma-assisted Nanosphere Lithography (NSL). Single layer of close-packed polystyrene nanoparticles were fabricated using convective self-assembly NSL. The diameters of the nanospheres were reduced by oxygen plasma etching. After the subsequent silver deposition and nanosphere lift-off processes, silver nanotriangles arrays were obtained. The side length of these nanotriangles increases with the increasing plasma treatment time, while the inter-particle distance decreases with increasing time. The average gap can be as small as 10 nm by control of the nanosphere sizes and the oxygen plasma treatment time. Each two nanotriangles form one pair of bowtie antenna with sub-100 nm gap. Plasmonic coupling of the bowtie nanoantenna arrays are very strong and were confirmed theoretically and experimentally through 3D finite-difference-time-domain method and optical absorption measurements, respectively. This fabrication technique is not only cost effectively but also very fast, which should be very attractive for future developments of new nanoantenna applications.

7911-40, Session 8

Experimental analysis of optical resonance transmission properties of subwavelength hole arrays in optically thick metal films

M. Najimimaini, F. Vaseli, B. Kaminska, Simon Fraser Univ. (Canada); J. J. L. Carson, Lawson Health Research Institute (Canada)

The optical resonance transmission properties (wavelength, peak, and spectral bandwidth of transmission resonance) of arrays of sub-wavelength holes (nano-holes) in a metal film are governed by surface plasmon (SP) effects and therefore depend highly on the dielectric constants of the metal and surrounding materials. To further characterize this effect, we studied the optical resonance transmission properties of nano-hole arrays fabricated in metallic films of different composition. Large nano-hole arrays for a range of hole periodicities (spacing between adjacent holes) in a square lattice arrangement were fabricated in the various optically thick metal films (Au, Ag, and Al) on a Pyrex substrate using Electron Beam Lithography (EBL). The optical transmission spectra of each array was measured in the visible and near infrared region (450 nm - 850 nm) using a setup consisting of an inverted optical microscope, a photometer, a monochromator, and a photomultiplier tube.

The optical resonance transmission peaks related to the excitation of (1,0) and (1,1) SP modes from the Pyrex side were clearly observed in the optical transmission spectra of all nano-hole arrays. Analysis of the optical resonance peaks revealed that the center wavelength, efficiency, and bandwidth depended on the nano-hole array periodicity and the composition of the metal film. Also, we observed that the transmittance of optical resonance peaks were larger for the nano-hole arrays in Ag and Au films in near infrared regime compared to the arrays in Al film.

7911-41, Session 8

Effect of adhesion layer on optical resonance transmission properties of nanohole arrays in an optically thick gold film

M. Najimimaini, F. Vaseli, B. Kaminska, Simon Fraser Univ. (Canada); J. J. L. Carson, Lawson Health Research Institute (Canada)

Various methods for fabricating an array of sub-wavelength holes (nano-hole array) requires a thin metal adhesion layer between the gold film and the Pyrex substrate. Titanium and chromium have been commonly used to form the adhesion layer. The adhesion layer is of importance during nano-hole array fabrication by Electron Beam Lithography, since this method is considered an aggressive fabrication processes and may cause the gold film on the Pyrex substrate to peel off resulting in corrupted nano-structures. To avoid this effect a sufficiently thick adhesion layer is deposited between the gold film and the Pyrex substrate. However, the selection of the thickness and material of the adhesion layer must be optimized to ensure the transmission efficiency of the nano-hole array is maximized.

In this paper, we present experimental analysis on the effect of adhesion layers (chromium and titanium) between gold and the Pyrex substrate on the optical resonance transmission properties (wavelength, peak, and spectral bandwidth of transmission resonances) of nano-hole arrays in an optically thick gold film. Nano-hole arrays with different hole periodicities in a square lattice arrangement were fabricated in a gold film on the Pyrex substrate, but with various adhesion layers. The optical transmission spectra of sub-wavelength hole arrays were optically characterized and the optical resonance transmission properties of sub-wavelength hole arrays with the same geometrical parameters, but with various adhesion layers were analyzed and compared. The results revealed that the optical resonance transmission efficiency related to excitation of Surface Plasmon from Pyrex side had poorer transmission with titanium in the adhesion layer compared to chromium.
7911-56, Session 8

**Wafer-scale plasmonic substrates for SERS sensing of chemical and biological molecules**

A. Dhawan, H. Wang, T. Vo-Dinh, Duke Univ. (United States)

No abstract available