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TECHNICAL CONFERENCE ABSTRACT SUMMARIES

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Conference 9536A: Advanced Microscopy Techniques IV

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Part of Proceedings of SPIE Vol. 9536 Advanced Microscopy Techniques IV; and Neurophotonics II

9536-47, Session PD

High resolution 3D volumetric imaging of live tumor spheroids using Selective Plane Illumination Microscopy (SPIM)

Stylianos Psycharakis, Evangelos Liapis, Ilias Kyparissidis-Kokkinidis, Athanasios Zacharopoulos, Joseph Papamatheakis, Foundation for Research and Technology-Hellas (Greece); Jorge Ripoll, Universidad Carlos III de Madrid (Spain); Giannis Zacharakis, Foundation for Research and Technology-Hellas (Greece)

Tumor spheroids are in vitro cancer models of increasing interest for cancer evolution diagnostics and pharmaceutical intervention studies, as they more closely resemble real tumours compared to conventional monolayer cultures. However, optical imaging of tumour spheroids is technically challenging, since these are large and highly scattering specimens. On the other hand, Selective Plane Illumination Microscopy (SPIM) is a novel optical microscopy technique that was introduced to life sciences in 2004. Since then SPIM has turned out to be a powerful tool especially in the field of in vivo imaging. SPIM combines optical sectioning, the main characteristic of confocal and two photon microscopes, with multi-angle and multispectral imaging which are performed in optical and fluorescence tomography. Moreover, SPIM has the potential to overcome several of the challenges that prevent high resolution imaging of live tumor spheroids, since it combines deep penetration of light into the specimen, minimal phototoxicity and high image acquisition speed. In this study, we employed SPIM to investigate the response of MDA-MB-231 breast cancer spheroids to the chemotherapeutic agent doxorubicin.

9536-48, Session PD

Parallel and flexible imaging using multiphoton RESOLFT microscopy with spatial light modulator (SLM) control

Yi Xue, Christopher James Rowlands, Peter T So, Massachusetts Institute of Technology (United States)

High resolution imaging in three dimension is important for biological research. RESOLFT (Reversible Saturable Optical Fluorescence Transitions) microscopy is one technique can achieve lateral super-resolution imaging. Two-photon microscopy naturally generate high resolution in the longitudinal direction with less background compared to single photon excitation. In this paper, we combine these two methods to realize three-dimensional high-resolution imaging. Spatial light modulator (SLM) is used as a flexible phase mask of the microscopy. Multiple super-resolution focuses as an array or in arbitrary positions could be generated by phase retrieval. This microscopy by SLM control could applied to parallel two-photon RESOLFT imaging or multiple spots tracking in high-resolution.

9536-5, Session PWed

A three-dimensional particle tracking strategy using temporal focusing multiphoton microscopy and astigmatism imaging

Fan-Ching Chien, National Central Univ. (Taiwan)

A three dimensional single particle tracking strategy was reported by temporal focusing multiphoton excitation microscopy (TFMPEM) combined with astigmatism method to provide axial information for revealing three dimensional trajectories of each single particle in that excited optical sectioning volume without z-scanning. The temporal focusing multiphoton excitation produces a widefield illumination with minimum optical trapping force on the fluorospheres, whereas other conventional spatial focusing multiphoton excitation schemes induce optical trapping interference. It was demonstrated the dynamical ability by measuring the diffusion coefficient of fluorospheres in glycerol solutions with a position standard deviation of 14 nm and 21 nm in the lateral and axial direction and a frame rate of 100 Hz. The TFMPEM with astigmatism imaging has a great promise to explore dynamical molecular behavior of complex biological system, because of a fast frame rate, lower photobleaching, and efficient background rejection at deeper imaging.

9536-24, Session PWed

Non-linear imaging techniques visualize the lipid profile of C. elegans

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The non-linear techniques Second and Third Harmonic Generation (SHG, THG) have been employed simultaneously to record three dimensional (3D) imaging and localize the lipid content of the muscular areas (ectopic fat) of Caenorhabditis Elegans (C. elegans). Simultaneously, Two-Photon Fluorescence (TPEF) was used initially to localize the stained lipids with Nile Red, but also to confirm the THG potential to image lipids successfully. In addition, GFP labelling of the somatic muscles, proves the initial suggestion of the existence of ectopic fat on the muscles and provides complementary information to the SHG imaging of the pharynx. The ectopic fat may be related to a complex of pathological conditions including type-2 diabetes, hypertension and cardiovascular diseases. The elucidation of the molecular path leading to the development of metabolic syndrome is a vital issue with high biological significance and necessitates accurate methods competent of monitoring lipid storage distribution and dynamics in vivo. THG microscopy was employed as a quantitative tool to monitor the lipid accumulation in non-adipose tissues in the pharyngeal muscles of 12 unstained specimens while the SHG imaging revealed the anatomical structure of the muscles. The ectopic fat accumulation on the pharyngeal muscles increases in wild type (N2) C. elegans between 1 and 9 days of adulthood. This suggests a correlation of the ectopic fat accumulation with the aging. Our results can provide new evidence relating the deposition of ectopic fat with aging, but also validate SHG and THG microscopy modalities as new, non-invasive tools capable of localizing and quantifying selectively lipid accumulation and distribution.

9536-25, Session PWed

Third Harmonic Generation microscopy as a diagnostic tool for the investigation of microglia BV-2 and breast cancer cells activation

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Technology-Hellas (Greece) and Univ. of Crete (Greece); George Filippidis, Sotiris Psilodimitrakopoulos, Foundation for Research and Technology-Hellas (Greece); Haris Markomanolaki, Maria Kalognomou, Sofia Agelaki, Vasilios Georgoulias, Irene Athanassakis, Univ. of Crete (Greece)

Nonlinear imaging techniques have found growing use in fields ranging from fundamental physics to biomedicine. In THG microscopy, third harmonic light is generated at the focal point of a tightly focused ultra-short pulsed laser beam. Due to the coherent nature of the THG, no net signal is obtained when focussed inside a homogeneous, normally dispersive medium. However, when the nonlinear medium is not uniform, either in the refractive index or in the third order nonlinear susceptibility, the THG signal does not vanish, and significant THG output can be obtained. This nature of the THG process renders THG microscopy highly sensitive to in-homogeneities and the efficiency of signal generation strongly depends on the relative size of the in-homogeneity and the focal volume [1].

Several studies demonstrate the ability of THG measurements to image various biological samples. Particularly, THG microscopy yields detailed images of multicellular and single cell specimens without any prior treatment and with no observable damage [2]. THG presents high contrast among sub-cellular features, such as cell membrane, nucleus and intra cellular organelles (mostly lipid depositions), without the necessity of any staining preparation

In our study we attempted to evaluate cell activation by using THG imaging microscopy. Firstly BV-2 microglia cells were examined with or without activation by lipopolysaccharide in order to discriminate between control and activated cells based on the quantification of THG signals. Statistically quantification was accomplished in both mean area and mean intensity values of THG. The values for mean total area and mean THG intensity values have been increased in activated versus the non-activated cells.

Similar studies of quantification are underway in breast cancer cells for the exact discrimination on different cell lines.

Experimental Setup

The experimental apparatus consists of a custom made upright nonlinear microscope. The source is a femtosecond laser (t-pulse) operating at 1028 at a pulse repetition rate of 50 MHz and pulse durations 200 fs. The system consists of a pair of galvanometric mirrors that move two dimensional the laser beam to attempt xy scanning of the biological sample. Objective lens (N.A. 0.85, 32 X, Carl Zeiss, C-Achroplan) is used to focus the laser beam tightly on the sample. The back aperture of the objective lens is fulfilled by using a telescopic system which expands the spot of the laser approximately six times. The sample is fixed on a mechanical stage that is available to move in three dimensions and allows obtaining 3D images. This system provides the opportunity to collect simultaneously two different nonlinear signals that are generated in the focal volume. The signals can be collected by using two different photomultiplier tubes (PMTs), one in the transmission and another one in the reflection mode. The intensity values collected from PMTs are saved in a computer. The computer controls further the mechanical stage via a developed Labview program. Biological sample is placed between two round glass slides (70?m thick) separated by a spacer of approximately 100?m to avoid damage the sample. The setup is adjusted to scan 500x500 pixels THG or TPEF images where 30 scans are averaged for each image to increase signal to noise ratio. A CCD camera is used for observation of the biological specimens. Image J program is employed for viewing and processing the obtained data. The developed system provides the capability to combine two nonlinear image modes in a single instrument, so that complementary information is taken regarding the structure and function of biological samples [3].

Results

Third-harmonic microscopy is a general-purpose technique and mainly provides structural information. Nevertheless, it cannot give information on specific molecules or organelles. This problem could be solved, by combining third-harmonic imaging with specific fluorescence labeling. Using our suitable experimental setup, it is possible to perform third-harmonic and two-photon excitation fluorescence (TPEF) imaging with a single laser source. Using a single laser source is mostly desirable for the following reasons: firstly, the problem of chromatic aberration does not exist and therefore the third harmonic and the TPEF signals are generated exactly at the same focal depth. Secondly, the microscope system and its alignment are mostly simplified, by using only one laser beam and by employing two separate collection paths, in the forward direction for the THG and in the backward direction for the TPEF. So a single scan is sufficient to provide the combined multimodal image.

Resting and activated microglia BV2 cells were firstly stained with three different fluorescent dyes detecting lipid bodies (LBs), mitochondria or endosomes and processed simultaneously for THG and TPEF imaging measurements. The results showed that the major sub cellular source of high THG signal were the LBs, while poor co-localization was obtained with the mitochondria and late endosomes rab7 markers.

THG microscopy was further used to quantify the changes of mean total area and mean THG intensity values of LBs before and after activation of BV2 cells. The results showed that the mean total area, above a constant intensity threshold of THG signals which corresponds mainly to LBs, was ten times bigger when the cells were activated. The quantification was accomplished for N=40 unstained cells for each case, while the threshold and the irradiation conditions were constant for the comparison. Correspondingly, quantification of the intensity of THG signals in the same cells showed a 22% increase in activated as compared to non-activated BV2 microglia cells. The results presented that there is statistically significant difference both in case of mean number of pixels and mean THG values, indicating that activation not only induces the formation of a bigger amount of sub cellular structures, mostly lipid droplets, but also changes the chemical constitution of the LBs structures. THG imaging could distinguish the two different states of BV-2 cells (resting vs activated). Furthermore, quantification of signal's mean area and intensity values could potentially provide further information related to LBs chemistry, since THG microscopy provides images of unstained biological samples and the contrast arising from spatial variations in third order susceptibility and refractive index changes.

Undergoing research includes the use of THG imaging technology in breast cancer cell lines seeking to distinguish them from healthy cells. We anticipate that, the developed methodology will potentially be proved extremely useful as a novel diagnostic tool in order to understand inflammation as well as cell activation.

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9536-26, Session PWed

Multiphoton microscopic imaging for cancer analysis in a microengineered 3D cell culture platform

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Nonlinear optical imaging techniques including multiphoton and third harmonic generation (THG) microscopy has provided as a powerful tool to study biological functionality and structural analysis in live tissue even cell level and offers many advantages over conventional imaging techniques. The advantages of the multiphoton microscopy include the capability of observing unstained (label-free) biological samples and non-invasiveness due to the conservation of energy through the nonlinear process. The imaging depth in scattering samples is enhanced due to

the long excitation wavelength. Furthermore, THG can be enhanced through interfaces with inhomogeneous 1st or 3rd order susceptibilities, which makes it suitable in the study of transparent biological samples. Therefore, the application of these nonlinear optical approaches to the study of breast cancer models holds particular promise as these techniques can be used to image exogeneous fluorophores such as green fluorescent protein as well as intrinsic signals such as THG from collagen and endogenous fluorescence from nicotinamide adenine dinucleotide or flavin adenine dinucleotide. The application of multiphoton excitation and THG to a relevant cancer analysis regarding the tumor-stromal interaction, cellular metabolism, morphology, and cell signaling in various breast cancers such as MCF-10A, MCF-7, MB-231 (disease models) and MCF-12F (normal epithelial cell line) is here described as a comparable model. In addition, in advance of a conventional 3D cell culture, an innovative 3D cell culture platform has been microengineered to get higher resolution images, processing convenience, and high-throughput analysis. These integrated techniques will provide a unique analysis tool for cell and tissue engineering researchers.

9536-27, Session PWed

Nanosecond Two-photon excitation fluorescence imaging with a multi color fiber MOPA laser

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We present a robust all fiber based laser source in the extended near infrared for two photon microscopy. The laser is based on a master oscillator fiber power amplifier (MOFPA). The output of a narrowband CW 1064 nm laser diode is modulated with a 12 GHz electro-optic amplitude modulator. Sub-nanosecond pulses are generated and amplified up to kWs of peak powers in an ytterbium doped fiber amplifier (YDFA). By Raman shifting the 1064 nm light in the fiber using a second 1122 nm laser diode for seeding, the output can be switched from 1064 nm to 1122 nm and to 1186 nm. The fast shifting process is fully electronically controlled. The pulse length and modulation pattern can be chosen arbitrarily and through synchronized, time gated detection the signals can be multiplexed in time. One the one hand, this reduces noise from background light and on the other hand, it enables the straight forward implementation of multi-modal imaging modalities with a simple and reliable system. We show the characteristics of this excitation laser and present two photon excitation fluorescence (TPEF) images of plant leaves and algae acquired in epi-direction. This robust fiber laser makes it a good excitation source for future inexpensive non-linear microscopes and nonlinear imaging endoscopes and can further be used as an extension to already existing microscopes, since it provides easy synchronization to existing systems.

9536-28, Session PWed

Multimodal microscopy analysis of photonic structured layers of chitin-protein in squid internal shell

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Chitin is a linear homopolysacharide composed of N-acetylglucosamine chains. It is found in arthropod exoskeleton, molusk shells and fungal cell wall. We have performed a systematic analysis of the chitin-protein complexes and organization in the internalized and non-calcified shell in squids. Although not developing as a calcified shell, the internal shell or pen performs and important function in body structure and resistance. By using transmission electron microscopy, we identified a layered structure, which revealed a series of fracture planes. Close examination and morphometric analysis unveiled an angular variation between the repeating in-plane fracture lines associated with the basic chitin microfibril protein complexes described by others [1]. Transmission electron microscopy allowed the confirmation of repeating optical units with preferential alignment and organization, fitting well with published repeating units revealed by X-ray diffraction studies. Under femtosecond pulsed laser imaging we could detect transmitted and reflected SHG, which were mutually exclusive, creating alternating pattern resembling cholesteric crystals. On further inspection of the 3D structure, the transmitted and reflected SHG were shown to originate from individual and intercalating layers. This phenomenon was interpreted as a differential interaction of the light produced by SHG with a birefringent material with a very organized distribution pattern. We discuss the implications of the present findings to other structures exhibiting SHG such as collagen fibers.

9536-29, Session PWed

The influence of sample fixation on Collagen SHG at 1060 nm to 1300 nm excitation

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Motivation

Nowadays the gold standard for the assessment of fibrosis is the classification of the severity by an experienced pathologist after staining. In the clinical environment mainly the hematoxin and eosin staining as well as picrosirius (PS) red stain and Masons trichrome (MT) are used. PS-red and MT offer the possibility to increase the detection of collagen fibres by the usage of polarized light.

For intravital detection of pathological tissue these staining procedures are not an option. Multi-photon microscopy (MPM) has proven to be a useful tool in classic research environment. Nevertheless the usage of MPM in clinical environment is rarely seen. The first step towards a better understanding of the usefulness of MPM would be a catalogue of detectable pathologies. For this we need to clarify which fixed and stored tissue samples are still in a condition to reflect the structure of living tissue. Therefor we compared in this study differently fixed samples of human and murine tissue with and without known pathologies. To evaluate the possibility to use these as a basis for a database for in vivo measurement and detection of diseases by endoscopy or during invasive surgery, we compared structural changes of collagen in differently fixed samples at different wavelengths.

It is generally assumed that non-linear microscopy in the range form 1060 nm to 1300 nm is less damaging than shorter wavelength. Lower absorption in combination with reduced scattering gives a deeper penetration into the tissue and overall higher laser safety is increased in clinical applications, since 2-photon excitation does not reach the regime of UV and blue light tissue damage. 3-photon excitation does not reach the excited states of DNA. Additionally, clinical usage should also benefit by the availability of compact femtosecond fibre lasers in this wavelength range. As a bonus feat for all-fibre endoscopic applications this spectral range shows low dispersion and absorption in silica

Aim of this study is to compare SHG excited at wavelengths from 740 nm – 1000 nm and 1060 nm - 1300 nm by collagen in fresh, fixed and frozen tissue.

Materials and Methods

Samples were provided under the restrictions of the ethical approval committee of Schleswig-Holstein. Human tissue samples were taken during surgical interventions of clinical necessity from patients with an aortic aneurism.

Human tissue was taken from aortic root from patients with different clinical phenotypes (n= 8). Samples were analysed directly after biopsy and after freezing to -80°C.

Additional measurements were performed with murine trachea after explanted and submerging in physiological pH buffer solution and after fixation in para-formalin solution (4 % m/m in PBS). Before measurements the samples were rinsed 3 times in PBS for 10 min.

The microscope used was a TriM Scope 2 (LaVision Biotec, Bielefeld, Germany). The scan frequency was 600 lines per second. The laser power used at wavelengths 1060 nm to 1300 nm was below 100 mW. At 740 nm to 800 nm the laser-power was below 30 mW. For the separation of the fluorescence in four spectral channels we used dichroic mirrors with transmission/reflection edges at 435 nm, 495 nm and 560 nm. The detection was performed with H7422 GaAsP-Photo-Multiplier-Tubes (Hamamatsu Photonics, Hamamatsu, Japan). Excitation from 700 nm to 1000 nm was done with a tuneable Ti:sapphire laser (MaiTai, Spectra Physics, Santa Clara, CA , USA). Above 1060 nm excitation light was supplied by an Insight DeepSee laser (Spectra Physics). Zeiss Multi-Immersion 20x NA 0.8 dipping objective was used for imaging.

Dichroic transmission properties, excitation power and detector efficiencies were taken into account to calculate the effective SHG intensity. Background intensity was subtracted from measurement. Data was analysed using ImageJ (National Institutes of Health, USA) and Matlab (MathWorks, Natick, MA, USA) with Bioformats (University of Dundee & Open Microscopy Environment, UK) plugin. Fibre detection was done using customized Matlab software.

Collagen fibre angle were calculated from SH generation excited by two orthogonally polarized laser beams, derived from the CLOUD scanner of the microscope. Laser power was measured and adjusted with a linear polarizer and a laser power-meter beforehand. Collagen fibres were imaged at a depth between 0 and 100 μm below epithelium in case of trachea and between 0 and 200 μm below endothelium in case of aortic tissue.

Results

We found a peak in SH generation efficiency at about 1100 nm. Since collagen shows fluorescence emission at about 530 nm the peak in efficiency might be resonant to this electronic transition. The width of this peak still needs further exploration.

Short-term fixation in para-formalin influenced neither collagen structure nor autofluorescence.

Autofluorescence of cells was increased after 24-hour fixation in para-formalin. On the other hand collagen structure and elastin autofluorescence still remained largely unchanged.

Freezing of the aortic tissue destroyed did as expected destroy the major part of cells. Collagen SHG was greatly reduced with 800 nm excitation, while longer wavelengths still showed SHG. In the overlay images typical undulating collagen fibres are much more clearly distinguishable from autoflourescence signal at 1100 nm excitation The mechanisms of this phenomenon, except lower autofluorescence excitation at NIR2 wavelengths, remain to be investigated.

9536-30, Session PWed

How an oncogene alters the morphology of an immature hematopoietic cell: a diffraction phase microscopy study

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The principle of diffraction phase microscopy (DPM) relies on both off-axis and common path principles in combination with fast acquisition rate and high temporal sensitivity. This non intrusive optical method is particularly suited for a real time study of the structure and dynamics of living cells. To retrieve the optical phase from transparent samples we propose to generalize Fourier filtering methods thanks to a two-dimensional (2D) space-scale analysis with the Morlet wavelet transform. Thanks to the Wavelet Transform Modulus Maxima (WTMM) method we compute directly the local optical phase gradients, escaping fastidious phase unwrapping computations. We demonstrate the performance of this method on the characterization of living hematopoietic stem cells by the reconstruction of their tomography. The spherical shape of these cells allows us to define a multi-shell model and to partition different compartments of the cell with different refractive indices. Comparing healthy and cancer cells (we use a model of immature cells of the chronic myelogenous leukemia -CML) we show that the transduction of the oncogene of the CML into these immature cells produces a modification of their internal biomaterial concentration, that could be explained by a variation of their internal osmotic pressure.

9536-31, Session PWed

In vivo characterization of early stage ionizing radiation-induced skin injuries in a mouse model using two-photon microscopy

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Detecting the range of ionizing radiation damage is difficult due to its recurring and unpredictable characteristics. We characterized early-stage changes of the skin by localized ionizing radiation in a mouse model. Two-photon microscopy (TPM) was used to investigate the skin damage at cellular levels. High doses of x-ray beam was locally irradiated in the right thighs of mice, and TPM visualized cellular changes in the epidermis and hair follicle, and blood vessel leakage in the dermis based intrinsic and extrinsic fluorescence by longitudinal imaging from day 1 to day 7. TPM showed decrease in cell density and increase in cell size and increased spacing among neighboring cells in the epidermis, destruction of cell clusters in the hair follicles, and leakage in the blood vessel. The cellular level changes between control and all irradiated group were clearly detected. This research may serve as a guide for diagnosis and treatment of radiation skin damage.

9536-32, Session PWed

An automated tool for 3D tracking of single molecules in living cells

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Since the behaviour of proteins and biological molecules is tightly related to the cell's environment, more and more microscopy techniques are moving from in vitro to in living cells experiments. Looking at both diffusion and active transportation processes inside a cell requires three-dimensional localization over a few microns range, high SNR images and high temporal resolution (ms order of magnitude). We developed an apparatus that combines different microscopy techniques to satisfy all the technical requirements for 3D tracking of single fluorescent molecules inside living cells with nanometer accuracy. To account for the optical sectioning of thick samples we built up a HILO (Highly Inclined and Laminated Optical sheet) microscopy system through which we can excite the sample in a widefield (WF) configuration by a thin sheet of light that can follow the molecule up and down along the z axis spanning the entire thickness of the cell with a SNR much higher than traditional WF microscopy. Since protein dynamics inside a cell involve all three dimensions, we included a method to measure the x, y, and z coordinates with nanometer accuracy, exploiting the properties of the point-spread-function of out-of-focus quantum dots bound to the protein of interest. Finally, a feedback system stabilizes the microscope from thermal drifts, assuring accurate localization during the entire duration of the experiment.

9536-49, Session PWed

Visual evoked potential and y band information flow of stereoscopic vision in brain networks

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This paper explores how the depth in stereoscopic vision influences the human brain. A stereo visual evoked potential (S-VEP) experimental system centered on an electroencephalograph and a 3D-TV was established. 4 images with different disparities were randomly displayed on 3D-TV. VEP waves and Granger causality were used to reflect the influence of disparity on human brains. 10 volunteers participated in this experiment. Results demonstrate that P270 component could be used to measure the disparity influence on human beings. Compared with other EEG frequency bands, ? band better reflects the brain change with the variation of the disparity. Combined with subjective feedbacks, it is concluded that people are more sensitive to the negative disparity. The result of Granger causality also provides relationships between acquisition channels under different disparity conditions, so that different disparities functioning on human beings could be exhibited and contrasted more clearly. The paper hopes to find the rule that how disparity influences human brains in virtue of these results.

9536-50, Session PWed

Atomic force microscopy enhanced with fluorescent lifetime imaging

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While confocal fluorescence lifetime imaging (FLIM) provides a significant amount of chemical information on the sample, its spatial resolution is diffraction limited. Therefore, it would be natural to combine FLIM with another high-resolution microscopy technique. One of the relatively new and established techniques for topographical imaging of both biological and non-organic samples at nanoscale resolution is atomic force microscopy (AFM). An advantage of AFM compared to other high-resolutions imaging techniques is that it can provide label-free topographical, electrical, magnetic and mechanical information. The correlation with fluorescence lifetime map provides an important tool for deeper investigation of a sample properties. The AFM imaging was performed with NTEGRA SPECTRA developed by NT-MDT. The AFM head has a maximum size of the view field of 120μm x 120μm with Z scan range of 10μm. All the images were collected using a tapping mode AFM silicon cantilever coated with TiN and having length of 125μm. The probe had a relatively low force constant 5.1N/m, which is preferable for study of biological objects, and resonant frequency 160 kHz. Fluorescence lifetime measurements have been done with Simple Tau 150 system, consisting of a single photon counting card SPC-150, detector control card DCC-100, and hybrid PMT (HPM-100-40). The excitation light from a picosecond diode laser (BDL-488-SMC) at 488nm wavelength, 50ps pulse width, and 50MHz repetition rate was focused on a diffraction-limited spot through the Mitutoya objective, NA=0.7, M=100. The fluorescence emission from a samples of E-coli bacteria, HEK cells and polymer blend was collected by the same objective.

9536-51, Session PWed

New frontiers in polarized light microscopy for live cell imaging

Rudolf Oldenbourg, Marine Biological Laboratory (United States)

We created the LC-PolScope, a modern polarized light microscope, by enhancing the traditional microscope with liquid crystal devices, electronic imaging and digital image processing, to reveal and measure the alignment of molecules over the whole field of view [1,2]. We expanded the LC-PolScope technique to include the measurement of polarized fluorescence of GFP and other fluorescent molecules, and applied it to record the remarkable choreography of septin proteins during cell division, displayed in yeast to mammalian cells [3]. We created the open-access platform OpenPolScope.org for the collection and dissemination of knowledge about the LC-PolScope technology, its applications and its further development. Most recently, we developed the MultiFocus PolScope by combining OpenPolscope technology and widefield multifocus microscopy for instantaneous 3D imaging capability [4].

Polarization analysis in microscopy combines the exquisite morphological detail available in modern microscope images with the submicroscopic resolution available with polarization analysis that reveals the alignment of molecular bonds, of fine structural form, and of fluorescent dipoles. Fluorescence polarization further combines the molecular specificity of fluorescent labeling with the structural specificity afforded by polarization analysis. In fact, most if not all contrast methods in light microscopy, when coupled with polarization analysis, can reveal new, vital information about the architectural dynamics in living cells and tissues.

9536-52, Session PWed

Comparison of 3D printed and regular lens performances on laser scanning confocal microscope

Firat Sansal, Bogaziçi Üniv (Turkey); Yigit Daghan Gokdel, Istanbul Bilgi University (Turkey)

This study aims to compare the performance of 3D printed lens and regular optic lens in laser scanning confocal microscope (LSCM). LSCM setup has been established in our lab and compared lenses are used in specified portion of LSCM. The data has been taken at the same rate and from exactly same point of target in order to obtain accurate comparisons. Thorlabs-LM9LP 658 nm pigtail laser diode which is driven by thorlabs-ITC4001 have been utilized. 20x 0.4 NA Olympus objective lens is placed after relay lens system in which 50 mm and 150 mm lenses are used. USAF 1951 resolution target which is comply with MIL-STD-150A standards used as target. 3D printed and regular lens with 100 mm focus length is placed before the pinhole which have 150 um aperture. Motorized mirror setup is used as scanner. A DC motor is attached to a rotating plate where the mirror was standing over the plate. 3D printed lens is fabricated by Istanbul Technical University. The results are showed that 3D printed lens focusing performance is very similar when compared with the regular lenses. However the intensity of light reduces. This reduction either could be due to the surface roughness or fabrication processes. Furthermore this results are quite enough to establish low cost disposable confocal microscopy techniques.

9536-1, Session 1

Shot noise limited detection in stimulated Raman scattering microscopy *(Invited Paper)*

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We demonstrate our shot noise limited set-up for Stimulated Raman Scattering (SRS) microscopy. In SRS, two different colored laser beams are incident on a sample. If the energy difference between them matches a molecular vibration of a molecule, energy is transferred from one beam to the other. By applying amplitude modulation to one of the beams, the modulation transfer to the other beam can be measured. The efficiency of this process is a direct measure for the number of molecules of interest in the focal volume. Combined with laser scanning microscopy, this technique allows for fast and sensitive imaging with sub-micrometer

resolution. Recent technological advances have shown an improvement of the sensitivity of SRS applications, but few show shot noise limited detection. We present a basic SRS set up with mainly commercial components and a custom built transimpedance amplifier that reaches shot noise limited detection from 0.45 mW to 60 mW of total power on the sample, which corresponds to biologically acceptable intensities.

Using SRS microscopy, we show the lipid distribution in zebrafish exposed to continuous light conditions. These animals have a high prevalence of adipocytes compared to control animals held in a normal day-night rhythm. The adipocyte prevalence is even higher than in animals fed on a high fat diet. The total lipid content of these fish is not significantly altered compared to the control, indicating a relocation of lipids, rather than an increased uptake.

9536-2, Session 1

In situ determination of collagen fibrils size via absolute measurements of second harmonic generation signals *(Invited Paper)*

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Type I collagen is a major structural protein in mammals that shows highly structured macromolecular organizations specific to each tissue. This biopolymer is synthesized as triple helix, which self-assembles into fibrils (diameter: 10-300 nm) and further forms various 3D organization. In recent years Second Harmonic Generation (SHG) microscopy has emerged as a powerful technique for the in situ investigation of the fibrillar collagen structures in tissues. However, this optical technique cannot resolve most of the fibrils. Moreover, it is a coherent multiphoton process so that quantitative measurements are highly challenging.

In this study, we correlated SHG and transmission electron microscopies to determine the sensitivity of SHG microscopy and calibrate SHG signals as a function of the diameter of the collagen fibril. To that end, we synthesized in vitro isolated fibrils with various diameters and successfully imaged the very same fibrils with both techniques, down to 30 nm diameter. We observed that SHG signals scale as the fourth power of the fibril diameter, as expected from analytical and numerical calculations. This calibration was then applied to diabetic rat cornea in which we successfully recovered the diameter of hyperglycemia-induced fibrils in the Descemet's membrane without having to resolve them. Finally we derived the first hyperpolarizability of a single collagen triple helix, which validates the bottom-up approach used to calculate the non-linear response at the fibrillar scale and indicates a parallel alignment of triple helices within the fibrils. These results represent a major step towards quantitative SHG imaging of nm-sized collagen fibrils.

9536-3, Session 1

Hyperspectral stimulated Raman microscopy with two fiber laser sources

Matthias Eibl, Univ zu Lübeck (Germany); Sebastian Karpf, Wolfgang Wieser, Thomas Klein, Ludwig-Maximilians-Univ. München (Germany); Robert A. Huber, Univ. zu Lübeck (Germany)

A fast all fiber based setup for stimulated Raman spectroscopy based on a rapidly wavelength swept cw-laser is presented. The applied Fourier domain mode locked (FDML) laser is a fiber ring laser, providing a continuously changing wavelength output over time. This fast swept source allows us to rapidly change the wavelength and, thereby the energy difference to a single color pump laser. The pump laser is a master oscillator power amplifier based on a fiber amplified laser diode and a Raman shifter. By controlled variation of the relative timing between probe and pump laser with an arbitrary waveform generator, the Raman signals are encoded in time and they are directly acquired with a synchronized, fast analog-to-digital converter. This setup is capable of acquiring rapidly high resolution spectra (up to 0.5 cm-1) with shot noise limited sensitivity over a broadband (750 cm-1 to 3150 cm-1) spectral region. Here, we show the performance of this system for imaging in the CH-stretch region around 3000 cm-1 and in the fingerprint region around 1600 cm-1. We present hyperspectral images of a plant stem slice with molecular contrast of lignin and a lipid representative as well as images of PS (polystyrene) and PMMA (poly(methyl methacrylate)) beads with an acquisition speed of 18 μs per spectral point.

9536-4, Session 1

Towards two-photon lensless endoscopes: inter-core group delay compensation in a multi-core fiber

Esben R. Andresen, Institut Fresnel (France); Siddharth Sivankutty, Institut Fresnel, CNR, Aix-Marseille Univ. (France); Géraud Bouwmans, Olivier Vainvinq, Univ. des Sciences et Technologies de Lille (France); Laurent Gallais, Serge Monneret, Hervé Rigneault, Institut Fresnel (France)

A lensless endoscope consists of an optical fiber which functions as the endoscope probe and which, importantly, has no elements whatsoever attached to the tip in closest proximity to the sample. This concept allows unprecedented miniaturization of the probe to the fundamental limit - the width of the fiber itself. All necessary optical elements required are located at the other end of the fiber, and endoscopic imaging is achieved by employing wavefront shaping elements to control the spatial phase.

We recently demonstrated a two-photon lensless endoscope. To activate two-photon fluorescence, ultra-short pulses are required. In a lensless endoscope setting, this obliges us to control not only the spatial phase but also group delay.

In this contribution we present a new experimental concept suitable for partially compensating the inter-core group delay dispersion in a multi-core fiber.

First we map out the group delays of all the 169 single-mode cores of a multi-core fiber using phase-shifting spectral interferometry and find the group delays distributed with standard deviation 123 fs in a 30 cm long multi-core fiber.

We then detail and apply the compensation scheme based on two wave front shapers with which we narrow the group delay distribution to 65 fs.

We quantify the performance gain in a lensless endoscope with 150 fs laser pulses as excitation and discuss possible generalizations of the concept.

9536-5, Session 1

Structural characterization of human cornea under inflation using polarization-resolved SHG microscopy

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Polytechnique (France); Jean-Marc Allain, Marie-Claire Schanne-Klein, Ecole Polytechnique (France)

Second Harmonic Generation (SHG) microscopy is an attractive technique for the investigation of the cornea. Fibrillar collagen organized in lamellae within the stroma exhibits strong SHG signals without any preparation or staining of the tissue. Nevertheless, regarding biomedical issues, quantitative information must be obtained to monitor structural modifications related to pathologies.

We therefore implemented polarization-resolved SHG (P-SHG) microscopy to measure two quantitative structural parameters at two different scales. The first parameter probes the micrometer scale organization and provides the probability density of the collagen lamellae orientations through the whole corneal thickness. The second parameter probes the local anisotropy at a nanometer scale. It quantifies local disorder of the collagen fibrils within each lamellae.

Moreover, we combined P-SHG microscopy with mechanical assays. Human corneas were imaged under inflation to mimic increasing intraocular pressure (IOP) as in glaucoma. Microscopic organization and surface strains of the cornea were then studied for each loading step.

We showed that collagen lamellae are mainly oriented along two orthogonal directions as expected in human corneas. Furthermore, the proportion of lamellae oriented along these directions varies with increasing pressure. This reorientation of the collagen lamellae is correlated with the surface deformation along the same direction.

In conclusion, combination of optical and mechanical measurements in a multiscale approach provides clues about the relationship between the microstructure of the corneal stroma and its mechanical properties.

9536-6, Session 2

Subnuclear foci quantification using high-throughput 3D image cytometry *(Invited Paper)*

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Double strand breaks (DSB) are considered as the most genotoxic type of DNA damage. DSB uniquely form gamma-H2AX foci and hence they are used to quantify DBS using fluorescent imaging assays. However most of such experiments are of low throughput in terms of imaging and image analysis techniques. Most of the studies still use manual counting or classification and they are limited to counting a low number of foci per cell (~ 5 foci per nucleus) as the process is extremely labour intensive. Therefore we have developed a high throughput instrumentation and computational pipeline specialised for subnuclear foci quantification for DNA damage for cultured cells. A high throughput image cytometer , developed in house, was used to image a population of cells with highly clustered foci inside cell nuclei, in 3D, in submicron resolutions and a 3D extended maxima based image processing algorithm was adapted to quantify the number of foci per cell nucleus. Imaging speeds as high as 800 cells/second in 3D were achieved by using HiLo wide-field depth resolved imaging and a remote z-scanning technique. Our results suggests that while most of the other 2D imaging and manual quantification studies can count only up to about 5 foci per nucleus our method is capable of counting more than 100 foci per nucleus. Moreover we show that 3D imaging and 3D data mining is significantly superior compared to all the 2D techniques.

9536-7, Session 2

High-throughput super-resolution imaging for mapping the whole mouse brain

Christopher J. Rowlands, Edward S. Boyden, Peter T. C. So, Massachusetts Institute of Technology (United States)

Any attempt to simulate the mammalian brain is hampered by the fact that obtaining the 'connectome', a description of the location of every neuron and synapse in the brain, is an unsolved problem. Attempts to obtain the connectome for commonly-studied animals such as c. elegans, drosophila, the zebrafish and mice are primarily based around scanning-electron microscopy, as the required spatial resolution in order to distinguish one dendrite from another is around 50nm or less. Unfortunately, the lack of specificity in SEM imaging means that it is necessary to image with a resolution on the order of a few nanometers in order to resolve the features of interest morphologically, rather than by labelling them; this lowers the total volume throughput, and drastically increases the data storage requirements to unrealistic levels. We present an alternative instrument design based on super-resolution microscopy that offers an unprecedented potential 1 gigapixel per second throughput with a predicted 50nm isotropic spatial resolution, sufficient to obtain a complete mouse connectome within a year.

The instrument is a highly parallel RESOLFT design, able to image 1 million 3D super-resolved spots simultaneously. In-depth optical characterizations have been performed in order to demonstrate that the constructed system is working with the anticipated resolution; data is presented and discussed, and further steps to demonstrate that the system works on real biological samples will be discussed. Finally, the prospects for imaging the mouse brain within a year will be summarized.

9536-8, Session 2

Common fluorescent proteins for single-molecule localization microscopy

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Super-resolution techniques for breaking the diffraction barrier are spread out over multiple studies nowadays. Single-molecule localization microscopy such as PALM, STORM, GSDIM, etc allow to get super-resolved images of cell ultrastructure by precise localization of individual fluorescent molecules via their temporal isolation. However, only a limited number of fluorescent proteins with special characteristics (photoactivation/photoconversion) seem to be suitable for these methods. Here, we report the application of common red fluorescent protein for single-molecule localization imaging based on spontaneous intrinsic blinking. Ideally, blinking should occur at low illumination powers, with high photon budget and characteristic lifetimes of on- and off-states. After testing several standard red fluorescent proteins, we could find out the one that satisfied all these conditions. We studied fusions

with cytoskeletal proteins in HeLa cells. Imaging was performed on commercial TIRF microscope equipped with EMCCD camera. We could observe spatially and temporarily separated bursts at the end of the bleaching series with millisecond timescale and moderate illumination power on both living and fixed cells. To process raw data we applied BALM/gSHRiMP and bSOFI algorithms allowing for analysis of densely labeled samples. As a result, high-resolution images of cytoskeleton structure were obtained. Essentially, there is no need of any additives or special buffers to induce switching of tested red fluorescent protein. Another advantage is that a single laser wavelength is usable both for on/off blinking and imaging. Moreover, the use of low-intensity yellow-green light for irradiation reduces phototoxicity and provides long-term live-cell imaging.

9536-9, Session 2

Uniquely identifying cell orientation and sarcomere length in the intact rodent heart with oblique plane remote focussing microscopy

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In cardiac imaging, the spacing between sub-cellular sarcomere structures is of great importance to physiologists in constructing models of cell function. Making accurate measurements of the sarcomere length (SL) presents a significant imaging challenge owing to the size of the SL (~2μm) and its naturally low variability (<10%), requiring a high level of precision to determine subtle changes between heart disease models. Moreover, measurements of SL from traditional two-photon imaging have so far been ambiguous to within a factor of cos(?), where ? is the inclination of the tissue with respect to the focal plane.

By remotely focussing a customised two-photon microscope, it is possible to image heart cells at two oblique angles within 200ms. The oblique images uniquely resolve the tissue inclination ambiguity and reduce the variance of SL measures by as much as 23%. This improved precision is crucial in discerning between pathological models of chronic hypertension. As well as improving measurement precision, the distribution of ? across the field of view provides additional structural information which can be related to disease morphology. To validate this new imaging protocol, the value of ? calculated from the oblique planes provided the input to a rigid model cell which was used to predict the appearance of the cell in the conventional focal plane. The comparison of the model to the image data provided a confidence metric for our measurements. Finally, by considering the optical transfer function, the range of cell orientations for which the method is valid could be calculated.

9536-10, Session 2

Dual collection mode optical microscope with single-pixel detection

Angel David Rodriguez Jimenez, Pedro J. Clemente Pesudo, Mercedes Fernández-Alonso, Enrique Tajahuerce, Jesús S. Lancis, Univ. Jaume I (Spain)

Despite systems that scan a single-element benefit from mature technology, they suffer from acquisition times linearly proportional to the spatial resolution. Significant per-pixel dwell times limit real-time performance to low resolution. A promising option is to use global illumination strategies while preserving a single-pixel detector. The sample is obtained by imaging the scene through microstructured masks implemented onto a programmable spatial light modulator.

Digital micromirror devices (DMDs) allow to create binary patterns useful in techniques such as structured illumination microscopy (SIM), single pixel imaging (SPI), compressive sensing (CS), and multidimensional imaging, to list only a few. Diamond pixel layout DMDs have the advantage that the incident beam and the output beam are in a horizontal plane, when the DMD is placed vertically. This allows a more precise alignment and a setup parallel to the table. However this pixel architecture creates a problem when images are sent in a regular orthogonal pixel manner.

In this work we have developed a single-pixel optical microscope that provides both reflection and transmission images of the sample under test by attaching a diamond pixel layout DMD to a commercial inverted microscope. Our system performs simultaneous measurements of reflection and transmission modes. Besides, in contrast with a conventional system, in our single-element detection system both images belong, unequivocally, to the same plane of the sample. Furthermore, we have designed an algorithm to modify the shape of the projected patterns that improves the resolution and prevents the artifacts produced by the diamond pixel architecture.

9536-11, Session 2

Recent developments in light sheet ultramicroscopy imaging techniques

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Ultramicroscopy-UM allows 3D-vizualization of biological specimens with ?m-resolution. The spatial intensity distribution of laser beam illuminating the specimen and the thickness of light sheet have the foremost impact on the quality of image in UM. In this paper, we investigate and compare different designs for generating light sheet and their optical characteristics (i.e. the length and diameter of the line of focus, spatial intensity distribution along the light sheet). A new design that can be used for both static as well as scanning light-sheet microscopy is presented. Furthermore, we compare the effects of the incident beam intensity distribution, Gaussian and Flattened-Gaussian beam, on the output beam profiles.

In this paper, a combination of numbers of achromatic aspherical optical elements is used to produce a thin laser light sheet. The optical characteristics have been improved and the laser energy is used more efficiently as there is no truncation of the beam. The specimen is kept at the focus of last cylindrical lens and the length of the line of focus can be varied by changing the position of other lenses.

Using the modified system we achieved a marked improvement in presenting fine details in in reconstructions of representative biological specimens. Results are presented that demonstrate an excellent agreement between theory and experiment.

9536-12, Session 3

Multimodal CARS-based nonlinear optical microscopy and optical coherence tomography *(Invited Paper)*

Angelika Unterhuber, Tschackad Kamali, Medizinische Univ. Wien (Austria); Sunil Kumar, Yaron Silberberg, Weizmann Institute of Science (Israel); Wolfgang Drexler, Medizinische Univ. Wien (Austria)

Optical coherence tomography (OCT) and coherent anti-Stokes Raman scattering (CARS) are well-established non-invasive techniques for biological imaging. OCT enables morphologic visualization of larger volumes of a tissue with a penetration depth of up to 2mm. CARS can probe specific molecular vibrations without the need of fluorescent labeling, hence preventing any interference with the cellular functions or photobleaching. Conventional implementations of CARS microscopy to image one molecular vibrational mode (narrowband CARS) or several molecular vibrational modes (multiplex CARS) are not able to completely remove the non-resonant background, which arises due to the electronic oscillations. Furthermore, spectral resolution is dependent on the probe pulse. Imaging molecular vibrations in the fingerprint region needs high spectral resolution and complete removal of the non-resonant background. A method which allows for complete suppression of the non-resonant background with high spectral resolution and easy integration of second harmonic (SHG) and third harmonic (THG) is presented here. OCT performs a large prescreening of the specimen to choose a zoom in region for chemical mapping with CARS and nonlinear microscopy.

Simultaneous extraction of structural and chemical ('morphomolecular') information is obtained with a single laser source. Excitation of the molecular vibration is performed by impulsive stimulated Raman scattering. The time resolved CARS signal and the background free CARS spectrum can be acquired online. The novel nonlinear optical imaging system with straightforward integration of wide-field OCT makes it a nearly complete optical imaging platform for versatile label-free and noninvasive bio-imaging.

9536-13, Session 3

Identification of malaria infected red blood samples by digital holographic quantitative phase microscope

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Microscopy is still considered as the gold standard for diagnosis of malaria, which is one of the most widespread and potentially fatal diseases found especially in Africa and Asia and. Its correct diagnosis is essential for proper medication and cure. Using a bright field microscope a trained health care professional identifies malaria in samples treated with chemicals which gets attached to malaria parasites and hence changes the absorption profile of the cell. In developing countries, due to lack of sufficiently trained technicians, good quality instruments and chemicals, visual identification of malarial RBCs may become unreliable. So malaria diagnostics could benefit from the use of easy-to-use instrument which does not require intervention from a technician.

Interferometric techniques can be used to yield quantitative phase contrast images of objects under investigation. Digital holographic microscopy is such a technique, providing a means for effective three-dimensional imaging of micro-objects. Digital holograms directly provide the phase information of the object, from which its thickness profile can be reconstructed. One of the biggest advantages of the technique is numerical focusing, allowing one to garner information at different object planes. Also digital holographic interferometric microscopy allows comparison of phases, making it possible to obtain phase information about the object after compensating for the phase due to aberrations. This is done by subtracting the phase reconstructed from the hologram recorded without the object in the field of view from the phase reconstructed using the hologram recorded with the object in the field of view. The cell thickness profile is obtained from this phase information using refractive index values of the cell and the surrounding medium. Also by numerical focusing additional information about the thickness profile at various cell planes, which might be useful in their identification could be obtained. Here the use of digital holographic microscopy for automatic identification of malaria infected blood samples is described. The identification is carried out by computing the thickness distribution of the cells as well as by comparing shape profiles at different axial planes. Discrimination parameters were obtained from the thickness profiles reconstructed at several planes. A correlation algorithm was then used to compare the shapes of the cells and determines whether the cell is infected by malaria. Both the comparison of thickness distribution and shape were fast and were found to provide accurate enough identification of infected samples.

9536-14, Session 3

Principles of orientation-independent DIC microscopy and its application for refractive index measurement

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Conventional differential interference contrast (DIC) microscope shows the two-dimensional distribution of optical phase gradient encountered along the shear direction between two interfering beams. Therefore, the contrast of DIC images varies proportionally to the cosine of the angle made by the azimuth of the phase gradient and the direction of wavefront shear. The image contrast also depends on the initial phase difference (bias) between the interfering beams. It is therefore necessary to examine the unknown object at several azimuth orientations and mechanically adjust bias of the DIC prism.

To overcome the limitations of available systems, we have developed an orientation-independent differential interference contrast (OI-DIC) microscope, which allows the bias to be modulated and shear directions to be switched rapidly without mechanically rotating the specimen or the prisms. New compact OI-DIC beam-shearing assembly fits into the existing slot of research Olympus microscopes and does not require the microscope modification. The assembly consists of two standard DIC prisms with a liquid crystal polarization rotator in between. Another liquid crystal cell is employed for modulating the bias. Within a second, the microscope captures a set of raw DIC images at the orthogonal shear directions and different biases.

The OI-DIC technique can be employed with available high-NA objective lenses providing the high-resolution map of the optical path length (OPL). In the report we describe principles of computing the OPL gradient and OPL maps. Then the obtained OPL map is used to measure the refractive index.

9536-15, Session 3

Shearing interference microscopy for tomography of living cells

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The research of living cells internal structure has the greatest interest for biology and medicine. For optically transparent cell (phase sample) this problem can be solved by combining the tomography, interferometry and microscopy. Usually tomographic projections are the phase images of the sample retrieved from the interferogram.

The tomographic interference microscopy for 3D refractive index spatial distribution measurement has been proposed. The current trend in the interference microscopy is the usage of an incoherent light in order to reduce the speckle noise. The short coherence length of light leads to the necessity of using the common path or shearing interferometry. The main disadvantage of shearing interferometry is the impossibility to restore the phase image of the sample, but only the one-dimensional derivative of phase image in the direction of shear. However it is known that the tomogram reconstruction can be made by the projections derivatives. This feature of tomographic reconstruction algorithm allowed us to use the shearing interferometry for obtained projections data.

The experimental setup is based on lateral shearing phase shifting interference microscope with low coherent illumination. For phase image reconstruction from interferogram a phase shifting technique was used.

To acquire projections of the sample at different angles the angular scanning system of the probing beam was used in the range of 120° at 100X, 1.30 NA oil immersion objective. The total quantity of 2D projections was 140. The iterative algorithm for limited-angle tomographic reconstruction was used. Tomograms of a single human blood cell (erythrocyte) are presented.

9536-16, Session 3

Tomographic phase microscopy using optical tweezers

Mor Habaza, Barak Gilboa, Yael Roichman, Natan T. Shaked, Tel Aviv Univ. (Israel)

We present an external interferometric module integrated with optical tweezers for obtaining quantitative phase maps of biological samples from a wide range of angles. This tomographic phase microscopy

approach enables full 3-D refractive-index reconstruction. Information about the internal refractive index of biological samples is an important asset, since it is one of the basic characteristics of every matter. Tomographic phase microscopy measures quantitatively the 3-D distribution of refractive-index in biological cells. Previous approaches measured the transmitted field from multiple angles, as implemented by either illumination beam rotation or sample rotation. In the first case, the concept is to rotate the illumination beam, while the specimen and optical setup are fixed. However, the angular range is limited in the approach. In the second case, rotating the sample makes it possible to cover the entire angular range, but it is difficult to fix the exact axis of rotation and the rotation inevitably perturbs the specimen while trying to keep the sample in focus. Therefore, the use of this method is restricted to specially prepared specimens, typically containing viscous fluid instead of a natural medium. In the first time to our knowledge, we have developed a close-to-common-path, off-axis interferometric system that enables a full-rotation tomographic acquisition of a single cell using optical tweezers. This system enables to acquire phase information of a single cell from all different angles, while maintaining the native surrounding medium. We experimentally compare two reconstruction algorithms: the filtered back-projection method and the Fourier diffraction method.

9536-17, Session 3

Quantitative phase microscopy for the study of bacterial cell growth

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The growth of bacterial populations is a quite well known process, and the modes of action of antibiotics are well determined at the molecular scale. However, the growth of single bacterial cells and the effects of antibiotics at the cellular level is still very poorly understood due to the lack of techniques. The aim of this work is to study bacterial cell growth of single cells using a newly developed optical Quantitative Phase Imaging (QPI) method, Diffraction Phase Microscopy with white light (wDPM). This interferometric technique provides quantitative phase maps from which dry mass density maps and the cellular dry mass can be extracted via a simple linear relationship and integration over the region of interest. wDPM exhibits very high stability and spatial and temporal sensitivities, thus allowing a precise and non-invasive measurement of the changes in dry mass of many individual living cells over time and in various conditions. We use this technique to investigate single-cell growth in the presence of antibiotics at different concentrations. Here we show that wDPM is well suited for this study by presenting some preliminary results on the growth rate of Escherichia coli growing on an agar substrate at 37 °C. We find a growth rate proportional to the mass, indicating an exponential growth of this species.

9536-18, Session 3

Lensfree video microscopy: high throughput monitoring and cell tracking of 2D cell cultures

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By focusing on simplicity, cost, feasibility, field of view, and time-lapse in-incubator imaging, we developed a lensfree video microscope based on digital in-line holography. This system has opened up new perspectives, enabling real time monitoring of thousands of cells, simultaneously, directly inside the incubators. This permitted us to perform label-free cell based assays to study the major cellular events – cell adhesion and spreading, cell division, cell division orientation, and cell death. In order to extend the analysis of the datasets produced by lensfree video microscopy, we have implemented a cell tracking algorithm to combine and correlate cell motility to the previously developed devised metrics. In this way lensfree video microscopy becomes a unique platform to perform cell motility study: (i) the field of view of approximately 30mm2 contains thousands of cells and allows to track cells over several millimeters (ii) ability to perform real-time monitoring for extended time period (>1week) (iii) possibility of quantifying not just cell migration but also other events (cell division, cell death, differentiation, etc.) that occur simultaneously, (iv) ability to provide both macroscopic and microscopic information, in order to monitor cell population kinetics. We will present comprehensive datasets obtained with different cell types and culture conditions (e.g.: primary cells, human stem cells, fibroblasts, endothelial cells, epithelial cells, 2D/3D cell culture, etc.).

9536-19, Session 3

Wide-field lensfree imaging of tissue slides

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Pathologist examination of tissue slides provides insightful information about a patient's disease. Traditional analysis of tissue slides are performed under a binocular microscope by pathologists, which requires staining of the tissue slide and delays the examination. We present a simple cost-effective lensfree imaging method to record 2-4μm resolution wide-field (10 mm? - 5 cm?) images of stained and unstained tissue slides. To our knowledge, our method is the first technique that enables wide-field lensfree imaging of such dense samples. Multiple holograms are recorded with different wavelength illumination, and a multispectral algorithm is used to retrieve both amplitude and phase. Our method can be used to retrieve images of stained tissue slides. For such absorbing object, the useful information is included in the modulus of the reconstructed complex field. Our method can also be applied to retrieve images of unstained tissue slides, where the useful information is in the retrieved phase. Slides from various tissues can be reconstructed, e.g. lung, small intestine, kidney, ganglion,... This technique is much cheaper and compact than a conventional microscope and could be made portable. Moreover, it enables wide field unstained tissue slides imaging, which could quickly provide useful information, for example on frozen section biopsies, when a rapid diagnosis is needed during surgery.

9536-20, Session 4

Increased metabolic activity detected by FLIM in human breast cancer cells with desmoplastic reaction: a pilot study

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Introduction: In breast cancer (BC), desmoplastic reaction, assembled primarily by fibroblasts, is associated with worse prognosis, but the

reason of this fact remains still unclear. In this context, nonlinear optics microscopy, including Fluorescence Lifetime Imaging Microscopy (FLIM), has provided advancement in cellular metabolism research. In this paper, we aim to differentiate the tumor cell metabolism with or without fibroblasts contact, in patients with BC. Methodology: Unstained paraffin sections of 14 patients with invasive ductal breast carcinoma were analyzed with FLIM methodology to NAD(P)H and FAD fluorescence lifetime on a Confocal Upright LSM780 NLO device (Carl Zeiss AG, Germany). Quantification of the fluorescence lifetime and fluorescence intensity was evaluated by SPC Image software (Becker & Hickl) and ImageJ (NIH), respectively. Redox ratio was calculated by dividing the FAD fluorescence intensity by NAD(P)H fluorescence intensity. Statistical analysis was performed in the R. Results: BC cells in contact with fibroblasts presented a significantly lower NAD(P)H (p=0.04) and FAD (p=0.04) fluorescence lifetime. Furthermore, redox ratio was also lower in these tumor cells (p=0.01). Conclusion: Our results suggest that contact of BC cells with fibroblasts increase their metabolic activity, which might explain the worse prognosis of cases associated with higher peritumoral desmoplastic reaction, characterized by increased numbers of fibroblasts.

9536-21, Session 4

Hardware friendly bi-exponential fluorescence lifetime imaging algorithms and phasor approaches

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New hardware-friendly fluorescence lifetime imaging (FLIM) algorithm has been proposed capable of resolving bi-exponential decays.The proposed algorithms are non-iterative offering direct calculation of lifetimes and therefore suitable for real-time applications. The proposed algorithms were implemented on a field programmable gate array (FPGA) and tested on both an FPGA synthesized data and FLIM images of Hela cells obtained by a two-photon FLIM microscope. Fourier transform has been performed on the analysis results obtained by the proposed method to check how the proposed method performs. Combing the proposed method with the widely used phasor approaches show great potential for high-speed FLIM analysis.

9536-22, Session 4

Characterization of atherosclerotic arterial tissue using combined SHG and FLIM microscopy

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Atherosclerosis is among the most widespread cardiovascular diseases and one of the leading cause of death in the Western World. Characterization of arterial tissue in atherosclerotic condition is extremely interesting from the diagnostic point of view, especially for what is concerning collagen content and organization because collagen plays a crucial role in plaque vulnerability. Routinely used diagnostic methods, such as histopathological examination, are limited to morphological analysis of the examined tissues, whereas an exhaustive characterization requires immune-histochemical examination and a morpho-functional approach. Non-linear microscopy techniques offer the potential for providing morpho-functional information on the examined tissues in a label-free way. In this study, we employed combined SHG and FLIM microscopy for characterizing collagen organization in both normal arterial wall and within atherosclerotic plaques. Image pattern analysis of SHG images allowed characterizing collagen organization in different tissue regions. In addition, the analysis of collagen fluorescence decay contributed to the characterization of the samples on the basis of collagen fluorescence lifetime. Different values of collagen fiber mean size, collagen distribution, collagen anisotropy and collagen fluorescence lifetime were found in normal arterial wall and within plaque depositions, prospectively allowing for automated classification of atherosclerotic lesions and plaque vulnerability. The presented method represents a promising diagnostic tool for evaluating atherosclerotic tissue and has the potential to find a stable place in clinical setting as well as to be applied in vivo in the near future.

9536-23, Session 4

Quantitative confocal fluorescence microscopy of dynamic processes by multifocal fluorescence correlation spectroscopy

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Abstract

Quantitative confocal fluorescence microscopy without scanning is developed for the study of fast dynamical processes via massively parallel Fluorescence Correlation Spectroscopy (FCS). The potential of this approach is demonstrated using live salivary glands from Drosophila third instar larvae expressing a fluorescently-tagged transcription factor. This approach paves the way for quantitative characterization of physiologic processes in live cells/tissue with a sub-millisecond temporal resolution (presently 21 μs/frame) and single-molecule sensitivity.

Instrumental design

Single light beam generated by a continuous wave (CW) 488 nm frequency-doubled diode laser was expanded and focused by a plano-convex lens through a diffractive optical element (DOE) designed to split the single laser beam into 32?32 beams. A 32?32 foci illumination matrix was formed at the rear port image plane of the microscope. The relay optics of the rear port, dichroic mirror, and the C-Apochromat 63?/1.2 W Corr objective transfer the illumination matrix image into the focal plane of the objective. The Zeiss Filter Set 38 HE for enhanced Green Fluorescent Protein (eGFP) consisting of an excitation band pass filter EX BP 470/40 nm (central wavelength/bandwidth), long pass dichroic mirror with a cut-off wavelength of 495 nm, and an emission band pas filter EM BP 525/50 was used throughout. Fluorescence was detected using a SPAD camera, containing a photosensitive chip and a 16-bit photon counter based on a Field Programmable Gate Array (FPGA) [1-4]. The photo sensitive area of the chip consists of 32x32 circular SPADs that are 20 ?m in diameter. The distance between adjacent diodes in the same row/column is 100 ?m. Since the aperture of every SPAD in the camera acts as a pinhole positioned in a conjugate focal plane with respect to the illumination matrix, confocal configuration is achieved for all 32x32 foci.

Auto-correlation and cross-correlation analysis

Raw data collected by the SPAD camera, consisting of 131000 frames acquired every 20.74 μs that yield 1024 fluorescence intensity fluctuation traces recorded over 2.7 s, were stored in the camera's internal memory, transferred to the computer and subjected to correlation analysis to yield

auto- and first- and second-order cross-correlation curves (ACC and CCC, respectively) for all 32?32 pixels in an image frame. For this purpose, the so-called multi-tau algorithm was used [5-7]. The CCCs are calculated for two SPADs of the camera designated as the “first” or “second” order neighbors of the reference pixel. Massively parallel calculations of ACC and CCC was achieved using a graphics processing unit (GPU). An ACC amplitude was estimated from the value of the autocorrelation function at 103.7 μs, and the characteristic decay time of the ACC from its full width at half maximum. These values are plotted in fFMI images to show the spatial distribution of ACC amplitudes and decay times across the sample.

Results

The fFMI instrument was used to characterize under ex vivo conditions the nuclear dynamics of a synthetic transcription factor that interacts with chromosomal DNA in live salivary glands from Drosophila third instar larvae expressing an mCitrine-tagged Sex combs reduced dimeric transcription factor (mCitrine-(Scr)2) and bearing a multimeric specific binding site of Scr (fkh250con) in the genome [8-10].

Conclusion

The novel approach to confocal microscopy that is presented here marks the beginning of a new era in the application of fluorescence microscopy imaging for quantitative study of fast dynamical processes. This approach, which retains all advantages of confocal microscopy, including the ability to control depth of field, improved SNR by elimination of out-of-focus light and the capability to produce 3D reconstruction of specimen by optical sectioning; is empowered by the abolishment of scanning, allowing a 100-fold and in the future even a 1000-fold improvement in temporal resolution of confocal microscopy. The underlying FCS analysis provides quantitative information about the local concentration and the mobility of molecules across the specimen, which cannot be deduced from fluorescence imaging by CLSM. The possibility to measure local concentrations and mobility of biomolecules is essential for understanding the spatio-temporal integration of molecular interactions in live cells/tissues. This approach is therefore of particular interest for biomedical research applications, as it holds the capacity to map the dynamic landscape of biomolecules activity in live cells/tissue with single-molecule sensitivity and sub-millisecond temporal resolution.

9536-33, Session 5

Molecular and cellular degeneration of myelin in the spinal cord *(Invited Paper)*

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Myelin plays an essential role in the nervous system and its disruption in diseases such as multiple sclerosis can lead to neuronal death, thus causing irreversible functional impairments. Understanding myelin biology is therefore of fundamental and clinical importance, but no tools currently exist to describe the fine spatial organization of myelin sheaths in vivo. We have developed a modality called polarization-sensitive CARS imaging to extract the molecular order of myelin, and we combine this with large-scale automated segmentation of spinal cord images to characterize its molecular and cellular organization.

9536-34, Session 5

Monitoring brain response with diffuse optical systems during focal brain injury in intact mouse head

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Multispectral imaging based on projection of structured near infrared illumination at different spatial frequencies and orthogonal diffuse reflectance spectroscopy were applied to quantitatively assess brain function before, during, and after the onset of focal traumatic brain injury in intact mouse brain. Injury was induced in anesthetized male mice by weight-drop device using cylindrical metal rod striking the mouse's head. Following data analysis, we were able to show a series of hemodynamic and morphologic changes over time including higher deoxyhemoglobin, reduction in oxygen saturation, cell swelling, etc., in comparison with baseline measurements. In addition, a comparative evaluation between two different neuroprotective drugs namely hypertonic saline and minocycline given post-injury was investigated; Minocycline was found to improve hemodynamic outcome while hypertonic saline decreased brain water content assume to inhibit the increase in intracranial pressure.

9536-35, Session 5

A hyperspectral time resolved DOT system to monitor physiological changes of the human brain activity

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Diffuse Optical Tomography (DOT) is a growing area of research in the field of biomedical optics and neurosciences. Over the past 20 years, technical development allowed a more and more accurate detection of the brain activation, both spatially and in the calculation of the variations of chromophores's concentrations such as Hemoglobin, cytochrome c oxidase, etc. In particular, time resolved systems are able to distinguish between superficial layers (skin, skull) and deep layers (brain) allowing the differentiation between the systemic response and the response of the brain.

In order to increase the accuracy of the brain's activation detection, and to obtain its broadband optical characterization, we have developed a Hyperspectral Time Resolved DOT system. It is composed of a compact supercontinuum laser within the picosecond range for the source part and of an ICCD camera coupled with an imaging spectrometer for the detection part. This allows a simultaneous detection of the spatial (up to 70 reception points), time resolved (minimum gate width 200ps, minimum delay shift 10ps), and spectral information (from 500 to 900 nm).

Through the information acquired by our system, we've been able to retrieve, to our knowledge, the first spectrum of the hemodynamic response of the human brain activity. We are also focused on the detection of the Fast Optical Signal.

9536-36, Session 5

Characterization of cerebral hemodynamics during obstructive sleep apnea

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In obstructive sleep apnea (OSA) syndrome, breathing is repeatedly interrupted during sleep because of upper airway obstruction. Episodes of apnea cause profound, repeated changes in cerebral hemodynamics including periods of hyper- and hypo-perfusion and intermittent hypoxia. It is hypothesized that these changes may play a role in increased risk of cerebrovascular conditions such as ischemic stroke. These events are very complex in nature and are affected by many confounding factors. A better understanding of the apnea effects on the cerebral hemodynamics may allow the development of personalized management strategies. In this work, we developed and applied a hybrid frequency domain (FD), near-infrared diffuse optical spectroscopy (NIRS-DOS) and diffuse correlation spectroscopy (DCS) system with a custom probe to follow the apnea-induced microvascular blood oxygenation, blood volume and blood flow changes in the frontal lobes. Sixteen patients were measured and 794 different OSA events were recorded. We were able to obtain good signal-to-noise ratio and, therefore, able to characterize blood oxygenation, blood volume and blood flow changes during each apnea episode. We then grouped apneas according to the duration, parametrized the typical curves and investigated correlations with other demographyc and systemic parameters (measured by polysomnography). We demonstrated that hybrid diffuse optics can be utilized for sleep studies and that we were able to characterize cerebral hemodynamics during each apnea episode.

9536-37, Session 5

Investigation of dendritic spines by STED Nanoscopy

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1. Introduction

Parkinson's disease (PD) and dementia with Lewy bodies (DLB) have been associated with the formation of amyloid-rich structures, so called Lewy bodies, which consist primarily of ?-synuclein, in the brain of patients, and which consequently lead to dopaminergic neuron loss. Interestingly, ?-synuclein-rich aggregates are found primarily at the presynapse, but the presynapse in PD patients remains relatively intact. In contrast to this, dendritic spines start to retract and change their morphologies, as shown by post mortem analysis of patient's brains [4]. Thus, it remains to be elucidated how ?-synuclein can exert these morphological changes in dendritic spines.

A challenge in the current study is that dendritic spines have relevant substructures – such as the spine neck – with widths below the resolution limit of conventional microscopes [3] and thus an optical super-resolution

microscope, with resolution below 100 nm and compatible with living cells is required. Furthermore, this system should be compatible with other, e.g. GFP-based sensors to permit the concomitant study of downstream signalling events that may be related to a deformation of dendritic spines. A custom stimulated emission depletion (STED) microscope [1] compatible with red dyes and fluorescent proteins is developed to address these pertinent questions in the field. This technique is fast and can therefore be used with dynamic structures such as live cells. The photophysical properties of the recently developed red fluorescent protein mNeptune2.5 [2] are investigated for STED microscopy and primary rat neurones overexpressing this protein are imaged.

Optical nanoscopy has been used to study the growth and structure of protein aggregates both in vitro [5, 6] and in cells [7, 8]. In this work we extend that by applying optical nanoscopy to better understand the effect of these aggregates on the morphology of cells and therefore effects on function.

2. Development of STED microscope

A custom pulsed STED microscope is developed to work with red dyes and red fluorescent proteins. Since both excitation and depletion are in the red, or near infra-red, STED images can be acquired with minimal cross-talk or photobleaching of other – blue shifted - fluorophores, for instance those used to localise aggregates or to sense other downstream effectors. STED excitation at 592 nm or 640 nm is generated by pumping a photonic crystal fibre with the depletion laser. The depletion laser is a 760 nm pulsed laser. The beam profile is shaped into the vortex beam required for STED using a spatial light modulator (SLM). An SLM permits the user to modify the phase of the beam and thus provides the ability to correct for aberrations induced, both, by the sample and the optical path as well as to assist with the coalignment of the excitation and depletion beams. The excitation and depletion beams are recombined in a confocal microscope (RESOLFT, Abberior instruments). This system incorporates excitation lasers at 405 nm and 488 nm as well as detectors suitable both for mNeptune2.5 and green fluorophores, for instance GFP

The STED was characterised first on 40 nm dark red fluorescent beads. A typical resolution of approximately 80 nm was observed with these samples. The system was also tested on imaging aggregates of ?-synuclein, a protein aggregate investigated in this study, labelled with ATTO 647N.

3. STED imaging of dendritic spines

We will use rat embryonic neurons which will be transfected with mNeptune2.5 [2]. Few red fluorescent proteins have been reported for STED [9] but mNeptune2.5 not only fluoresces in the red but has both a high quantum yield and low photobleaching [2] and is therefore an ideal candidate for STED. The photophysical properties of this protein for STED are then investigated. The photobleaching and resolution in mammalian cells overexpressing this protein are measured on this microscope and it is verified that dendritic spines can be resolved in neurons labelled with this protein both in confocal and in STED microscopy. Following on from this it is verified that correlative confocal imaging at 488 nm is compatible with STED microscopy with mNeptune2.5. This permits correlative imaging either with different green-labelled sensors, or amyloid proteins. Dendritic spines are imaged in the presence or absence of ?-synuclein and the morphology measured at different time points.

4. Conclusion

A custom STED microscope has been built for the imaging of morphological changes in dendritic spines. This system uses the new red fluorescent protein mNeptune2.5 [2] and is compatible with confocal imaging of green dyes and green fluorescent proteins. This system can therefore be used to image dendritic spines with super-resolution and either protein aggregates present in the sample labelled with other green dyes or GFP-based sensors. By measuring the morphology of dendritic spines over time one may understand how protein aggregates, such as consisting of ?-synuclein, can finally lead to neurodegeneration and thus may pave the path to neuroprotective strategies.

9536-38, Session 5

Optical mapping of neuronal activity with cellular resolution on a brain-wide scale

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Dissecting brain-wide neuronal networks that are activated in response to a stimulus or during the execution of a task is an important step to obtain a more comprehensive view of brain activity. However, cellular-resolution activation maps of the whole brain have not yet been obtained because of technical limitations in current imaging methods. Indeed, state-of-the art techniques to image brain activity patterns are limited by coarse resolution and/or restricted field of view. Here, we present a method to quantify neuronal activity in the entire mouse brain with cellular resolution, based on a combination of genetics, optical imaging and computer science.

As an indicator for neuron activation, we used the immediate early gene Arc, whose expression is known to be activity-regulated. We monitored the expression of this gene using a transgenic mouse strain in which a destabilized version of the Venus fluorescent protein is expressed under the Arc promoter. To map dVenus expression in the whole brain with cellular resolution, fixed mouse brains were chemically cleared and imaged with a custom-made confocal light sheet microscope optimized for cleared specimens. The whole-brain reconstructions obtained were subsequently analyzed to automatically localize the position of each fluorescent neuron, using a novel computational approach called semantic deconvolution.

The combined approach presented here allows quantifying the amount of Arc-expressing neurons throughout the whole mouse brain, opening the possibility to study brain-wide neuronal activation patterns associated with complex behaviors and tasks.

9536-44, Session PS

In vivo investigating the correlation between the reduced scattering coefficient and intracranial pressure by using near-infrared spectrum

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Traumatic brain injury (TBI) is a serious threat to adult population. In this paper, we studied the in vivo feasibility and reliability of using reduced scattering coefficient (u's) as an objective measurement of intracranial pressure (ICP). We developed a near infrared spectrum (NIRs) measurement system to obtain u's and a ICP measurement system for rat models. The wavelength range of light source was from 360 nm to 2000 nm. The u's could be deduced with the former empirical equation. The pressure transducer was used in the ICP system and the diameter of the ICP probe was ~4 mm. TBI rat models were built by Feeney's free-falling method. The mannitol with the dose of 0 g/kg, 0.25 g/kg and 2 g/kg were injected for the control and treatment, respectively. At the same time, the u's and ICP of TBI rat models were continuously acquired in vivo during the treatment and no treatment process, respectively. The data at some time points was studied and the correlation of u's and ICP was acquired. The experimental results showed that u's was associated with actual ICP in rat models and the correlation was linear. Our studies demonstrate that optical parameters, such as u's, might be potentially used for evaluating TBI patients in clinical.

9536-45, Session PS

Novel near infrared sensors for hybrid BCI applications

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Using dual modalities is thought to enhance the overall performance and information transfer rate in BCI usage. NIRS is emerging as a noninvasive, mobile, silent, low cost and relatively easy to use acquisition method for brain signals and was applied for BCI purposes as well. This study's goal is to develop a low cost, portable, accurate and comfortable NIRS module that can be used simultaneously with EEG in a dual modality system for BCI.

The sensing modules consist of EEG electrodes (at the positions FPZ, FP1 and FP2 in the international 10-20 setting) with eight custom made NIRS channels, positioned on the prefrontal cortex area with two extra channels to measure and eliminate extra-cranial oxygenation. The NIRS sensors were designed to guarantee good sensor-skin contact, without causing subject discomfort, using springs to press them to the skin instead of pressing them by cap fixture. Two open source software packages were modified to carry out dual modality hybrid BCI. The experimental paradigm consisted of a mental task (arithmetic task or text reading) and a resting period. Both ?HBO2, and EEG signals showed an increase during the mental task, but the onset, period and amount of that increase depends on each modality's characteristics. The subject's degree of attention played an important role especially during online sessions. The sensors can be easily used to acquire brain signals from different cerebral cortex parts. The system serves as a simple technological test bed and will be used in stroke patient rehabilitation purposes.

9536-46, Session PS

Optical neural stimulation modeling on degenerative neocortical neural networks

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Neurodegenerative diseases usually appear at advanced age. Medical advances make people live longer and as a consequence, the number of neurodegenerative diseases is growing. There is still no cure for these diseases, but there are different techniques that improve patients' condition. Some of these techniques induce cerebral activity via electromagnetic fields. In this work Optical Neural Stimulation (ONS) has been studied. ONS stimulates noninvasively the outer regions of the brain (neocortex), but surgical intervention is necessary in deeper brain regions. In order to find out how ONS affects neuronal activity, mathematical models of neural networks have been studied. The results of these studies show the response of a neural network when it is stimulated via different optical radiation sources. This model allows choosing the appropriate light source and its parameters for stimulation of different areas of neocortex.

9536-53, Session PS

Modified Beer-Lambert law for blood flow

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The modified Beer-Lambert law is among the most widely used approaches for analysis of near-infrared spectroscopy (NIRS) reflectance signals for measurements of tissue blood volume and oxygenation. Briefly, the modified Beer-Lambert paradigm is a scheme to derive changes in tissue optical properties based on continuous-wave (CW) diffuse optical intensity measurements. In its simplest form, the scheme relates differential changes in light transmission (in any geometry) to differential changes in tissue absorption. Here we extend this paradigm to the measurement of tissue blood flow by diffuse correlation spectroscopy (DCS). In the new approach, differential changes of the intensity temporal auto-correlation function at a single delay-time are related to differential changes in blood flow. This modified Beer-Lambert law scheme for flow facilitates real-time flow monitoring in any geometry, enables flow monitoring in geometries with non-diffusive light transport, and when combined with probe pressure modulation, is well suited for filtering superficial tissue contamination in the measured optical signals. The scheme has been validated in vivo in a pig experiment.

9536-39, Session 6

Cellular interactions in disease: nonlinear optics for in vivo studies *(Invited Paper)*

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We are interested in studying the contribution of multiple physiological systems to disease initiation and progression, with applications in neurodegenerative disease, cardiovascular disease, and cancer. We hope to study how the vascular, immune, inflammatory systems and cells native to a tissue interact in these diseases in vivo. To meet this challenge we use multiphoton microscopy in whole animals which provides sufficient spatial and temporal resolution to quantify cellular dynamics. We also now have tools to produce targeted disruption with cellular-scale precision by using femtosecond laser ablation. We used these tools to piece together a picture of how occlusion or hemorrhage of small blood vessels in the brain affects the health and function of nearby neurons, and thus contributing to cognitive decline. In brain, we have been investigating the relationship between Alzheimer's disease and dysfunctional microvasculature. Vascular health is increasingly being recognized as a critical factor in Alzheimer's. In addition to neuroscience applications, these tools also provide opportunities in a variety of in vivo systems. In mouse gut, the use of multiphoton microscopy enables us to study the stability of stems cells in the crypts of the intestine. We can disrupt this stability by using femtosecond laser ablation to remove single cells and observe the resultant changes in cell-cell interactions important for stem cell maintenance. Microvascular function of the heart has been difficult to study because the motion in a heart beat obscures high-resolution imaging, but is also essential for blood flow. We have recently developed methods to apply multiphoton microscopy in the beating mouse heart.

9536-40, Session 6

Adaptive aberration correction for whole brain imaging

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One of the most promising applications of light sheet microscopy (LSM) is the reconstruction of macroscopic biological specimens with microscopic resolution without physical sectioning. To this aim, LSM is combined with clearing protocols based on refractive index matching which render the tissue transparent. Recently objectives suitable for LSM have become commercially available which have been designed for use with clearing solutions and typically also feature a correction collar for the compensation of a certain amount of spherical aberration. However, a complete correction of system induced aberrations requires the ability to also correct for residual spherical aberration and higher order aberrations introduced by the sample mount and slight misalignment of the optical system. Additionally aberrations introduced by the sample itself degrade image quality. Adaptive optics provides a means to compensate for these aberrations and is capable of restoring diffraction limited imaging where possible. Here we describe the correction of optical aberrations by introduction of a micro-electro-mechanical systems (MEMS) deformable membrane mirror into the detection path of a LSM. The aberration

correction is determined in a wavefront sensorless approach by rapidly altering the mirror shape with a random search algorithm until the fluorescence signal intensity is improved. The improvement in image quality is demonstrated in murine brain which has been optically cleared using a modified CLARITY protocol. Increasing image quality in large 3D data sets is critical for fully automated post processing for example in cell counting or vasculature segmentation.

9536-41, Session 6

A versatile new technique to clear mouse and human brain

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Large volumes imaging with microscopic resolution is limited by light scattering. In the last few years based on refractive index matching, different clearing approaches have been developed. Organic solvents and water-based optical clearing agents have been used for optical clearing of entire mouse brain. Although these methods guarantee high transparency and preservation of the fluorescence, though present other non-negligible limitations. Tissue transformation by CLARITY allows high transparency, whole brain immunolabelling and structural and molecular preservation. This method however requires a highly expensive refractive index matching solution limiting practical applicability. In this work we investigate the effectiveness of a water-soluble clearing agent, the 2,2'-thiodiethanol (TDE) to clear mouse and human brain. TDE does not quench the fluorescence signal, is compatible with immunostaining and does not introduce any deformation at sub-cellular level. The not viscous nature of the TDE make it a suitable agent to perform brain slicing during serial two-photon (STP) tomography. In fact, by improving penetration depth it reduces tissue slicing, decreasing the acquisition time and cutting artefacts. TDE can also be used as a refractive index medium for CLARITY. The potential of this method has been explored by imaging a whole transgenic mouse brain with the light sheet microscope. Moreover we apply this technique also on blocks of dysplastic human brain tissue transformed with CLARITY and labeled with different antibody. This clearing approach significantly expands the application of single and two-photon imaging, providing a new useful method for quantitative morphological analysis of structure in mouse and human brain.

9536-42, Session 6

Raman-based imaging uncovers the effects of alginate hydrogel implants in spinal cord injury

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The treatment of spinal cord injury by use of implants that provide a permissive environment for neuron growth is in the focus of the research for regenerative therapies. The effects of alginate hydrogels implants in rat models of surgically-induced spinal cord injury were investigated at different time-points by using Raman-based, label-free techniques. Raman microspectroscopy followed by chemometrics allowed the mapping of the different degenerative area, while multimodal coherent anti-Stokes Raman (CARS) microscopy (e.g. the combination of CARS, two-photon fluorescence and second harmonic generation on the same platform) enabled to address the morphochemistry of the tissue at cellular level. The information retrieved with the different techniques is in agreement and complements itself. The regions of injury (characterized by demyelination and scarring) could be retrieved and the distribution of key tissue components was evaluated by Raman mapping, demonstrating that the alginate hydrogel remain in the lesion up to six month after implantation and has positive effects both on preserving the nervous tissue and decreasing scarring. CARS microscopy provided the micromorphology of lipid-rich tissue structures and allowed discerning myelinated axons that were preserved in contact with the implants. Therefore, these findings have the potential to contribute to new strategies for monitoring and manipulating the scarring process induced in SCI and improving the effectiveness of therapies.

9536-43, Session 6

Visualization of hemodynamics and light scattering in exposed brain of rat using multispectral image reconstruction based on Wiener estimation method

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We investigate simple and rapid multi-spectral imaging to visualize hemodynamics and light scattering in the exposed brain using the spectral reflectance images reconstructed from a single snap shot of RGB image by the Wiener estimation method. In the proposed method, the multi-spectral reflectance images of in vivo exposed brain are reconstructed from the digital red, green blue images using the Wiener estimation algorithm. The Monte Carlo simulation-based multiple regression analysis for the absorbance spectra is then used to specify the absorption and scattering parameters of brain tissue. In this analysis, the concentration of oxygenated hemoglobin and that of deoxygenated hemoglobin are estimated as the absorption parameters whereas the scattering amplitude a and the scattering power b in the expression of mus'=a*lambda^(-b) as the scattering parameters, respectively. The spectra of absorption and reduced scattering coefficients are reconstructed from the absorption and scattering parameters, and finally, the spectral images of absorption and reduced scattering coefficients are estimated. In order to confirm the possibility of the method to evaluate pathophysiology of cerebral cortex, we performed in vivo experiments for exposed rat brain during CSD evoked by the topical application of KCl.

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9537-1, Session 1

Strategies for isolation and enrichment of bacteria for subsequent Raman spectroscopic identification *(Invited Paper)*

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Raman microspectroscopy is an attractive tool for the identification of bacterial species. By combining a conventional microscope with a Raman spectrometer the investigation of single bacteria cells is enabled. Due to this high sensitivity culture steps can be omitted entirely. This is a huge advantage over current standard methods for the detection of bacteria, which often require time consuming cultivation. Raman spectra of bacteria cells, suitable for identification purposes, can be acquired within seconds. Next to the rapid availability of the results, Raman spectroscopy also provides a high specificity. The Raman spectrum of a bacterial cell reflects the chemical composition of the whole cell content and thereby allows distinguishing between different species or even strains.

Regarding real-world samples it has to be considered that sample preparation steps will be necessary before the actual Raman measurements can be conducted. In most cases the cells need to be isolated from their surrounding matrix beforehand, in order to prevent the components of the sample from interfering with the Raman spectra of the bacteria, which would hamper the identification process. Within this contribution a selection of Raman compatible particle and chip based approaches for the isolation of whole microbial cells will be introduced. A key aspect of these techniques is the choice of suitable capture probes for the bacteria.

Acknowledgement

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9537-2, Session 1

Time-resolved study of microorganisms by Raman spectroscopy

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The main goal of our investigations is to focus on the basic mechanisms of microorganisms (bacteria and yeast) exposed to stress factors by time-course Raman spectroscopy. This study provides an insight into the mechanism of antimicrobial agents or targeted stress factors on different species metabolism in vivo, in real-time and label free. We also focused on evaluation of biofilm time course formation in order to detect this virulence factor in a particular strain. Biofilm formation can proceed through different mechanisms in different time ranges. Thus, its evolution is different corresponding to the observation time.

9537-3, Session 1

Lipid distribution imaging in zebrafish with stimulated Raman scattering microscopy

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The lipid distribution in whole 15 day old zebrafish is imaged using Stimulated Raman Scattering (SRS) microscopy. SRS microscopy allows for imaging of all lipids in the fish in 3D, showing the adipocytes but also total lipid content within the animal. This provides a powerful new tool for biomedical research of healthy function and disease. Although fluorescence labeling and imaging have become increasingly sophisticated, fluorescent labels are often large compared to the molecule of interest. This makes labeling without perturbing the biological function difficult or impossible. SRS imaging uses the intrinsic vibrational modes of molecules, making labeling unnecessary. We present a basic SRS set-up with mainly commercial components ideal for label free imaging of biological systems. A custom built transimpedance amplifier allows shot noise limited detection over a broad window of biologically relevant laser powers.

Zebrafish exposed to continuous light conditions have a very high prevalence of adipocytes compared to control animals held in a normal day-night rhythm. The adipocyte prevalence is even higher than in animals fed on a high fat diet. Whole-animal SRS imaging shows that the total lipid distribution in the rest of the 24 hour light fish is similar to that of control fish, but with more and larger lipid deposits in the visceral region. The total lipid content of these fish is not significantly altered compared to the control, indicating a relocation of lipids, rather than an increased uptake. SRS microscopy is a new label-free platform for studying the lipid distribution in whole model organisms.

9537-4, Session 1

Raman and fluorescence microscopy to study the internalization and dissolution of photosensitizer nanoparticles into living cells

Claudia Scalfi-Happ, Rudolf W. Steiner, Rainer Wittig, Univ. Ulm (Germany); Susanna Gräfe, biolitec AG (Germany); Anastasia Ryabova, Victor B. Loschenov, BioSpec (Russian Federation) and A. M. Prokhorov General Physics Institute (Russian Federation)

1. Summary

Photodynamic therapy (PDT) is a promising anticancer treatment with low risk of systemic toxicity [1]. A photosensitizing dye is applied locally and undergoes a photochemical reaction upon illumination. The efficacy of PDT is dependent on the uptake of the photosensitizer by a tumor or inflammatory tissue environment. Recently, nanoparticles of aluminium phthalocyanine were considered as promising photosensitizer formulation [2]. These nanoparticles show no fluorescence as long as the photosensitizer is in crystalline form while fluorescence appears upon dissolution.

Confocal Raman microspectroscopy has developed into a powerful tool for the investigation of living cells and biological samples [3, 4].

In a previous study we investigated the role of lipids in the discrimination between Caco-2 colon carcinoma cell line and the rat intestine epithelial cell line IEC-6 by confocal Raman microscopy [5] with the alpha300 R

Raman microscope (WITec GmbH, Germany).

In this work we applied the Raman-microspectroscopic approach to follow the cellular uptake of photosensitizer nanoparticles in their crystalline, non-fluorescent form. Crystalline nanoparticles with different size made from the hydrophobic porphyrin-derived photosensitizer were applied to either L929 murine fibroblasts or to J774A.1 murine monocytes/macrophages. The cellular colocalisation with lipids and the influence on the cytochrome C signal from mitochondria were analysed in dependence on application time.

In a further step, the dissolution process of the nanocrystals with increasing fluorescence signal was evaluated by fluorescence microscopy.

These investigations will help understanding the effect of photosensitizer particle size on cellular uptake and the differences in internalisation mechanisms of the studied cell lines.

9537-5, Session 1

Rapid screening test for porphyria diagnosis using fluorescence spectroscopy

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Introduction

Porphyrias are rare genetic metabolic disorders, which result from deficiencies of enzymes in the heme biosynthesis pathway. As a result, different types of porphyrins and heme precursors accumulate in erythrocytes, liver, blood plasma, urine and stool. Patients with acute hepatic porphyrias can suffer from acute neuropathic attacks, which can lead to death when undiagnosed. Therefore, a rapid screening test is required to allow for identification of these patients. In this study, fluorescence spectroscopic measurements were conducted on blood plasma and phantom material, mimicking the composition of blood plasma of porphyria patients. Means to differentiate the occurring porphyrins despite their initial spectral overlap were evaluated.

Materials and methods

Protoporphyrin-IX, Coproporphyrin-I and -III, Uroporphyrin-I and -III were dissolved in Dulbecco's PBS with human serum albumin (HSA), as well as in human blood plasma. Fluorescence spectroscopic measurements were done using a cuvette-based fluorometer (Fluoromax-2, Horiba).

Results

In this study, it was shown that the fluorescence characteristics of porphyrins in human blood plasma could be reproduced when dissolving them in PBS with HSA. Although Uro- and Coproporphyrin show virtually identical excitation and emission peaks for pH=7.4 (buffered in PBS), pH<2.0 shifts the excitation maxima so that a difference of 5nm is apparent.

Discussion

The application of fluorescence spectroscopy as screening test for porphyrias in blood plasma has been described in the literature. However, literature identifying the different types of porphyrins under various conditions is heterogenic. Therefore, this study may help to establish a rapid screening test with spectroscopic differentiation of the occurring porphyrins.

9537-6, Session 1

Multi-spectral interrogation for surface plasmon resonance sensing in complex media

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In this contribution, a multi-spectral imaging technique is introduced to enhance the performances of surface plasmon resonance systems in complex biosensing applications. A 5-LEDs light source is used to perfrom spectral interrogation in real time and in combination with a 2D imaging capabitility, providing robust and multiplex measurements of biomolecular interactions on a biochip surface. The developed system allows the study of antigen-antibody affinity within the context of cancer biomarker analysis in diluted serum.

Surface plasmon resonance (SPR) sensing is a well-established technology in the field of biomolecular studies. With the benefit of a label-free measurement, SPR systems allow multiplex, real time detection of biological species on a biochip format. To provide such performances, most SPR sensors operate in a so-called reflectivity interrogation mode, consisting in measuring the reflectivity changes of a light beam reflected by a thin gold film, the plasmonic biochip. This particular configuration requires both fixed wavelength and angle of incidence, chosen close to the optimal sensitivity of the SPR phenomenon. The choice of these operating conditions is directly dependent on the surface chemistry deposited on the gold layer as well as on the running buffer used during the experiment. As a consequence, this interrogation mode suffers from a limited dynamic range as well as from data dispersion, especially when it comes to real life applications such as biomolecular detection in complex media.

These limitations can be overcome by performing either an angular or a wavelength interrogation on the plasmonic biochip. Both methods provide accurate measurements within a large dynamic range, through the monitoring of the SPR shift. Although commercial SPR systems are currently available in the angular interrogation configuration, the spectral counterpart has not been implemented at the industrial level.

In this contribution, we propose a compact, low-cost illumination device easily adaptable to an existing SPR module. Based on five electronically-switchable light-emitting diodes (LEDs), the developed prototype allows spectral interrogation to be performed in combination with 2D imaging and real time analysis, providing high quality data along with a multiplexing capability. The performances are tested within the study of cancer antigen-antibody affinities in diluted serum.

The SPR phenomenon can be observed at the interface between a thin metal film (usually a gold layer deposited on a glass substrate) and a dielectric medium, such as water, by a coupling between an incident light beam and the free electrons of the metal. The resonance condition being directly dependent on the refractive index of the dielectric medium, any modification taking place at the vicinity of the metal surface will strongly affect the coupling and consequently the optical properties of the reflected beam.

Performed at a fixed angle of incidence, spectral interrogation consists in measuring these changes on the reflectivity spectrum: the shift of the spectral position of the plasmon resonance is then directly proportional to the amount of biomolecules adsorbed on the biochip surface. Most commonly, such measurement is achieved by directing the reflected light to a spectrometer, providing high resolution and extended dynamic range. However, because of this implementation, only a limited portion of the biochip can be analyzed.

An alternative solution is based on a white light source combined with a monochromator, in order to scan the incident wavelength and record the corresponding reflectivity image of the biochip. In previously published work [1], we have shown that only a limited number of scanning wavelengths is needed to achieve higher performances compared to reflectivity interrogation. Typically, using five interrogation wavelengths and a robust fitting model, the resonance profile can be accurately reconstructed from the discrete reflectivity values, in real time and for every region of interest of the biochip. Moreover, the dynamic range as well as data quality are considerably improved.

Following these preliminary results, we developed a compact and low-cost multi-spectral source using five LEDs [2]. The device relies on computer-controlled five-current source combined with a conventional plasmon resonance imaging (SPRi) system. Spectral interrogation is performed using five LEDs that are sequencially switched on/off with a typical current of 50 mA for each LED. To improve the stability of each interrogation wavelength, 10 nm FWHM bandpass filters are used in combination with the LEDs. The multi-spectral emission is collected by

optical fibers combined in a bundle. The output beam is then directed on the gold biochip using the Kretschmann configuration, and imaged on a CCD camera. The reflectivity images, recorded sequencially at each interrogation wavelength, allow SPRi to be performed in real time.

The stability of the multi-spectral illumination device is a key factor for the precision of the measurement. Through a rigourous characterization of the noise sources, we have identified the limits of our system and optimized its performances to meet the requirements of a potential "end-user".

With a dynamic range of 8x10^{-3} RIU and a resolution of 5x10^{-6} RIU, the developed setup exhibits better performances, in terms of robustness, as compared to the classical reflectivity interrogation method. In particular, the large dynamic of the measurement allows the analysis of targets in complex media. Current experiments focus on the study of four potential cancer biomarkers, and the affinities of the respective antibodies, in diluted serum.

In response to a real need for robust, compact and reliable systems to perform biomolecular interaction analysis, we present a five-LEDs SPRi system allowing spectral interrogation, data multiplexing and real time analysis. The developed prototype exhibits high performances and provides better data quality compared to conventional existing SPRi systems based on reflectivity interrogation.

9537-7, Session 2

Minimally invasive translation of targeted fluorescence guidance in gastrointestinal endoscopy *(Invited Paper)*

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Used more extensively than radiology methods, endoscopic white-light imaging is the major diagnostic method in gastrointestinal tract diseases. Despite its wide clinical acceptance, the method is limited by the overall limitation of human vision, i.e. the lack of sensitivity to sub-surface activity or to particular physiological or molecular disease features. Fluorescence molecular guidance in the near-infrared (NIR) can significantly improve these performance characteristics providing a "red-flag" technique for small lesion detection and characterization. However, this potential is currently challenged by the lack of commercially available endoscopes capable of acquiring NIR fluorescence images at video-rates and the regulatory difficulties for human use of targeted contrast agents. We previously addressed both challenges by the development of a platform technology and tracer and in this study we further elaborate onto our platform technology by the implementation of an alternative endoscopic imaging method based on a miniature fiber bundle. This fiber bundle outperforms the optical features of our earlier developments but it is still long and thin enough to be inserted through the accessory channels of conventional gastrointestinal videoscopes. Therefore, it offers a minimally invasive way for the translation of the technology, i.e. no additional time burden or impact to current work flow in conventional color endoscopy is provoked.

9537-8, Session 2

Development of a time-gated fluorescence lifetime microscope for in vivo corneal metabolic imaging

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Metabolic imaging may provide information for early diagnosis of corneal diseases since metabolic disorders are associated with several corneal pathologies. This imaging technique is based on the fluorescence of metabolic co-factors flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NADH) molecules and can be implemented using fluorescence lifetime methods. Being the most exterior eye structure, cornea is prone to non-invasive optical assessment.

We are developing a time-gated fluorescence lifetime microscope for in vivo corneal metabolic imaging. The microscope is based on the Time-Gated Fluorescence Lifetime Imaging (FLIM) technique. Structured illumination is used for improving optical sectioning and lateral resolution. This technique is based on the projection of shifted sinusoidal patterns in the sample plan followed by image reconstruction. We use a Digital MicroMirror Device (DMD) with 685x608 switchable mirrors to project the sinusoidal patterns.

We present results concerning the microscope performance and images of both ex vivo and in vivo corneas. Ex vivo images are from Wistar rats corneas and from human helathy (not suitable for transplantation) and diseased corneas obtained from the Department of Ophthalmology of the Coimbra University Hospital. In vivo imaging was performed in Wistar rats.

9537-9, Session 2

Rapid diagnostic imaging and pathologic evaluation of whole core biopsies at the point-of-care using structured illumination microscopy

Mei Wang, Hillary Z. Kimbrell, David B. Tulman, Joyce Ward, J. Quincy Brown, Tulane Univ. (United States)

Prostate core needle biopsy is often performed to diagnose prostate cancer, where typically 8-14 cores are obtained in a single biopsy session. However, the lack of suitable tools to locate the cancer within the prostate means that, even with the saturation biopsy protocol, initial biopsy may fail to reveal cancers in up to 30% of men. Diagnostic screening of biopsy tissue is also a concern in biospecimen banking for research purposes. In this work we demonstrate the potential of video-rate structured illumination microscopy for accurate, high-throughput, non-destructive diagnostic imaging and remote web-based evaluation of fluorescently stained prostate biopsies in point-of-care timeframes.

We conducted a pilot blinded review study, in which the study pathologist reviewed the randomly numbered VR-SIM images and the H&E slides from 32 frozen prostate biopsies. The accuracy of the pilot study is 90.6%. With the encouraging result from the pilot study, we are extending our study to fresh human prostate biopsies. We have recently implemented rapid autofocus into the system, which allows the microscope objective to automatically track the topography of the tissue and yields even better quality images. We have also established a ultra-high-resolution web-enabled streaming image viewer based on the Internet Image Protocol, which allows multiple pathologists to rapidly review the VR-SIM images and provide a diagnosis from any location using only a web browser. The speed and ease of use of the system could be features in favor of adoption for rapid on-site biopsy imaging and digital pathology consultation using web-enabled image viewers.

9537-11, Session 2

TERS tracking of head-deuterated lipids and invasomes in human stratum corneum

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Friedrich-Schiller-Univ. Jena (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany)

The aim of this research is to understand the penetration mechanism of invasomes (flexible liposomes; phospholipid vesicles) in the stratum corneum (SC) and learning about interactions between invasomes and SC. These lipid vesicles having a size between 50 and 200 nm show best penetration behavior– and are therefore not resolvable by diffraction limited lateral resolution of standard IR- and Raman microscopy. TERS is a promising tool since it is a high-resolution method that provides chemical specificity. The contrast between skin and liposomal lipids - which are very similar in their natural state – is improved by using head-deuterated lipids for invasome preparation. The differentiation by TERS can be achieved utilizing the C-D band at around 2100 cm-1 in the 'cell silent' range of the TERS spectra. The results demonstrate the capabilities of TERS as a technology to investigate vesicle penetration into highly complex SC samples.

9537-83, Session PD

Quantitative evaluation of lipid concentration in atherosclerotic plaque phantom by near-infrared multispectral angioscope at wavelengths around 1200 nm

Daichi Matsui, Katsunori Ishii, Kunio Awazu, Osaka university (Japan)

Atherosclerosis is a primary cause of critical ischemic diseases like heart infarction or stroke. A method that can provide detailed information about stability of atherosclerotic plaques is required. We focused on spectroscopic techniques that can evaluate the chemical composition of lipid in plaques and a novel angioscope using multispectral imaging at wavelengths around 1200 nm for quantitative evaluation of plaques have been developed. The angioscope consists of halogen lamp, indium gallium arsenide (InGaAs) camera, 3 optical band pass filters transmitting wavelength 1150, 1200, 1300 nm, an image fiber having 0.7 mm outer diameter, and an irradiation fiber which consist of 7 multimode fibers. Atherosclerotic plaque phantoms with 100, 60, 20 vol.% of lipid volume concentrations were prepared and measured by the multispectral angioscope. The acquired datasets were processed by spectral angle mapper (SAM) method. As a result, simulated plaque areas in atherosclerotic plaque phantoms that could not be detected by an angioscopic visible image could be enhanced. In addition, quantitative evaluation of atherosclerotic plaque phantoms based on lipid volume concentration was performed up to 20 vol.% of lipid volume concentration. The potential of a multispectral angioscope at wavelengths around 1200 nm was indicated for quantitative evaluation of stability of atherosclerotic plaques.

9537-12, Session 3

Detection of hypercholesterolemia using hyperspectral imaging of human skin *(Invited Paper)*

Matija Milanic, Asgeir Bjorgan, Norwegian Univ. of Science and Technology (Norway); Marcus Larsson, Tomas Strömberg, Linköping Univ. (Sweden); Lise L. Randeberg, Norwegian Univ. of Science and Technology (Norway)

Hypercholesterolemia is characterized by high blood levels of cholesterol and associated with increased risk of atherosclerosis and cardiovascular disease. Xanthelasma, is a subcutaneous lesion appearing in the skin around eyes. Xanthelasma is related to hypercholesterolemia. Identifying micro- xanthelasmas can provide a mean for early detection of hypercholesterolemia and prevent onset and progress of disease.

The goal of this study was to investigate the spectral and spatial characteristics of hypercholesterolemia in facial skin. Optical techniques like hyperspectral imaging (HSI) might be a suitable tool for such characterization as it provides spatial and spectral information simultaneously.

In this study a 3D Monte Carlo model of lipid inclusions in human skin was developed to create synthetic hyperspectral images in the spectral range 400-1000 nm. Four lesions with diameters 0.12–1.0 mm were simulated for 3 different skin types. The simulations were analysed using four algorithms; the spectral Angle Mapper, Minimum Noise Fraction transform (MNF), and the 2- and 3-layer diffusion approximation. The simulated lesions were detected by all methods, but the best performance was obtained by the MNF algorithm.

The results were verified using data from 11 volunteers with cholesterol levels. The face of the volunteers was imaged by a LCTF system (400-720 nm), and the images were analyzed using the previously mentioned algorithms. The identified features were then compared to the known cholesterol levels of the subjects. Significant correlation was obtained for the MNF algorithm only.

This study demonstrate that HSI can be a promising, rapid modality for detection of hypercholesterolemia.

9537-13, Session 3

In vivo estimation of epidermal thickness, melanin and hemoglobin concentrations, oxygen saturation, and scattering using diffuse reflectance spectroscopy

Ricky Hennessy, Will Goth, The Univ. of Texas at Austin (United States); Mia K. Markey, The Univ. of Texas at Austin (United States) and The Univ. of Texas M.D. Anderson Cancer Ctr. (United States); James W. Tunnell, The Univ. of Texas at Austin (United States)

Diffuse reflectance spectroscopy (DRS) is a technique that can be used to noninvasively measure the properties of skin. Typically, skin is approximated as a homogenous slab of tissue; however, this assumption can lead to significant errors in the estimated skin properties. Using of a two-layer model can improve the accuracy of the parameters and can also provide additional diagnostic information such as epidermal thickness. In this study, we collected diffuse reflectance spectra between 430 and 700 nm from the palms, cheeks, forearms, backs, and calves of 80 subjects using a fiber-based probe customized for skin applications. An inverse two-layer model based on the Monte Carlo lookup table method was used to extract epidermal thickness, melanin and hemoglobin concentrations, oxygen saturation, and scattering from the collected spectra. The extracted values agreed with known values for average epidermal thickness in human skin with an average estimated epidermal thickness of 90 ?m. Additionally, estimated melanin concentration was significantly lower on the palm, which agrees with known skin physiology. These results show that we can successfully use DRS to estimate epidermal thickness, melanin and hemoglobin concentrations, oxygen saturation, and scattering in skin. The same method could also be used for the noninvasive diagnosis of skin pathologies.

9537-14, Session 3

Accessing deep optical properties of skin using diffuse reflectance spectroscopy

Anne Koenig, Commissariat à l'Énergie Atomique (France); Blandine Roig, CEA-LETI (France); Jimmy Le Digabel, Gwendal Josse, Pierre Fabre Dermo-Cosmétique (France); Jean-Marc Dinten, MINATEC (France)

Diffuse reflectance spectroscopy (DRS) has been widely used to determine the optical properties of biological tissues in different applications. We have developed a low-cost optical instrument based upon the spatially resolved diffuse reflectance spectroscopy to detect in vivo delayed hypersensitivity reaction (occurrence of an erythema

and an induration) either in a clinical environment or at a point of care. The temporal or frequency methods require an expensive and complex instrumentation thus we chose to design a spatially resolved probe in order to measure diffuse reflectance at various wavelengths (400-900nm). Our objective here is to demonstrate the potential of DRS and of our instrument to analyze multilayered tissues such as skin. In this paper, we firstly introduce the system and the employed methodology. Then, we present results on homemade multilayered phantoms. To conclude, in vivo skin measurements that were carried out on volunteers exhibiting face redness or rosacea are presented.

9537-15, Session 3

Multidimensional spectroscopic data fusion improves precancerous tissue discrimination based on spatially resolved autofluorescence and diffuse reflectance spectroscopy

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This paper proposes a new approach to process spatially resolved bimodal spectroscopic data applied to mouse skin precancerous stages non-invasive diagnosis. In this field, the development of efficient methods of extraction of discriminant features followed by supervised classification step is of a crucial importance.

Our idea is to exploit the spatial resolution dimensions (3 in the present study) to take advantage from the fact that each collecting fiber provides a complementary piece of information for the discrimination. The purpose of acquiring data at three different source-to-detector distances is to combine complementary spatially resolved information i.e. data from three sources on one single skin spot. Such spatial resolution allows to probe skin at three different mean depths in order to get knowledge from the various layers of skin dermis and epidermis.

The first step of our method proposed here consists in applying (i) 2D discrete cosine transform to extract discriminant spectral features from autofluorescence excitation emission matrices and (ii) mutual information to select relevant features from diffuse reflectance spectra. In the second step, these feature sets, which capture information from each collecting fiber (among 3) per modality, is independently classified by one versus all decomposition involving support vector machines, creating a multiclassifier system. In the last step, classification results are fused using the first combination rule of belief function theory to produce one final classification. The proposed method improves overall classification accuracy over independent classifiers. The best recognition rate obtained is 86.1%.

9537-16, Session 3

Towards the application of diffuse optics in the management of hematological malignancies: can diffuse optics probe the bone marrow?

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The abnormal, uncontrolled production of blood cells in the bone marrow causes hematological malignancies that alter bone marrow hemodynamics by mechanisms such as angiogenesis. Non-invasive methods that measure changes in hemodynamics in the bone marrow have a potential impact on diagnosis, prognosis and in treatment monitoring. To this end we have applied diffuse optical spectroscopy to evaluate the feasibility of the noninvasive measurements in the healthy manubrium as a site rich of bone marrow. We have combined time resolved spectroscopy (TRS) and diffuse correlation spectroscopy (DCS) for simultaneous measurement of microvascular blood volume, blood oxygen saturation and blood flow. We have recruited thirty-two subjects and measured four locations on the manubrium of each subject to characterized the optical (absorption and scattering coefficients) and physiological properties (hemoglobin concentration, oxygen saturation and blood flow index) of the healthy human bone marrow. We also investigate the effect of the location of the probed site on the manubrium, body mass index, gender, thickness of the overlying tissue and age on the distribution of the measured parameters. This study is the first step in the path of applying optics for the studies on bone marrow cancer.

9537-17, Session 3

Diffuse optical characterization of the human thyroid

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Thyroid nodules have a palpable nodule prevalence around 5 % in women and 1 % in men. Approximately 5-15 % of thyroid nodules turn out to be thyroid cancer, which is the most common malignant tumor of the endocrine system. Standard ultrasound screening together with ultrasound-guided fine needle aspiration biopsy (FNAB) of the suspicious nodule have limited effectiveness, specificity and sensitivity. It is hypothesized that vascularity and microvascular hemodynamics may be potential biomarkers for different types of thyroid nodules which may improve the sensitivity and specificity of the method. This led us to propose diffuse optical methods that are sensitive to the hemodynamics of the microvasculature to characterize the healthy thyroid gland as a first step towards introducing them for thyroid cancer screening. We have used time-resolved diffuse optical spectroscopy and diffuse correlation spectroscopy to compare the healthy hemodynamics and metabolism of the thyroid glands and neck muscles of twenty-two healthy volunteers. The results show a clear differentiation of the muscle and the thyroid gland, indicating high vascularization of the gland. We will discuss the distribution of the parameters, their relationship to the anatomy and physiology of the patients and introduce anecdotal measurements on thyroid nodules.

9537-48, Session PTues

Non-invasive skin fluorescent spectroscopy: effect of blood saturation in evaluating main metabolic biomarkers

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High demand in reliable bedside or general practices diagnostic devices for monitoring main body parameters in parallel, like blood perfusion, tissue oxygen saturation, and tissue metabolic activity invigorates physicists for developing compact non-invasive systems based on advanced laser technologies: laser Doppler Flowmetry , tissue oximetry and fluorescent spectroscopy, etc. In particular tissue fluorescence can give physicians vital information on diabetes complications, stomach ulcer prognosis, drug toxicity and cancer diagnostics and in general can demonstrate individual wellbeing during emotional or sport stress recovery.

Light passing through the biological tissue can bring comprehensive information about structure and metabolic activity which can be significantly misinterpreted due to individual variety in tissue blood saturation. To minimise the blood shading effects (higher or lower) initial fluorescent data have to be analysed regarding to blood oximetry readings. This can only be performed with multimodal system acquiring complex physiological and biochemical signals from thebody. Here we present results in using multifunctional non-invasive system, LAKK-M, developed by LAZMA (Moscow) where the laser Doppler Flowmetry, blood and tissue oximetry and fluorescent spectroscopy techniques are simultaneously incorporated.

One of most important features of LAKK-M is capability to specifically assay the range of tissue fluorophores: NADH, Flavins, elastin, carotenoid, pyridoxine, riboflavin, porphyrins, etc. The implementation of four LED or laser light emitters, UV (365nm), blue (450nm), green (535nm) and red (633nm) has significantly improved system selectivity in excitation of the fluorophores in vivo. Flavins which can now be selectively exited with 450 nm laser and gave more distinctive fluorescence spectrum avoiding NADH fluorescence misrecognition when exited by only UV light for more precise NADH/FAD ratio calculation in further development of the diagnostic criteria.

Initially for calibration of the modernized LAKK-M device for the NADH/FAD redox ratio as well as other tissue chromospheres we used true solutions of above mentioned biochemicals. Test measurement showed high data reproduction and matched fluorescence parameters of fluorophores well known for skin tissue.

In vivo measurements of skin bio-fluorescence have demonstrated complex spectra after UV, Green, Blue, and Red excitation. These emitters applied separately allowed to distinguish fluorescent peaks more distinctively for individual fluorophores like NADH, elastin, flavins, carotenes, pyridoxine, and porphyrins. Measuring skin fluorescence and blood saturation before and after occlusion (3 min) we discovered significant changes in chromophores fluorescence pointing on necessity to use rather relative to skin nutritive blood flow parameters (blood perfusion (Im) or blood velocity (Vb)) fluorescence values then absolute ones.

9537-49, Session PTues

Detection of early caries by laser-induced breakdown spectroscopy

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To improve sensitivity of dental caries detection by laser-induced breakdown spectroscopy (LIBS) analysis, it is proposed to utilize emission peaks in the ultraviolet. We newly focused on zinc whose emission peaks exist in ultraviolet because zinc exists at high concentration in the outer layer of enamel. It was shown that by using ratios between heights of an emission peak of Zn and that of Ca, the detection sensitivity and stability are largely improved. It was also shown that early caries are differentiated from healthy part by properly setting a threshold in the detected ratios. The proposed caries detection system can be applied to dental laser systems such as ones based on Er:YAG-lasers. When ablating early caries part by laser light, the system notices the dentist that the ablation of caries part is finished. We also show the intensity of emission peaks of zinc decreased with ablation with Er:YAG laser light.

9537-50, Session PTues

Self-controlled zeptomolar detection of cancer marker using nano ELISA-assisted plasmonic biosensor

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For the last decade, research advancements on localized surface plasmon resonance (LSPR) have made the LSPR a sensitive tool for the applications of bio-medical diagnosis and environmental monitoring owing to its high sensitivity, label-free detection, small sample volumes, simple instrumentations and real-time detection. The LSPR biosensors are sensitive to changes in the refractive index near the interface between a metal nanostructure and an ambient medium. We have utilized the LSPR biosensor for detection of biological molecules.

In this study, we demonstrate a highly sensitive detection of AFP(?-fetoprotein) protein (liver cancer marker) in human serum using the LSPR biosensor.

Gold nanodot array (NDA) on a glass wafer were fabricated by UV NIL. After the NIL process using a film stamp and the removal of residual layer via oxygen plasma etching, metal films were deposited using an electron-beam evaporator, followed by the lift-off step. Consequently, the gold NDA was realized on 5-inch glass wafer and the pitch, diameter and height of nanodot were 300nm, 150 nm and 20 nm, respectively.

We employed observation of LSPR spectra via back-reflection, which provides a stable measurement of LSPR because a probe light does not pass a bio-sample. In addition, one channel among two flow channels was used a control channel, the NDA surface in which was modified with bovine serum albumin, not antibody. Therefore, the NDA in this control channel does not response for the AFP antigen in serum and hence, any additional reference sample is not required for quantification of antigen. After antigen-antibody reaction, the precipitation reaction of 5-bromo-4-chloro-3-indolyl phosphate p-toluidine/nitro blue tetrazolium (BCIP/NBT), catalyzed by alkaline phosphatase, was achieved. As a result, the enzyme-catalyzed precipitates were accumulated only on the surfaces of gold nanodots (“Nano-ELISA”), which led to a drastic increase in local refractive index on the surface of the nanodot and amplification of change in LSPR peak wavelength. Consequently, we could detect AFP in 50uL human serum with limit of detection (LOD) of 0.7 zeptomole (7x10-22 mole).

This method also offers a sensing platform for studying interactions between protein-protein and DNA-protein and could also be easily adapted for ultra-sensitive detecting other biomarkers and pathogens etc.

9537-51, Session PTues

The influence of silver nanoparticles and ions on light-scattering properties of bacteria cells Desulfuromonas acetoxidans

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Concentration changes and relative content of bacterial cells of D.acetoxidans in the intervals of sizes 0,2 - 2,0 μm under the influence of nanoparticles and ions silver have been investigated. Correlation between these changes of light-scattering properties of bacterial cells and growth abilities of bacteria D. acetoxidans under influnce of silver nanoparticles and ions has been shown. The intensity of processes the change of indexes of the antioxidant system the cells of D.acetoxidans at influence of silver nanoparticles and silver nitrate was the aim with work. The influence of various concentrations of silver nanoparticles and silver nitrate on enzymatic activity of catalase and reduced glutathione synthesis by D. ?cetoxidans cells under their cultivation with fumarate addition and with absence of sulphur has been determined. Specific catalase activity increased with enhancing of concentration and duration of bacterial cultivation under the addition of this salt. The highest specific catalase activity was determined on the second day of bacterial growth under the influence of all concentration range of investigated metal salt. The reduced glutathione content under silver nitrate and silver nanoparticles exposure varied depending on the cultivation time and metal concentration. The maximum reduced glutathione content has been observed. The mechanism of action the silver on bacterial D.acetoxidans cells has been description.

9537-52, Session PTues

Wet chemical synthesis of quantum dots for medical applications

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With this work we want to show the progress of our research related to the interaction of quantum dots in living cells.

9537-54, Session PTues

Assessment of mango fruit ripening using fluorescence spectroscopy

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We are presenting non-invasive assessment of mango ripening using fluorescence spectroscopy. Fluorescence spectra have been acquired from peel of Dusehri mango, a popular cultivar of Pakistan, using a blue LED at 460 nm as an excitation source. It has been observed that instead of chlorophyll fluorescence peak intensity at 680/740 nm, carotenoids fluorescence peak intensity at 540 nm can be used for fruit ripening/maturity assessment. Similar observations have been found in fluorescence spectra of Dusehri pulp using the same experimental setup. It has been further demonstrated that for Langra mango, where peel remains green even at fully ripped stage, chlorophyll based maturity assessment gives false prediction. However, the carotenoid fluorescence signatures are more reliable spectroscopic markers and give more accurate prediction about the ripening of Langra.

9537-55, Session PTues

Two dimensional spectral camera development for cartilage monitoring

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Summary

In a joint research project with the University Medical Center Ulm, the University of Applied Sciences Ulm is developing a bioreactor for tissue engineering of facial cartilage with a spectral camera for monitoring cartilage growth and composition. This camera will record low intensity fluorescence spectra for each position of a cartilage implant in the spectral range from 380 to 500nm to identify cartilage compounds. Currently it is possible to record spectra of a line of several hundred points. The development of a 2-D system for recording of ca. 100 x 100 spectra has just started and produced preliminary results. After optimization of the optical setup, first spectra of cartilage will be recorded and analyzed.

Introduction

Facial cartilage defects can lead to grave psychological or physiological consequences for the patient. Defects should ideally be replaced with autologous cartilage material and tissue engineering is an appropriate solution to produce this personalized implant from the patient´s own cells [1, 2]. However, tissue engineering is a new research field with many experiments necessary on the path to suitable implants. Cartilage samples must be analyzed for their quality but existing examination methods destroy the sample, so it can´t be implanted. The goal of this project is to develop a spectral camera to identify cartilage compounds like collagen I & II, elastin and glycosaminoglycan by fluorescence spectra, since no commercial system offers sufficient sensitivity in the essential blue and violet spectral region. The development is based on an idea of Habel et al. [3, 4]. The first step is a 1-D spectral camera for analyzing one line on a cartilage sample. The next step will be an extension to a 2-D system by changing aperture and grating and performing a more complex image data processing.

Experimental Setup and Results of the 1-dimensional Spectral Camera

Fluorescence excitation of the cartilage samples is either performed by a 340nm or a 365nm LED (Fig. 1). The first lens creates an image of the sample on a slit aperture. By a two lens system this slit is imaged on a PE-cooled black and white CCD chip. A blazed line grating between both lenses causes a diffraction pattern. From this pattern a complete spectrum can be calculated for every position of the slit or the line sample (Fig. 2a). This means that this approach delivers 550 fluorescence spectra in the region of 360 to 510nm (Fig. 2b) with a spectral resolution of 2 - 3nm with a high NA of 0.3.

Development of 2-dimensional Spectral Camera and first Results

For the 2-D setup the slit aperture and the line grating were exchanged by a rectangular aperture and a cross grating. This results in diffraction patterns like the one in Fig. 3 of an energy saving lamp. With the Expectation–maximization algorithm of [3] spectra like the one in Fig. 4 can be calculated for more than 100 x 100 positions on cartilage samples.

Conclusions

A 1-D spectral camera for low intensity cartilage fluorescence detection has been developed. A 2-D variant for more than 100 x 100 spectra is under construction. The preliminary results demonstrate that this technical approach is working, but the system has to be optimized for the spectral region of 380-500nm, where most cartilage components show their peak fluorescence emission.

In a future step the cartilage composition of different samples will be calculated by a chemometric analysis and compared to laboratory results.

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9537-57, Session PTues

Synthesis and study of bio-conjugated GaO(OH) nanoparticles : a potential candidate for usage in modern medical imaging techniques

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68Ga is a positron emitting radioisotope that is effectively used for specific applications in modern imaging techniques (like Positron Emission Tomography(PET). We synthesized uniform sized bio-conjugated Gallium Oxide hydroxide nanoparticles conjugated with ?-cyclodextrin, that can be suitably doped with 68Ga. The compound is envisaged to be useful for cancer diagnosis using molecular imaging techniques(like Positron Emission Tomography(PET)). We also studied the structural and optical spectroscopic properties of the synthesized nanoparticle.

GaO(OH) nanoparticles were synthesized through a simple precipitation technique using a wet chemical route at physiological pH(7-7.5). This can be easily taken up by the living system due to the similar chemical properties of FeIII and GaIII [1] . However, in order to selectively allow the malignant cells to take up the nano particles, GaO(OH) was conjugated with ?-cyclodextrine[2], a giant sugar molecule that is rapidly taken up

by the dividing cells. It is worth to mention, that the surface conjugation of the synthesized nanoparticles also enhanced the water solubility of the nanoparticles, that is envisaged to be used as a drug. We have also studied the photo-absorption and emission spectra of GaO(OH) and bio-conjugated GaO(OH).

?-cyclodextrine was selected for the surface conjugation of the nanoparticles for its high water-solubility, bio-compatibility, easy uptake by the dividing cells, and good renal clearance in animals[2].

GaO(OH) and bio-conjugated GaO(OH) was first studied with FTIR-spectroscopy, IR-spectroscopy and X-ray diffraction. The FTIR spectrum clearly revealed that the bio-conjugated nanoparticles carried a different spectral signature, than the bare ?-cyclodextrine or GaO(OH), through the changes in the –OH bending and stretching mode frequencies. The X-ray diffraction pattern of GaO(OH) and GaO(OH)-CD were found to be very different, confirming the successful bio-conjugation of the nanoparticles. Further UV-Vis spectroscopy revealed that the spectra of GaO(OH) and bio-conjugated GaO(OH) has considerable differences in their spectra, with a shift of ?max . The wider FWHM of the absorption peak in bio-conjugated GaO(OH) entails a non-uniform distribution of sizes of the nanoparticles; whereas the bio-conjugated nanoparticles showed a more or less uniform size distribution. The same was also verified from the TEM studies. In case of bio-conjugated GaO(OH), the size was found to be between 5-6nm and the crystallinity was ascertained through the fringe structure. Bandgap measurement of the nanoparticles were also performed. GaO(OH) had a bandgap of 5.28eV whereas bandgap for bio-conjugated GaO(OH) was found to be 5.37eV. The fluorescence spectrum of GaO(OH) and bio-conjugated GaO(OH) was also studied. The emission spectra for both the compounds had peaks centred at 238nm and 274nm. But quenching of intensity for the 274nm peak was observed after conjugation of ?-cyclodextrine with the nanoparticle. However, the intensity of the 238nm peak remained unchanged. This phenomenon has been further studied for resonance energy transfer through Froster distance. We infer, the quenching of intensity is due to conjugation of surface electrons in the conduction band of GaO(OH) by ?-cyclodextrine via Froster resonance energy transfer. This is another proof of the conjugation of GaO(OH) with ?-cyclodextrine.

Further, in vitro studies of the cytotoxic effects of the surface conjugated nanoparticles are in progress.

9537-58, Session PTues

Terahertz time-domain spectra of biological tissues

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The terahertz is an electromagnetic wave with non-ionization and low average power, therefore it is thought not to be hazardous to biological tissues. The terahertz waves can excite the vibration mode and rotation mode of macromolecules, and both amplitude and phase information of samples which are detected and contrasted could be gotten. Compared with visible and infrared light, it also has the advantage of low scattering and that the surface character and depth pattern of samples can be acquired at the same time, which makes the terahertz detection in the field of biomedicine possible to measure the tissue in vivo without any damage.

With the development of terahertz radiation and detection technology, intermolecular transition models can be detected. DNA, RNA and protein molecules' vibration frequency lie in terahertz range, and information of these molecules can be gotten coherently using terahertz radiation. Terahertz imaging can measure skin inflammation, such as dermatitis, eczema and psoriasis, etc. It is reported that people in Japan and England image human tissues in vivo and vitro by the technology of terahertz radiation and distinguish diseased, normal and inflamed tissue in quality. THz-CT imaging has the spatial resolution of sub-millimeter, and it is possible to replace X-CT with THz-CT imaging. In 2004 and 2006, various cancer types and organs were studied by Anthony J. Fitzgerald et al, which means that THz imaging can be used to give the tumor margins in fresh tissues. However, terahertz imaging can analyze the difference between tumor tissues and normal tissues at present.

In this article, THz transmission and reflection spectrums of samples are tested and analyzed based on THz-TDS(Terahertz time Domain System), including glucose solution and different kinds of biological tissue, etc. The optical relations of different samples in reflective system are acquired, and formulas and spectrum results in two kinds THz systems are contrasted and discussed.

The transmission system is based on the Z-3 Time Domain Spectrometer, in which the mode-locked Mai-Tai laser generates femtosecond pulse with 800 nm center wavelength, pulse width less than 120 fs, pulse power 1w and repeating frequency 80 MHz. The frequency of chopper is 1.1 kHz. Incident beam with 45° angle arrive at the p- <100> of GaAs-on- InAs crystal after the chopper, which produces THz pulse with the frequency range from 0.2 to 3 THz. The pulse beam through a parabolic mirror (PM) focuses on the sample. The THz transmitting beam of sample is finally on the <110> ZnTe crystal together with the probing beam. Polarization change of output light contains the sample information of amplitude and phase based on the linear electro-optic Pockels effect, which is detected by balanced photodiodes. The delay line with the 200 Hz saw tooth oscillating scans the entire THz pulse in order to obtain the THz field as a function of delay and amplitude. To eliminate the influence caused by the absorption of water vapor, the terahertz beam path is completely put into the nitrogen environment to keep a relative humidity less than 4% and enhance the SNR.

Terahertz reflection system is familiar with the one above and sample information is detected in upper reflective mode.

Data processing methods of transmission mode are based on the physical models of acquiring the THz optics parameters of samples. The refractive index, the absorption coefficient and the extinction coefficient with the change of frequency can be calculated.

In reflective system, the refractive index and extinction coefficient of quartz slice should be known for further learning optical characteristics of sample. Then, the refractive index and extinction coefficient of sample are deduced too.

By measuring glucose solution THz spectrum, the transmittance of the different concentration sample is obtained. With concentration rising, the transmittance of the samples increased in a linear manner and the change rate of transmittance is 28.7 /%. Using this relation, the concentration of unknown solutions can be deduced.

The refractive indexes at different frequencies are decreased with the increase of the glucose concentration, which has a good negative linear relationship between them, which coincides with the existing solution theory model.

The spectrum characteristics of different biological tissues with different thickness are acquired, and refractive indexes, absorption coefficients of several tissues at 0.51THz and 0.99 THz are given. The conclusion is that thickness has basically no effect on the refractive index of bio-tissues, and absorption increases with the increase of thickness. Different tissues have different refractive indexes in the THz regime, so different tissues can be distinguished quantitatively in the experiment. In addition, the double Debye model of sample is given, and the approximate parameters of tissue are obtained based on its dielectric constant curve with the change of frequency. These works have potentially practical value in distinguishing among cancer tissues, diseased tissues and normal tissues in quantification.

9537-59, Session PTues

Optical methods for monitoring of the demineralization process of bone grafts during their manufacture

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Demineralization process is an important step in obtaining of bone biomaterial for replacement of bone tissue defects. Clinically proven, the regeneration rate is higher in the transplantation of bone partially demineralized biomaterial. In turn, correctly selected of demineralization degree provides the perfect combination of osteoinductive and osteoconductive properties for successful bone regeneration.

36 and 39 samples of three-dimensional material of solid cortical and cancellous bone tissue of «Lioplast» ® series (TU-9398-001-01963143-2004) with different degrees of demineralization were used as objects

of study. Bone was placed in HCl solution of 1.2 normality to obtain the demineralized biomaterial.

In this paper, Raman spectroscopy (RS) was used as an effective method for studying the mineral component of bone biomaterial. Change of the mineral composition of the samples also was monitored by scanning electron microscopy. Raman spectra were processed in program Mathematica'8. The method of polynomial approximation was used to suppress the fluorescence background and differentiate the Raman spectra. It is also important to take into account the engraftment of bone biomaterial, so an additional control for the process of recipient cell integration into the bio-carrier was realized using confocal fluorescence microscopy.

As a result of experiments, the Raman spectra for the cortical and cancellous human bone with different degree of demineralization were obtained. Shown, that the demineralization process can be controlled using the ratio of (??4)3- and amide I, as well as the ratio of (??3)2-substitution B- and A-type. Depending on demineralization degree, spectral features of bone tissue collagen were investigated by analyzing the spectral region 1200-1460 cm-1 and 2880-3000 cm-1. Raman spectroscopy results of change in the content of the mineral component with demineralization degree were compared with the results of scanning electron microscopy.

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Combined analysis of whole human blood parameters by Raman spectroscopy and spectral-domain low-coherence interferometry

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INTRODUCTION

Blood tests are one of the most fundamental medical exams, because the specific changes of its chemical composition (such as the presence of disease markers or elevated/decreased levels of some compounds) are associated with specific pathological conditions. Moreover, the hematological parameters may reflect on the overall health state of a patient, as well as help in deciding further examinations which lead to a diagnosis. Thus, fast and accurate detection of blood parameters is of utmost importance in medical practice. Monitoring blood parameters is performed during medical procedures such as surgeries, dialyses, or on a daily basis by chronically ill patients, as well as from time to time for regular health check-ups. Therefore, the employment of optical technologies, which offer fast and reliable measurement will bring significant improvement in clinical as well as routine blood analysis. We suggest that the appropriate combination of several optical methods should allow for simultaneous analysis of multiple components in whole blood without the need for conventional sample processing, such as centrifuging and adding reagents.

The objective of this study is the investigation of blood parameters by complementary optical methods: Raman spectroscopy (RS) and spectral-domain low-coherence interferometry (SD-LCI). Simultaneous use of these optical methods provides additional information on sample properties, because low-coherence interferometry measures optical properties (refractive index, dispersion) of the investigated object, while Raman spectroscopy gives information about its molecular composition. Combined analysis of Raman spectra and LCI interferograms shall provide more valuable information than that obtained by each method alone, because of the synergic effect when using complementary methods. In this case, both methods exploit their advantages, while their drawbacks are alleviated, since they are sensitive to different parameters of the same sample. Such parameters might have the same underlying origins, therefore the mutual relationship between chemical and physical properties may be investigated and understood.

METHODS

For the preliminary investigations we have utilized two separate set-up for LCI and RS, since the integration of those two modalities into a single set-up is a complex engineering task, worthy of its own research. Therefore the measurements were conducted on separated set-ups, while the focus of the research lies in the analysis of spectra from different modalities and assessing the usefulness and validity of undertaken approach.

For spectral-domain low-coherence interferometric measurements, a reflection-mode fiber-optic Fabry-Perot interferometer was implemented. The fibre-optic Fabry-Perot interferometer was formed by the uncoated end surface of the single-mode fiber and the silver mirror, with reflectivity 0.2 and 0.995, respectively. The fiber is attached to a translation table equipped with a differential adjuster changing the cavity length. In this set-up the interference occurs in the cavity formed between the beams reflected from the fiber-end surface and a parallel mirror. The beam propagates through the sample, placed between these surfaces, and undergoes phase-shift, and consequently spectral band shift, which depend on the refractive index of a sample. The implemented system comprises of four broadband SLD sources with center wavelengths of 810 nm, 970 nm, 1310 nm, and 1550 nm, a fiber-optic coupler, an adjustable Fabry-Perot cavity formed between the fiber-end and a mirror surfaces mounted on a micromechanical stage, and an optical spectrum analyzer (OSA) (Ando AQ6319, ANDO Japan) with wavelength resolution better than 20 pm and a dynamic range of 70 dBm [1-3]. The optimum–maximum value of visibility of the measured spectra has been performed for the cavity length of 200 ?m.

The Raman spectroscopy was performed on a system based off a pre-commercial Raman spectrometer Ramstas developed by the VTT – Technical Research Centre of Finland [4-6]. Excitation wavelength, of 830 nm, provides a tradeoff between the reduction of fluorescent background and the Raman spectra intensity for biological samples such as blood. The continuous wave excitation signal from a diode laser, equal to 100 mW on the sample, is delivered by a fiber optic probe with embedded laser line filter. The working distance of the probe was set at 2.5 cm. Collected scattering signal is transmitted to the spectrograph by the collection part of the probe with dielectric low-pass filter. The L-shaped axial transmissive spectrograph is equipped with holographic transmission grating with high throughput which is connected to thermoelectric cooled (250K) CCD array detector. The spectral resolution of the setup equals about 8 cm 1. Due to the complex and distorted nature of the Raman spectra of biological samples, chemometric pre-processing of the raw spectra was applied for de-spiking, de-noising, background removal and normalization of the spectra.

EXPERIMENT

A vast number of 2-mL whole human blood samples were collected by the Gda?sk Blood Donor Centre from a wide and representative group of healthy blood donors with variable parameters, most notably the hematocrit level in the samples varied from 30% to 50%. The Gda?sk Blood Donor Centre also performed reference measurement of the hematological parameters, such as hematocrit level in each blood sample using the standard procedures. The subsequent experimental process was performed carefully following all of the relevant laboratory procedures, especially controlling the temperature of blood samples. Both measurements were performed within 24 h from the donation to ensure correct and consistent results. For the Raman spectroscopy, 50 µl aliquots of blood were deposited in the form of a drop on an aluminum substrate and measured in a dark chamber. For the LCI measurements, the sample was introduced into the F-P cavity which filled it all up.

RESULTS

The measurements on LCI set-up were focused on the determination of relationship between the spectral separation of the interference fringes in the recoded interferograms and the amount of hematocrit in patients' blood. Validation of the measurements of HCT levels in blood was based on assumption that hematocrit changes cause changes in the real part of the refractive index of blood. Comparing the spectra with variable amounts of hematocrit it is possible to note the shift of the fringe pattern was indeed caused by the change in the refractive index of blood in the interferometer cavity. Experiments using blood samples from healthy volunteers yielded a linear relationship between the sensor's output and the HCT level in the range from 30% to 50%. The difference between the linearized sensor output and the hematocrit level measured with the reference method was below 1%, while the determination coefficient of the linear model was quite high (R2 = 0.978), which is close to value obtained during the validation of the sensor [7]. Moreover it was confirmed that despite of increase of imaginary part of the refractive index (signal losses) visibility of the fringes in interference spectra is sufficient to determine peak positions and the spectral separation. Based on the carried out experiment, it is safe to conclude that the relationship between the response of the investigated sensor and the HCT level measured by the standard method in healthy persons, is statistically

significant and proves the ability of LCI to measure HCT levels in whole human blood. Further step in the improvement of this method lies in the assessment of usability of the method also for pathological values of HCT. Also, the translation of the method for other blood parameters is under investigation.

Due to the strong absorption of blood, high scattering, and high levels of fluorescence background it was necessary to use background correction during measurements and pre-processing procedures to improve the quality of the Raman spectra. We have utilized a fast Fourier filtering for spike and noise removal, along with Savitzky-Golay smoothing. The spectra were normalized using sample normal variate (SNV) algorithm. Such prepared spectra were qualitatively analyzed, to locate and identify changes in the samples of whole blood from different patients. The analysis yielded information about the behavior of the spectra, which was exhibited by changes in the intensity of several spectral regions, caused by e.g. hemoglobin concentration, iron oxidation state, glucose. As there is no straight relation between change of spectra and blood parameters, two-modal multivariate calibration is applied.

We conclude, that a quantitative analysis may be carried out with grater dataset, which will allow statistical approach by employing multivariate models and classification algorithms [8-11], such as principle component analysis (PCA), partial least-square (PLS) regression, or support vector machines (SVM). Such approach will enable to determine the quantities of specific compounds in blood.

CONCLUSIONS

A series of in-vitro measurements were carried out to assess sufficient accuracy for monitoring of blood parameters. Vast number of blood samples from different donors with various parameters have been measured in order to achieve statistical significance of results and validation of the methods. Preliminary results indicate the benefits in combination of presented complementary methods and form the basis for development of a multimodal system for rapid and accurate optical determination of selected parameters in whole human blood. Future development of optical systems and multivariate calibration models are planned to extend the number of detected blood parameters and provide a robust quantitative multi-component analysis.

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9537-61, Session PTues

Contrast enhancement based on entropy and reflectance analysis for surgical lighting

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For surgical illumination, it is important not only to provide bright lighting but also to easily identify the living body tissues for surgery. Many researches on tissue contrast enhancement using LED lighting have been discussed, such as surgical retractor with RGB-WHITE LEDs and specific narrow-band endoscopic imaging to enhance tissue features. In this paper, we proposed a method to obtain optimal OR lighting for contrast enhancement. The whole procedure contained wavelength selection and image evaluation. A pilot study was done to select wavelengths based on the optical properties of hemoglobin (HGB) and results showed that light with the selected wavelengths (525, 450 and 615nm) gave the better tissue contrast than the commercial white LEDs in different CCTs (3000K, 4100K and 6000K). However, the analysis of HGB didn't take into account optical properties of background tissues. Then we selected wavelengths through spectral reflectance comparison between HGB and the background. And we found the wavelength band 450-550nm made the most contribution to the identification of tissues with blood. Then white light was mixed by color LEDs with 479, 506, 522 and 593 nm. Images of different tissues were captured under compounding light and commercial white light separately. Meanwhile, we created a new evaluation function integrated with entropy analysis and gray level contrast to analysis these images. We found that compounding light had better enhancement than white LEDs and would be helpful to identify tissue features.

9537-62, Session PTues

Luminescence monitoring of particle delivery into rat skin in vivo

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Upconversion microparticles [Y2O3:Yb, Er] and quantum dots (CuInS2/ZnS coated by PEG-based amphiphilic polymer) delivery into rat skin using the fractional laser microablation has been studied in vivo. The results were obtained by luminescence spectroscopy, OCT and histochemical analysis. We can conclude that upconversion microparticles are appropriate for deep imaging of skin.

9537-63, Session PTues

Development of a movable diffuse reflectance spectroscopy system for clinical study of esophageal precancer

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Diffuse reflectance spectroscopy (DRS) has been used to determine optical and physiological properties of superficial tissue. This study aims to develop a rapid and non-invasive method for the detection of esophageal precancer by in-vivo measurements of diffuse reflectance spectra through an endoscope. We constructed a movable imaging spectrograph-based system and a contact probe consisting of fibers with several source-to-detection separations (SDS) to measure spatially-resolved diffuse reflectance spectra from superficial tissue. We designed a perpendicular probe and an oblique probe in which the distal end of the fibers was beveled at 45 degree. The goal is to estimate optical properties of mucosa and investigate correlations between the estimated properties and precancerous changes in the mucosa. A previously developed iterative curve-fitting GPU-based inverse Monte Carlo model was used to extract optical properties from measured spectra. We validated the system with two-layer tissue phantoms, took in vivo measurements on the buccal mucosa of four normal volunteers, and extracted optical parameters including the reduced scattering coefficient in the epithelium and stroma, the absorption coefficient in the epithelium and stroma, oxygen saturation, concentration of hemoglobin, concentration of collagen, and thickness of epithelium. In results, the calibrated spectra of two-layer phantom are compared with the simulated spectra obtained by

Monte Carlo forward model using phase functions calculated from the Mie theory, and the percentage root-mean-square errors (RMSE%) between experimental and simulated spectra are around 16-20%. In the in-vivo experiments, the RMSE% between measured spectra and best-fit spectra of four volunteers were 10%, 17%, 13%, and 13%.

9537-64, Session PTues

Fluorescence ratiometric classifier for the detection of skin pathologies

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Detection of pre-malignant lesions in skin could improve in reducing the 5 year patient mortality rates and greatly improve the quality of life. Current gold standard for the detection of skin pathologies is a tissue biopsy and followed by a series of steps before it is examined under a light microscope. The disadvantage with this method is its invasiveness. Light based biomedical point spectroscopic techniques offers an adjunct to invasive tissue pathology. In this context, we have implemented a simple multiplexed ratiometric approach (F470/F560 and F510/F580) based on fluorescence at two excitation wavelengths 378 nm and 445 nm respectively. The emission profile at these excitation wavelengths showed a shift towards the longer wavelengths for melanoma when compared with normal and nevus. At both excitation wavelengths, we observed an increased intensity ratios for normal, followed by nevus and melanoma. This intensity ratios provide a good diagnostic capability in differentiating normal, nevus and melanocytic skin lesions. This method could be applied in vivo because of the nature of the simplicity involved in discriminating normal and pathological skin tissues.

9537-65, Session PTues

Estimating the relative concentrations of nicotine amide adenine dinucleotide and flavin adenine dinucleotide in urothelial carcinoma using multivariate curve resolution

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In the recent years, several clinical studies by different research groups have demonstrated the potential fluorescence spectroscopy for the detection of epithelial pre-cancers. This technique provides inputs related to biochemical and morphological composition of a tissue when tissue changes its state. The current gold standard technique for the detection of urothelial carcinoma is white light cystoscopy. On the downside, this method is invasive, causing trauma to the patient and also misses flat tumors such as carcinoma in situ. In order to address these limitations we have implemented fluorescence spectroscopy at two different wavelengths 378 and 445 nm to probe nicotine amide adenine dinucleotide and flavin adenine dinucleotide in urothelial carcinoma. Several studies have indicated that the composition of these fluorophores are found to change during the progression of tumor. We intend initiate a study to explore the relative changes in the concentrations of these two fluorophores using multivariate curve resolution (MCR) between normal and tumor bladder tissues. MCR measures the intrinsic spectral profile the constituents in a turbid multi-component media like biological tissue when there is a no further priori knowledge is available. Our prospective initial findings suggest modulations in the fluorescence spectral profile at both excitation wavelengths which could be changes in the tissue optical absorption and scattering properties. The outcomes of this study will be presented further.

9537-66, Session PTues

Lensless imaging platform for visualization of subsurface pattern: design, and phantom experiments

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Adequate blood perfusion is essential for functioning of healthy tissue. Disturbance in structural and functional microcirculation of superficial microvasculature has an important prognostic value in serious clinical conditions such as septic shock, or cancer. Heterogeneity (maldistribution) of blood flow and reduction in capillary density increases the diffusion length of oxygen, creating hypoxic regions of poor tissue oxygenation. Hence, subsurface microvascular capillary density is an indicator of tissue perfusion. For clinical imaging and diagnostics of microcirculation pathogenesis, acquisition of large FOVs with high spatial resolutions to assess the margins of abnormal microvascular architecture becomes a critical clinical task. The goal of this research is the development of a portable, inexpensive, lensless imaging platform for visualization of subsurface capillary network. Based on a CMOS chip and fiber-optic conduits (FOC), in-lab designed imaging unit was tested on microfluidic phantom with embedded narrow periodic channels (CH) filled with hemoglobin extracted from red blood cells. Images obtained from the surface of the phantom support the feasibility of application of lensless CMOS imaging chip-FOC device for imaging of subsurface structures via dense optical media.

9537-67, Session PTues

Metabolites of Fusarium oxysporum cause structural and Raman scattering changes in immunocompetent rats

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The secondary metabolites produced by fungi are important because can produce biological response even in the absence of microorganism. Fusarium oxysporum is the second most common cause of invasive infections in immunocompromised patients and the increased incidence of immunocompetent patients infected by Fusarium has been an important subject. The aim of this work was to monitor by Raman spectroscopy and histopathological study the response of the cutaneous tissue in immunocompetent rats, after the contact with metabolites produced by Fusarium oxysporum. A crude extract (CE) taken from the fungal culture was intradermally injected in rats. After three, six, 12 and 24 hours, samples of the skin was collected and analyzed by FT-Raman or processed for paraffin embedding and HE staining to histologic study. The most relevant degenerative changes were observed at six and 12 hours. In these periods the inflammatory and degenerative response was more intense, with loss of epidermis, vascular hyperemia, disorganization of the dermal matrix. The spectral region between 3040-2750 cm-1 showed most significant differences. The most prominent spectral differences,

after injection of CE, occurred within 6 hours when the dermis spectra were similar to epidermis characterized for absence of peaks at 3010 cm-1 and 2892 cm-1, and decrease the intensity of the peak at 2852 cm-1 compared to the peak 2862 cm-1. The similarity between the dermis and the epidermis spectra suggests that CE may have caused physicochemical changes. The secondary metabolites stimulated, even in the absence of the fungus, inflammatory response and important physicochemical changes.

9537-68, Session PTues

Binding of cationic porphyrins to zeolite nanoparticles

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In photodynamic therapy of tumors and photodynamic inactivation of bacteria nanoparticles of zeolites are widely used to enhance the effectiveness and targeted delivery of photosensitizers. The first attempts to combining of zeolites with porphyrins showed that porphyrins are associated with outer surface of zeolites better than encapsulates at the internal pores. Further studies showed that cationic porphyrins well encapsulates in the inner region of zeolites by ion exchange mechanism, whereas anionic porphyrins are not embedded, and the neutral only in a trace amounts. Via spectral methods the binding of nanoparticles of zeolite with a number of cationic porphyrins we have investigated. For study of the mechanism of binding the zeolite nanoparticles with porphyrins we selected 5 types of cationic porphyrins so that they differed by hydrophobicity (with the various peripheral groups), by the presence of hydroxyl groups (for studying the possible hydrogen-bond with the surface of the zeolite), by various central metal atoms (Zn and Ag), by various provisions of the lateral functional group (third or fourth position in the pyridyl ring). To study the ionic bond the zeolite with porphyrins we investigated also the interaction of nanoparticles with anionic photosensitizers - chlorin e6 and Al-phthalocyanine. Binding studies of these 7 porphyrins gives grounds for assuming that the main mechanism of binding zeolite nanoparticles with porphyrins is an ionic bond. We investigated the binding of such nanocomposites to Gram (+) microorganisms (St. aureus) and was shown that the efficiency of such compounds is higher compared with a free porphyrins.

9537-69, Session PTues

Scale-selective analysis of myocardium polarization images in problems of diagnostic of necrotic changes

Yuriy A. Ushenko, Yuriy Fedkovych Chernivtsi National Univ. (Ukraine)

By tradition, the processes of transforming optical radiation of phase-inhomogeneous objects and media are considered, as a rule, in a statistical approach (theory of radiation transfer, Monte-Carlo modeling). Among the most spread traditional methods for studying the scattered light fields, one can separate the following independent directions: “scalar” (photometry and spectrophotometry) and “vector” (polarization nephelometry, Mueller-matrix optics). Using these approaches, determined are interrelations between the sets of statistical moments of the 1-st to 4-th orders, correlation functions, fractal dimensionalities that characterize phase-inhomogeneous or rough surfaces and coordinate distributions for phases, azimuths and ellipticity of polarization in their laser images.

In parallel with traditional statistical investigations, formed in recent 10 to 15 years is the new optical approach to describe a structure of polarizationally inhomogeneous fields in the case of scattered coherent radiation. The main feature of this approach is the analysis of definite polarization states to determine the whole structure of coordinate distributions for azimuths and ellipticities of polarization.

This work is aimed at ascertaining the possibilities to diagnose and classify phase-inhomogeneous layers (PhIL) of myocardium of the patients who died of coronary artery disease (CAD) and acute coronary insufficiency (ACI) by determination values and ranges for changing the statistical (moments of the 1-st to 4-th orders), correlation (autocorrelation functions) and fractal (logarithmic dependences for power spectra) parameters that characterize coordinate distributions for polarization-singular states in PhIL laser images.

1. Analyzed in this work are the main physical mechanisms providing formation of polarization singularities in laser images of PhIL with surface, subsurface and bulk light scattering.

2. Offered are statistical, correlation and fractal parameters for polarization-singular estimating the optical properties inherent to PhIL of all types.

3. Determined are the ranges for changing the set of criteria that characterize distributions of the amount of polarization-singular states in laser images, which enabled us to realize both “intergroup” classification and differentiation of optical properties related to PhIL of various types.

4. We have demonstrated diagnostic efficiency of the wavelet analysis applied to coordinate distributions for polarization maps in laser images of patients with CAD and ACI.

9537-70, Session PTues

System of polarization correlometry of polycrystalline layers of bile in the differentiation of systemic pathologies

Olexander V. Dubolazov, Yuriy A. Ushenko, Yuriy Fedkovych Chernivtsi National Univ. (Ukraine)

Among diverse optical-physical methods of diagnosing the structure and properties of the optical-anisotropic component of various biological objects a specific trend has separated – multidimensional laser polarimetry.

Our research is aimed at designing the experimental method of Fourier’s laser polarimetry of human bile layers for the sake of diagnosing and differentiating cholelithiasis with underlying chronic cholecystitis and diabetes mellitus of degree II by means of the statistical analysis of the polarization filtered out scattered coherent radiation in a distant (Fourier) zone of diffraction with due regard for linear and circular birefringence of biological crystals.

A model of generalized optical anisotropy of human bile is suggested and a method of polarimetric of the module and phase Fourier of the image of the field of laser radiation is analytically substantiated, that is generated by the mechanisms of linear (a phase shift between the orthogonal components of the amplitude of a laser wave) and circular (the angle of rotation of the polarization plane) birefringence of polycrystalline networks with a wavelet - diagnosis and differentiation of cholelithiasis against a background of chronic cholecystitis.

A set of diagnostic criteria and diagnosis and differentiation of cholelithiasis against a background of chronic cholecystitis is identified and substantiated:

- statistical moments of the 3rd and 4th orders, which characterize the distributions of wavelet coefficients of Fourier phase of the image of a polarization inhomogeneous laser image of polycrystalline network of bile.

9537-71, Session PTues

System of multivariate laser polarimetry of phase and amplitude anisotrophy of biological layers

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Among various opticophysical methods of diagnosing the structure and properties of the optical anisotropic component of various biological

objects a specific trend has been singled out - multidimensional laser polarimetry of microscopic images of the biological tissues with the following statistic, correlative and fractal analysis of the coordinate distributions of the azimuths and ellipticity of polarization in approximating of linear birefringence polycrystalline protein networks. At the same time, in most cases, experimental obtaining of tissue sample is a traumatic biopsy operation. In addition, the mechanisms of transformation of the state of polarization of laser radiation by means of the opticoanisotropic biological structures are more varied (optical dichroism, circular birefringence). Hereat, real polycrystalline networks can be formed by different types, both in size and optical properties of biological crystals.

Our research is aimed at developing experimental method of the Fourier polarimetry and a spatial-frequency selection for distributions of the azimuth and the ellipticity polarization of blood plasma laser images with a view of diagnosing prostate cancer.

A method of polarization mapping of the optico-anisotropic polycrystalline networks of the blood plasma albumin and globulin proteins with adjusted spatial-frequency filtering of the coordinate distributions of the azimuth and ellipticity of the polarization of laser radiation in the Fourier plane is proposed and substantiated.

Comparative studies of the effectiveness of direct methods of mapping and a spatial-frequency selection in differentiating polarization azimuth and ellipticity maps of the field of laser radiation converted by the networks of albumin - globulin crystals of the blood plasma in healthy people and patients with prostate cancer have been carried out.

A set of criteria of diagnosing prostate cancer based on the statistical (statistical moments of the 1st - 4th orders), correlation (correlation moments) and fractal (the slope of approximating curves and the dispersion of the distribution of extreme log - log dependences of power spectra) analysis of the spatial - frequency filtered polarization distributions generated by dendritic networks of albumin and globulin and spherolithic networks has been detected and substantiated

9537-72, Session PTues

Morphological and physicochemical analysis of spleen of Swiss mice infected with Paracoccidioides brasiliensis

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Paracoccidioidomycosis is the most common human systemic mycosis in Latin America caused by Paracoccidioides brasiliensis, a soil thermally dimorphic fungus. In natural paracoccidioidomycosis the spleen is not the preferred organ, but it is affected from the pulmonary focus, after fungal spreads via lymphatic and hematogenous route. The aim of the present work was to evaluate the evolution of paracoccidioidomycosis in the spleen of Swiss mice and correlate morphological factors with the expression of gp43 and with physicochemical analysis via FT-Raman of the infected organ. The animals were inoculated with P. brasiliensis and were euthanized one, two, 4 and 8 weeks after inoculation. Histopathological and spectroscopic analysis were performed in the spleen mices. Histological sections of spleen were stained with hematoxylin and eosin and periodic acid-Schiff for histopathological analysis. Non-fixed spleen fragments were analyzed using FT-Raman spectroscopy. According to colony forming unit the first and second weeks were the periods when infection was most intense. Inflammatory processes were observed in the entire infection period. The gp43 molecule was distributed throughout the splenic parenchyma, and immunostaining was constant in all observed periods. The main physicochemical changes of the infected spleen were observed in the spectral ranges between 1700 – 1510 cm-1 and 1500 – 1200 cm-1, area variation of protein content and -CH2 and-CH3 compounds associated to collagen, respectively. Overall infection period there was a direct proportional relationship between the number of colony forming unit and the physicochemical changes in the spleen of mice infected with Paracoccidioides brasiliensis.

9537-73, Session PTues

Spectral fiber sensors for cancer diagnostics in-vivo

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Review of fiber spectroscopy systems using all main spectroscopy methods in the range from 200nm to 16μm: fluorescence, diffused reflection, Mid IR-absorption and Raman scattering, - with the main focus on fiber probes design used in two areas:

1) In labs – for in-line monitoring of Multi-Organ Chips (MOC) and for tissue analysis in-vitro;

2) In clinics – realized with tiny probes for endoscopic or laparoscopic applications, including:

a) ATR-fiber probes made from innovative Polycrystalline IR-fibers (PIR-fibers), and

b) fluorescence, Near IR and Raman probes made from silica glass fibers.

The latest development in Mid IR-fiber optics extends the spectral range covered by biospectroscopical methods from UV-Vis-Near IR to Mid IR to 18μm (20.000 to 550cm-1). Up to now, all fiber systems configured for biomedical diagnostic applications using absorption/transmission, reflection, fluorescence and Raman-spectroscopy methods – were restricted to silica fibers with a transmission from 180 nm to 2.4 μm. Nowadays, IR-glass fibers, Polycrystalline PIR-fibers and Hollow Waveguides can also cover the Mid IR-range up to 18μm, including the “finger-print” region where specific absorption bands of molecular vibrations are concentrated. These fundamental vibrational bands in the MIR are 100-1000 times more intensive and defined compared to their 2nd & 3rd overtones found at shorter wavelengths (<2μm). Spectroscopy using ATR-probes for the analysis of tissue (with sensitive tips using Attenuated Total Reflection) is based on tissue absorption analysis in the range from 2 to 16μm.

Results obtained by ATR-absorption spectroscopy will be compared to Raman spectroscopy - as the both methods compliments each other in molecular vibration analysis. In difference with high tissue absorption in Mid IR typical laser wavelength used for Raman scattering excitation can propagate in tissue much deeper - providing the synergy advantage from these complimentary method combination in more specific and accurate cancer diagnostics.

9537-74, Session PTues

Spectroscopic fluorescence measurements as an intraoperative tool for glioma resection

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Gliomas are infiltrative cancers and are often not curable. Nowadays, 5-ALA-induced protoporphyrin IX (PpIX) fluorescence is widely used during surgical resection, but current methods have a low sensitivity. Our goal is then to create a non-invasive intraoperative tool based on fluorescence spectroscopy to help the surgeon to distinguish the tumor’s margins

We previously showed the presence of a peak at 620 nm added to the known one at 634 in PpIX emission spectra. We assumed that it is due to different states of the same chromophore, PpIX. From this, we determined measured parameters to distinguish the solid component of glioblastomas (which are high grade gliomas) from low grade gliomas and infiltrative component of glioblastomas on biopsies. Our goal is now

to create a device usable in vivo and study its feasibility and benefit.

This device uses multi-wavelength excitation to get the re-emitted spectrum though a probe set on the brain. The spectrum is fitted with reference spectra obtained in preliminary work. From the fit, concentrations of PpIX at both states are obtained.

In vitro experiments confirmed that the ratio decreases when the excitation wavelength increases, for a given solution. The ex vivo spectrum is more complex, including autofluorescence but follows the same trend.

9537-76, Session PTues

Reproducible high-resolution multispectral image acquisition in dermatology

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Multispectral imaging offers improved tissue discrimination compared to RGB imaging due to the formers ability to better resolve reflectance spectra of the tissues. The ability to distinguish between different tissue types can be of great use in improving medical services. Most applications of multispectral cameras in dermatology have focused imaging nevi, with regions of interest covering only several square centimetres.

When considering multispectral images of larger regions of interest, resolving increasingly finer details becomes not only a problem of the resolution of the sensor but of the entire image formation pipeline.

In this work we present a novel multispectral imaging framework consisting of both hardware and software components aimed at solving such problems. We focus on acquiring high-resolution multispectral images greater than 4 megapixel, on regions of interest exceeding 150 square centimeters.

We consider two main classes of factors that have an influence on image formation: those affecting color reproduction and those affecting the registration of the different spectral channels.

We model and correct for different sources of errors during the complete image formation process with the goal of reproducible quantitative imaging for dermatology. We use two datasets for validation: one of synthetic lesions using dye, acquired under controlled conditions at our offices and the other of psoriasis lesions acquired during regular consultation hours at the university clinic. We successfully validate our framework by evaluating our performance for color reproduction and accuracy by which we register the different spectral channels.

9537-77, Session PTues

Raman spectral library of prominent biomolecules towards identifying/ digital staining of diseased regions by monitoring disease specific marker molecules

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An ability to identify the molecular composition of the sample/area under interrogation makes it possible to isolate/detect margins of diseased tissues as well as aids in early detection of diseases. Optical spectroscopy/imaging has been one of the techniques which has a major impact in the field of optics. Recently, there have been many studies reporting the use of spectroscopic techniques towards diagnosing diseased conditions. The degree of multiplexing (e.g. detecting multiple analytes through a single spectral acquisition) and versatility of spectral techniques in molecular sensing domain has been well established in the literature. Specifically, Raman spectroscopy has the ability to detect individual molecules and is independent of excitation wavelength, allowing selection of application specific wavelengths while using the same data library measured at a fixed wavelength, making RS a prime candidate for label-free sensing and imaging. The most important feature of Raman spectra is that they are very sensitive biochemical markers due to the unique vibrational fingerprint spectra of the tissue. Raman spectroscopy is also very sensitive towards small molecular/ chemical changes, such as an increased nucleus-to-cytoplasm ratio, disordered chromatin, higher metabolic activity, and changes in lipid and protein levels. We propose forming spectral libraries and classification architectures to be utilized for obtaining operator independent diagnostics under ex-vivo and in-vivo conditions. The classification architectures are planned to be a blend of multivariate analysis of the spectral data as well as their transformation into feature space with prominent features defined as peak location, peak widths and relative peak-peak ratios.

9537-78, Session PTues

Temperature dependence of the fluorescence spectrum of ZnCdS nanoparticles introduced into subcutaneous adipose tissue in vitro

Irina Y. Yanina, Elena K. Volkova, Vyacheslav I. Kochubey, Alexander A. Skaptsov, Julia G. Konyukhova, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Valery V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation) and Institute of Precise Mechanics and Control (Russian Federation); Alexey Popov, University of Oulu (Finland)

Temperature dependence of the fluorescence spectrum of ZnCdS nanoparticles introduced into subcutaneous adipose tissue in vitro were studied. The 200-500 mm fat tissues slices were used. Overall heating of the samples from room to physiological temperature leads to stronger (in depth) and faster tissue morphology change at the similar other processing conditions. These data can help to detect the location of nanoparticles during fat cell photothermolysis.

9537-79, Session PTues

Paper-based surfaced enhanced Raman spectroscopy for drug level testing with tear fluid

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We demonstrate a paper-based surface enhanced Raman spectroscopy (SERS) measurement aimed at an easy and rapid therapeutic drug level testing using tear fluid as specimen. The SERS substrates were fabricated of common filter paper and gold nano-rods. In this study, we measured the Raman spectra of sodium phenobarbital (PB), an anti-epileptic agent aqueous solution with the paper-based SERS substrate. It was found that this paper substrate was effective in enhancement of the Raman scattering derived from PB. This result showed the potential of this substrate to drug level testing using tear fluid.

9537-80, Session PTues

Hyperspectral imaging applied to microbial categorization in an automated microbiology workflow

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Hyperspectral imaging (HSI) is being evaluated as a pre-selection tool to categorize and localize populations of microbial colonies directly onto

their culture medium, in order to facilitate the microbiology workflow downstream the incubation step. The categorization criteria were here limited to the diffuse radiance spectra acquired mostly in the visible region between 400 and 900 nm.

Although the diffuse radiance signal is much broader than the one acquired using vibrational techniques such as Raman and IR and limited to chromophores absorbing in the visible region, it can be acquired very quickly allowing to perform hyperspectral imaging of large objects (i.e. Petri dishes) with throughputs that are compatible with the needs of a clinical laboratory workflow. Moreover, additional cost reduction could possibly be achieved using application-specific multispectral systems. Furthermore, recent research has shown that good power of discrimination, at the species level, could be achieved at least for a low level of species.

In our work, we test different culture media, with and without a strong light absorption in the visible region, and report categorization results obtained when selecting end-member spectra according to a multi-parametric study (colonies, agar type). Results of categorization (e.g. at the species level) are presented using two types of supervised-categorization algorithms providing that they deliver subpixel fractional abundance information (Linear Spectral Unmixing type) or not such as Spectral Angle Mapping (SAM) and Euclidian Distance (ED) type. Interestingly the performance between the two classes of algorithms is dramatically different, a trend which is not always observed. An interpretation is proposed on the basis of the agar interference and the spectral purity of end-member spectra.

9537-81, Session PTues

Common-path spectral-domain optical coherence microscopy for real-time 3D image cytometry

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We built up a common-path spectral-domain optical coherence microscopy (OCM) with center wavelength around 870 nm to image a droplet of whole blood and plasma from a rabbit. The axial and transverse resolutions of the system are 1.71 microns (in tissue) and 0.77 microns, respectively. The 3D OCM imaging can be evaluated the concentration of platelets and blood cells.

9537-82, Session PTues

Non-contact high resolution Bessel beam probe for diagnostic imaging of corneal and trabecular mesh work region in eye

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Primary angle closure glaucoma is a major form of disease that causes blindness in Asia and worldwide. In glaucoma, irregularities in the ocular aqueous outflow system cause an elevation in intraocular pressure (IOP) with subsequent death of retinal ganglion cells, resulting in loss of vision. High resolution visualization of the iridocorneal angle region has great diagnostic value in understanding the disease condition which enable monitoring of surgical interventions that decrease IOP. None of the current diagnostic techniques include such as Goniophotography, Ultrasound biomicroscopy (UBM), Anterior Segment Optical Coherence Tomography (ASOCT) and EyeCamTM can image with molecular specificity and required spatial resolution that can delineate the trabecular meshwork structures. This paper in this context proposes new concepts and methodology using Bessel beams based illumination and imaging for such diagnostic ocular imaging applications. The salient features using Bessel beams instead of the conventional Guassian beam and the optimization challenges in configuring the probe system will be illustrated with porcine eye samples.

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9537-84, Session PTues

Optical biopsy during thyroid and parathyroid surgery

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Locating parathyroid glands is an important and challenging task during both thyroid and parathyroid surgery as the glands are small and similar to the surrounding tissue. Appropriate intraoperative technologies for detecting the parathyroid will reduce both the risk of damage to the glands and the operation times. In addition, it would be beneficial to detect the pathologic status of thyroid during the operation to avoid the unnecessary tissue removal and waiting times for histopathologic analysis. In this work potentials of fluorescence spectroscopy for detection of parathyroid autofluorescence in the near-infrared (NIR) region and optical coherence tomography (OCT) were investigated for application in parathyroid and thyroid surgery on tissue samples from a total of three patients.

OCT was capable of distinguishing the main tissue types encountered in thyroid surgery. The typical microstructure of thyroid was clearly visible in the OCT images. The NIR autofluorescence of parathyroid was easily distinguishable from that of fat and thyroid. However, the signal was very weak and collection needed a careful design of the system and setup. Further studies will include implementing OCT for identification and preoperative characterization of pathologic status in thyroid and NIR imaging for detection of parathyroid.

9537-85, Session PTues

Blood optical properties at various glucose level values in THz frequency range

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Number of patients with diabetes is rapidly growing every day (230 million in 2010, 460 million is projected by 2025). The disease is characterized by a sharply increased risk of complications such as diseases of the cardiovascular system - up to 20 times. The seriousness of complications straightly depends on the control of blood glucose levels. Now, the most common way of glucose measurement is made by glucometers that require making a finger puncture. Each puncture is negative for patient.

Using non-invasive techniques for measurement of glucose levels could reduce the amount of manipulations that can cause a risk for patients by 1500 per year. Various biomolecules have specific frequency signatures in the terahertz (THz) frequency range, which can reveal their presence and determine the concentration. Therefore, the optical properties of blood were studied in the THz frequency range in order to develop the method

of control the blood glucose level by time-domain THz spectroscopy. Dependences of refractive index on glucose concentrations and different times after blood sampling were obtained.

Double Debye model could be described by five Debye parameters: static dielectric, optical dielectric constant, intermediate constant and two relaxation times for fast and slow processes. Using this model the dispersion of complex dielectric permittivity of investigated object could be approximated with a high accuracy. The determination of these main parameters allows analyzing cellular processes in various biological objects. Moreover, by using the effective medium theory, the blood components concentrations of the samples were obtained. The blood was considered as a compound of elements: cloth (in transmission geometry), water and the blood components. Hence, using permittivity of each of these components obtained from the experiment and with the help of the special iteration algorithm the concentrations of each of the components were estimated.

9537-86, Session PTues

Cartilage analysis by reflection spectroscopy

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A cartilage bioreactor with analytical functions for cartilage quality monitoring is being developed. For determining cartilage composition, reflection spectroscopy in the visible (VIS) and near infrared (NIR) spectral region is evaluated. Main goal is the determination of the most abundant cartilage compounds water, collagen I and collagen II. Therefore VIS and NIR reflection spectra of different cartilage samples of cow, pig and lamb are recorded. Due to missing analytical instrumentation for identifying the cartilage composition of these samples, typical literature concentration values are used for the development of chemometric models.

In spite of these limitations the chemometric models provide good cross correlation results for the prediction of collagen I and II based on the visible spectra and the NIR reflection spectra prove to be suitable for determining the water content.

9537-87, Session PTues

Energy transfer efficiency in quantum dot/chlorin e6 complexes

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Here we present a spectroscopic analysis of complexes based on water-soluble CdSe/ZnS and ZnS:Mn/ZnS quantum dots (QDs) with second-generation photosensitizer chlorin e6. The CdSe/ZnS QDs are characterized by bright excitonic luminescence. In the case of ZnS:Mn/ZnS quantum dots excitonic luminescence is not observed, there is only the impurity manganese luminescence. In these systems QDs act as energy donor and chlorin e6 acts as an acceptor. CdSe/ZnS and ZnS:Mn/ZnS QDs have different resonant conditions with acceptor. The excited electronic states of chlorin e6 are in resonance with excitonic luminescence band of CdSe/ZnS QDs and with both, excitonic and manganese ion, luminescence bands of ZnS:Mn/ZnS QDs. Analysis of experimental data has shown that at low chlorin e6 concentration both systems provide highly efficient energy transfer from quantum dots to chlorin e6 (~ 50%). It was found that increase of chlorin e6 concentration in samples leads to a drop in intracomplex energy transfer efficiency in complexes with CdSe/ZnS QDs and it has no effect on energy transfer in ZnS:Mn/ZnS complexes. An appearance of new trap states due to complex formation could be a reason in difference of intracomplex energy transfer in CdSe/ZnS and ZnS:Mn/ZnS complexes.

9537-88, Session PTues

Detail enhancement in microscopy of human papillary thyroid carcinoma by acousto-optic spatial filtering

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We report a new method for detail enhancement in microscopy of transparent samples using analog image processing in coherent light. Experimental technique uses spatial filtering with an acousto-optic tunable filter (AOTF) in a telecentric optical system. The optical system is attached to the side port of a wide-field light microscope. The image is relayed with a telecentric lens, then filtered by means of an AOTF, and captured with a CCD matrix. This experimental setup can operate in two regimes depending on illumination of the sample. When white light from is used, the AOTF operates in hyperspectral imaging (HSI) mode. With laser illumination, adaptive spatial filtering of diffracted light takes place in the AOTF. Peculiar performance of the system is defined by the telecentric diaphragm. First, it provides normal incidence of the beams from each point of the image onto the AOTF. Thus, all fragments of the image through the field of view possess the same Bragg angle. Second, the telecentric diaphragm selects certain spatial frequencies of the input light.

As an object of the study we have chosen cytological smears after fine needle aspiration biopsy and histological sections of human papillary thyroid carcinoma. Cytological smears have been stained by Azur&Eosin; histological sections have been stained by Haematoxilin&Eosin. RGB wide-field images are compared with the single wavelength HSI of the same sample fragment and the coherent light images with spatial filtering. The results demonstrate detail enhancement in transparent samples when coherent illumination was used.

9537-89, Session PTues

Quantum dot-tetrapyrrole complexes as photodynamic therapy agents

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In this work, photophysical properties of complexes of conventional photosensitizers (Pc) for photodynamic therapy (PDT) with semiconductor quantum dots (QDs) were investigated in aqueous solutions and in living Erlich ascite carcinoma cells. In complexes with QDs an effective energy transfer from QDs to photosensitizer molecules allows to significantly increase photodynamic therapy effect due to increased generation of singlet oxygen. Spectral-luminescent study of complexes in aqueous solutions was performed that allowed ones to estimate potential efficiency of the complexes, based on the assessments of the intracomplex energy transfer and retention of the luminescence quantum yield (QY) of the photosensitizer. Increasing of the photosensitizer relative concentration in complexes resulted in sharp drop of the energy transfer efficiency and of the luminescence QY of the photosensitizer. This fact indicates that additional channels of nonradiative energy dissipation may take place in the complexes. Using nonconjugated complexes of Al(OH)-sulphophthalocyanine with CdSe/ZnS QDs in the aqueous solution as an example, we have demonstrated that aggregation of the Pc molecules on the QD surface leads to the appearance of the concentration dependence of the photophysical properties of the complexes. The model describing phthalocyanine QY of luminescence in complexes and intracomplex FRET efficiency as a function of the probability of Pc aggregates formation has been proposed that matched well with experimental data. We also demonstrated that use of methods of complex formation that prevent aggregation of photosensitizers allows high energy transfer efficiency

and Pc luminescence QY in complexes to be preserved in wide range of the relative Pc concentrations. Next in-vitro PDT test have shown that complexes of biocompatible ZnSe/ZnS QDs and chlorin e6 exhibit a twofold improved cancer cell photodynamic destruction effect against the Erlich ascite carcinoma cells when compared to that of free molecules because of efficient photoexcitation energy transfer and enhanced intracellular uptake of the Pc in the QD complex. We believe that our study allowed obtaining new information about the physical mechanisms of interaction of conventional PDT photosensitizers with semiconductor quantum dots in complexes.

9537-90, Session PTues

Optical sprctroscopy of cancerous tissues

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Simultaneous laser induced- breakdown spectroscopy (LIBS) and acoustic response techniques as well as Laser induced fluorescence (LIF) are applied to investigate the abnormal lymph tissues due to Hodgkin disease. The spectral shift in the emissive fluorescence of the cancerous tissues has been observed respect to the normal ones. Regarding LIBS, the concentrations of Ca and Na trace elements have been identified to be higher in the cancerous samples. In addition, the acoustic response of cancerous tissues has been elevated against healthy ones. The distinct differences in the spectra are taken into account for early and the rapid identification and diagnosis.

9537-91, Session PTues

Classification of neoplastic and pre-neoplastic lesions in the central bronchial tree using in vivo Raman spectroscopy

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Currently lung cancer has a poor prognosis with a five year survival rate of only 17%. This is due to most cases being detected at a later stage, where there is a lack of efficient treatment options. The ability to detect cancerous lesions early leads to a higher probability of the cancer being cured. However, the current detection methods for preneoplastic and neoplastic lung lesions have a low specificity, which in turn causes lesions to be biopsied when it is not necessary. There remains a need for real time in vivo diagnosis strategies at the bedside with better specificity while maintaining high sensitivity. Previously it was shown that Point Laser Raman Spectroscopy, when used in adjunct to current bronchoscopy techniques, is able to increase the procedure specificity by over 20% while maintaining high sensitivity. Here we present the findings of a larger scale single center study on the use of in vivo Point Laser Raman Spectroscopy as an adjunct modality to standard lung detection methods, along with new classification and spectral analysis.

9537-92, Session PTues

Combined autofluorescence and Raman spectroscopy method for skin tumor detection in visible and near infrared regions

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The problem of non-invasive monitoring of skin cancer requires innovate diagnosis method with high precision. In current work we used a combined Raman spectroscopy (RS) and autofluorescence (AF) method in visible and near infrared regions.

The laboratory setup combines principles of RS, and AF for skin tissues studies. The setup includes a thermally stabilized semiconductor laser (785 nm, 150 mW) for excitation of Raman spectra and autofluorescence in near infrared (NIR) region, solid-state laser (457 nm) for excitation of autofluorescence in visible region, and a spectrograph with a digital cooling CCD camera.

Ex vivo experiments were performed for 28 skin tissue samples (12 malignant melanomas - MM, 16 basal cell carcinoma - BCC, and 28 healthy tissues).

We propose to use a phase-type method for tumor detection and identification in RS studies. The most significant differences of Raman band intensities are located at 1320, 1450 and 1660 cm-1 from analyzed spectral range 1200-1800 cm-1.

In current research, lasers with excitation wavelength 457 and 785 nm were used for AF excitation. AF spectrums of each sample were normalized to the maximum in 550-750 nm range for blue laser (457 nm) and 790-910 nm ranger for NIR laser (785 nm).

Proposed Raman spectroscopy phase method allows to reach 75% of sensitivity for ex vivo malignant melanoma diagnosis and autofluorescence analysis in visible region allows to reach 83% of sensitivity. Combined AF and RS method for malignant melanoma detection in visible and NIR spectral ranges allows to reach 93% of sensitivity.

9537-93, Session PTues

Paper-based surfaced enhanced Raman spectroscopy for drug level testing with tear fluid

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In pharmacotherapy, the checking of the therapeutic drug concentration level in blood is important for the safety and effectiveness. We propose a drug level testing based on Raman spectroscopy of tear fluid. Tear fluid is considered as one of good samples to obtain biomedical information because it can be more easily and minimally-invasively collected than blood. Previous studies have reported that some drug concentrations in tear fluid correlate with those in blood. Therefore blood drug level is able to be estimated by tear fluid analysis.

Raman spectroscopy is an optical technique for biochemical analysis, that has some advantages including less sample volume, no need of preprocess, and regardless sample state. Our previous study showed the ability of paper-based Raman spectroscopy in drug detection. However, mainly due to the low scattering, Raman spectroscopy does not have sufficient sensitivity required for measurement of a small amount of drug secreted in tear fluid. Surface enhanced Raman scattering (SERS) is a technique that enhance the scattering light using metal nano-structure and the enhancement has reported to be as much as 14 orders of magnitude greater. Paper has great potential as a scaffold of SERS substrate due to its higher adsorption of nano-structures than conventional silicon substrate derived from its porous surface. Furthermore, cellulose paper is an ideal tool for safe and efficient tear

collection. Paper also has some good points such as flexibility, simple use, hygiene, disposability and inexpensiveness, so it is a suitable tool for practical use in clinical fields. Then we propose a method measuring the Raman spectra of the drug level in tear fluid collected in paper-based SERS substrate, for easy, rapid and high sensitive drug level testing. In this work, as a feasibility study, we fabricated a paper-based SERS substrate and measured a drug aqueous solution to explore the potential for drug analysis.

9537-18, Session 4

Tissue classification and diagnostics using a fiber probe for combined Raman and fluorescence spectroscopy *(Invited Paper)*

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Two different optical fiber probes for combined Raman and fluorescence spectroscopic measurements were designed, developed and used for tissue diagnostics. Two visible laser diodes were used for fluorescence spectroscopy, whereas a laser diode emitting in the NIR was used for Raman spectroscopy. The two probes were based on fiber bundles with a central multimode optical fiber, used for delivering light to the tissue, and 24 surrounding optical fibers for signal collection. Both fluorescence and Raman spectra were acquired using the same detection unit, based on a cooled CCD camera, connected to a spectrograph. The two probes were successfully employed for diagnostic purposes on various tissues in a good agreement with common routine histology. This study included skin, brain and bladder tissues and in particular the classification of: malignant melanoma against melanocytic lesions and healthy skin; urothelial carcinoma against healthy bladder mucosa; brain tumor against dysplastic brain tissue. The diagnostic capabilities were determined using a cross-validation method with a leave-one-out approach, finding very high sensitivity and specificity for all the examined tissues. The obtained results demonstrated that the multimodal approach is crucial for improving diagnostic capabilities. The system presented here can improve diagnostic capabilities on a broad range of tissues and has the potential of being used for endoscopic inspections in the near future.

9537-19, Session 4

Early diagnosis of tongue malignancy using laser induced fluorescence spectroscopy technique

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Cancer became a major concern in both developed and developing countries. Around 90% cancers are squamous cell carcinoma (SCC). SCC of the tongue is the most common oral malignancy. One of the important factors for successful therapy of any malignancy is early diagnosis. Although considerable progress has been made in understanding the cellular and molecular mechanisms of tumorigenesis, the lack of appropriate diagnostic methods for early diagnosis has led to increase in the mortality rate in various types of cancers. Spectroscopy techniques are extremely sensitive for the analysis of biochemical changes. These techniques can provide a clear picture of alterations that occur during the development of cancer. This is especially important in oral cancer, where “tumor detection is complicated by a tendency towards field cancerization, leading to multi-centric lesions” and “current techniques detect malignant change too late”, and “biopsies are not representative of the whole pre-malignant lesion”. Current work deal with in vivo fluorescence studies of tongue malignancy using home assembled Laser Induced Fluorescence (LIF) system. About >800 fluorescence spectra from tongue (top, tip, bottom and lateral) have been recorded from 330 subjects under normal (133), potentially malignant (154) and malignant (63) conditions by excitation with 325 nm CW He-Cd laser.Tongue top, tongue tip, tongue lateral, and bottom give spectra differing from each other. Under the above clinical conditions the fluorescence spectra are found to be noticeably different.

9537-20, Session 4

Topical fluorescent analogue for virtual hematoxylin and eosin histology in point-of-care ex vivo microscopy

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Histological assessment of freshly removed tissue specimens requires accurate and fast analysis in clinical procedures such as diagnostic biopsy and surgical tumor resection. Current histological assessment methods are either time-consuming or damage the tissue beyond the ability to re-analyze post-procedure. We demonstrate a novel dual-stain fluorescent analogue to brightfield Hematoxylin and Eosin for in-procedure histopathology that is both time-efficient and preserves the analyzed tissue for later analysis. H&E-like images are created from the combination of DRAQ5 and Eosin applied to human prostate tissue and animal muscle tissue under confocal microscopy. D&E images are pseduocolored to match H&E coloring, showing near-identical features to brightfield H&E of the same tissue. The histological accuracy, short staining time, and tissue preservation aspects of this dual-stain technique demonstrates its potential to be adopted for use in point-of-care pathology.

9537-21, Session 4

Autofluorescence spectroscopy for multimodal tissues characterization in colitis-associated cancer murine model

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The purpose of this project research is to assess mice colon wall, using three optical modalities (conventional endoscopy, confocal endomicroscopy and optical spectroscopy) and endoscopic MRI. The study is done in the context of inflammatory bowel disease and colorectal cancer which represent 13% of new cases of cancer, every, year in western countries.

An optical spectroscopic bench (fluorescence and reflectance) was developed and combined with a mini multi-purpose rigid telescope (Karl Storz®) and a confocal endomicroscope (Mauna Kea Technologies®). The optical modalities were assessed, first, in vivo on mice c57b6j. Then, in order to better understand the photons trajectories and the contribution of the interaction with the tissue, a second optical spectroscopic bench was designed (two channels of acquisition) and tested on phantoms (containing two layers of eosin and fluorescein solution). Finally, this new configuration was assessed in vivo on mice.

The preliminary results show the feasibility to combine such modalities in the same protocol. Conventional endoscopy is useful to depict inflammation along the colon wall. Confocal endomicroscopy show high-contrasted images from where it is possible to obtain biomarkers after computed some features (fractal dimension or textural features). Optical spectroscopy was able to provide biochemical information. Information gather by the two channels spectroscopy prototype show differences between those channels, which point that the interaction with the media observed is different. This work opens perspectives to better understand one of the most deadly cancer and bring, potentially, ways to develop therapeutic responses.

9537-22, Session 4

Influence of structural length-scale variations on azimuth-resolved light scattering patterns of inhomogeneous cell models

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Optical scattering provides an intrinsic contrast mechanism for the diagnosis of a wide variety of diseases. This has particularly led to the development of numerous scattering-based spectroscopic and imaging tools to detect early precancerous changes in tissues. There have also been a multitude of numerical studies targeted at delineating the relationship between cancer-related alterations in morphology and internal structure of cells and the resulting changes in their optical scattering properties. Despite these efforts, we still need to further our understanding of inherent scattering signatures that can be linked to precancer progression. As such, computational studies aimed at relating electromagnetic wave interactions to cellular and subcellular structures at different length-scales are likely to provide a quantitative framework for a better assessment of the diagnostic content of optical signals. In this study, we aim to determine the influence of structural length-scale variations on two-dimensional light scattering properties of cells. We numerically construct cell models with refractive index heterogeneities at different length-scales and we employ the finite-difference time-domain method to compute their azimuth-resolved light scattering patterns. The results indicate that changes in characteristic length-scale can significantly alter the two-dimensional scattering patterns of cell models. More specifically, the degree of azimuthal asymmetry characterizing these patterns is observed to be highly dependent on the length-scale. Overall, the study described here is expected to offer useful insights into whether azimuth-resolved measurements can be explored for diagnostic purposes.

9537-23, Session 4

Feasibility trial of using NIR reflectance spectrum method to monitor tumor microwave ablation

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The widely use of microwave ablation method to treat liver tumors brings growing interest on the study of efficacy assessment during microwave ablation. Optical approaches have been proved to be effective for the monitor of thermal damage. Ex vivo microwave ablation experiments were carried out on human liver tumor to study the feasibility of using optical properties for the monitoring of ablated tissue status. Near infrared (NIR) reflectance spectrum and reduced scattering coefficient (?s') on 690 nm were measured during the ablation in real-time. A rising trend of the spectrum intensity was observed in the experiment. ?s' showed an exponential growth during the ablation and remained stable after fully coagulation. Due to the sensitivity to tumor tissue thermal damage degree, ?s' is a potential efficacy assessment factor for the monitoring of microwave ablation.

9537-24, Session 5

Raman spectroscopic characterization and discrimination of inflammatory bowel disease *(Invited Paper)*

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Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's colitis (CC), affects nearly 2 million Americans, and the incidence is increasing worldwide. It has been established that UC and CC are distinct forms of IBD that have heterogeneous presentation within the colon and require different medical care. Currently, the distinction made between UC and CC is based upon inexact clinical, radiological, endoscopic, and pathologic features. A diagnosis of indeterminate colitis occurs in up to 15% of patients when UC and CC features overlap and cannot be differentiated. In these patients, diagnosis relies on long term follow up based on success or failure of existing treatment and recurrence of the disease. Thus, there is need for a tool that can improve the sensitivity and specificity for fast, accurate and automated diagnosis of IBD. Here we present colonoscopy-coupled fiber optic probe-based Raman spectroscopy as a novel diagnostic tool for IBD. This in vivo study of patients with existing IBD diagnoses of UC (N=15) and CC (N=26) aims to characterize spectral signatures of UC and CC. Samples are correlated with tissue pathology markers and endoscopic evaluation. Optimal collection parameters for detection have been identified based upon instrument design. The collected spectra are processed and analyzed using multivariate statistical techniques to identify spectral markers and discriminate UC and CC. The characterization of spectral markers to discriminate disease type and the development of a real-time classifier interface are vital steps towards accurate and automated in vivo detection of IBD during colonoscopy procedures.

9537-25, Session 5

Video-rate structured illumination microscopy (VR-SIM) for rapid assessment of fresh surgical margins

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Surgical removal of tumors is the frontline curative strategy for non-metastatic cancers of many organs; the success of the strategy depends on the ability to completely remove the tumor during the primary operation. However, positive surgical margins (PSMs), or tumor extending to the surface of the excised specimen, remain a significant problem in many procedures. To address the unmet clinical need for an imaging tool that can provide sub-cellular resolution images of large areas of excised surgical specimens in an intra-operative timeframe, we developed a video rate structured illumination microscopy (VR-SIM) system. In an ongoing study, 5 patients undergoing radical prostatectomy and 4 patients undergoing partial nephrectomy were enrolled into a clinical trial using VR-SIM to image the entire margin aspect surface for each specimen. VR-SIM images of the lateral prostate margins were characterized by primarily smooth muscle fibers, whereas images of the posterior prostate margins were characterized by fibrous stroma interspersed with scattered vessels and nerves. VR-SIM images of the renal parenchymal margins were characterized by glomeruli and renal tubules. The average imaging time for each margin was 9 minutes. All surgical margins were pathologically determined to be free of tumor at the time of image review, and each specimen was confirmed as negative for cancer at the margin on final histopathology. VR-SIM is a promising and practical method for assessing intra-operative prostate and renal margins during radical prostatectomy and partial nephrectomy that could translate into decreased PSMs and thus decreased need for adjuvant treatment modalities.

9537-26, Session 5

Morphological changes in the internal organs in rats with transplanted liver cancer PC-1 at intravenous injection of citrate stabilized iron nanoparticles

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The magnetic resonance imaging (MRI) and morphological examination of internal organs and tissues of 30 white outbred rats with transplanted liver tumor PC-1 were conducted after intravenous administration of hydrosols magnetite. Intravenous administration of citrate - stabilized iron nanoparticles in a dosage of 20 μg/kg and 16 mg/kg resulted in a disruption of blood filling of the organs, mainly due to the plethora. The dystrophic cell damage was observed in liver and in kidneys. The immunostimulating effects of iron nanoparticles on the white pulp of the spleen and peribronchial lymphoid follicles were fixed. In both dosage the nanoparticles were founded in the heart and brain, but only in dosage of 16 mg/kg the significant iron accumulation in the tumor was detected by methods of MRI and atomic absorption spectroscopy (AAS). It is possible to conclude that selected dosage is sufficient for accumulation of nanoparticles in tumor, but it does not cause significant changes in the internal organs.

9537-27, Session 5

Comparison of the simplified laterally uniform and geometrically realistic optical fiber probe-tissue interface in terms of Monte Carlo simulated diffuse reflectance

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Throughout the years, numerous applications of diffuse reflectance spectroscopy have demonstrated the usefulness of the method for determining the composition of various biological tissues. Several experimental setups for measuring the sample diffuse reflectance have been proposed, among which the setups utilizing optical fiber probes are frequently used. Generally, the optical fiber probes consist of a one or more source and detector fibers. In order to extract the optical properties from the acquired diffuse reflectance spectra, an accurate light propagation model, such as Monte Carlo (MC), is required. Although in principle the MC stochastic model is very accurate, it can significantly depend on the description of the probe geometry and the medium. Recent advancement in the computational power of graphics processing units (GPUs) has provided the means to explore more realistic geometries in the MC simulation and compare them to their commonly used simplified counterparts.

Optical fiber probes are commonly manufactured by arranging fibers in the desired source–detector layout and mounting them in a stainless steel tubing. By using MC simulations, we investigate the impact of the stainless steel fiber probe–medium interface on the diffuse reflectance spectra. For this purpose, a commonly used simplified laterally homogeneous interface with mismatched refractive index was compared to the extended interface description taking into account the fiber layout and the specular reflections from the stainless steel probe tip. The results show that the error introduced into the diffuse reflectance spectrum estimate by the simplified probe–medium interface can easily exceed 5%.

9537-28, Session 5

Fiber-optic technologies for advanced thermo-therapy applied ex vivo to liver tumors

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Thermal ablation, using radiofrequency, microwave, and laser sources, is used for treatment of hepatic tumors. We present optical fiber sensors that allow unprecedented capabilities for recording the biophysical phenomena occurring at the point of treatment. Distributed thermal sensors allow recording temperature with sub-mm spatial resolution, while a thermally insensitive pressure sensor allows recording pressure rise supporting advanced treatment of encapsulated tumors. The presence of miniature sensors that record the evolution of thermal ablation, and is ultimately capable of dynamically adjusting it; using laser ablation technology, it is possible to integrate the ablation device and the sensor in the same fiber. The main achievements on biophysical sensing are discussed, while the foundation for the advanced topics is presented.

9537-29, Session 5

Pressure effects on optical properties measured by Single Fiber Reflectance spectroscopy

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Many forms of elastic scattering spectroscopy have been used in non-invasive disease diagnosis. Measurements with spectroscopy systems such as Single Fiber Reflectance spectroscopy (SFR) require direct contact between the probe and tissue. Previous studies have reported effects of the probe pressure on the measured spectrum while it is difficult to compare and quantify the various results due to different spectroscopic techniques and pressure control geometries used. Quantitative measurement of optical properties under tightly controlled pressure gradient distribution within the optical sampling tissue volume is needed to study the pressure effect on tissue optical properties measured.

In our study Single Fiber Reflectance spectroscopy was used to extract ?_a and ?_s^,. The laboratory setup was designed to generate a one dimension pressure gradient throughout the optical sampling volume. The pressure applied on 5 mm thick tissue ranges from 0 to 200 mN/mm2.

Preliminary studies show that the scattering properties of chicken breast tissue follow the trend normally observed in biological tissue: a decrease of scattering coefficient with wavelength leads to a decrease in measured reflectance with wavelength. Under pressure different changes in the reflectance spectra are observed in different wavelength regions.

The study shows controlling the pressure applied by the fiber optic probe is necessary in order to obtain reproducible optical properties measurements. Moreover, it also shows the possibility to develop a novel in vivo diagnostic technique based on pressure dependent optical properties.

9537-30, Session 6

Lab-on-a-chip SERS towards clinical application: detection of levofloxacin in simulated body fluid *(Invited Paper)*

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Levofloxacin, a second generation fluoroquinolone antibiotic, is widely used for the treatment of infections caused by both gram positive and gram negative bacteria. As most antibiotics it has a concentration dependent bacteria killing properties. Therefore, a monitoring is important for increasing the efficiency and for reducing the costs of the applied treatments. More than 85% of the administered drug is excreted in urine unchanged, the detected normal urinary concentrations being around 0.1-0.8 mM after 24 hours from the intake.1

Among the newly developed bioanalytical tools, surface enhanced Raman spectroscopy (SERS) in combination with microfluidic platforms gained high interest. The lab-on-a-chip SERS (LOC-SERS) technology provides both automatic and reproducible measurement conditions with high sample throughputs. Additionally, information on a molecular level is provided with high sensitivity allowing the detection of analytes in trace amounts.

To the best of our knowledge, this is the first time when the detection of levofloxacin in simulated body fluid, namely simulated urine (SU), is reported. SU was prepared by using a modified protocol published by Yang et al.2 Besides different salts, the main chemical component is urea at a concentration of 0.8 M. As compared with SERS analysis carried out in pure aqueous solutions, a competition at the surface of the SERS active substrate is expected. Within this contribution, the competitive absorption at the surface of the Ag nanoparticles is assessed. Furthermore, results concerning the achieved limit of detection (0.14 mM) and the linear response (SERS signal vs. analyte concentration) will be presented.

Acknowledgement

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9537-31, Session 6

Surface enhanced Raman spectroscopy for the detection of environmental harmful biomedical substances

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The contamination of the water cycle with medical drugs such as hormones or antibiotics is hazardous for plants, animals and humans and lead on to multi resistant pathogens. As an example, sulfamethoxazole, an antibiotic for urinary tract infection is an often found contaminating drug in the waste water of hospitals. Therefore, a fast, easy-to-apply and cost efficient method is required to detect such contaminations in water and to purify sewage water subsequently.

Our method of choice is the molecular specific Raman spectroscopy. By doing so, the molecular fingerprint of each molecule is recorded; however with the drawback of low sensitivity [1]. For enhancing the inherent weak Raman signal, metallic nanostructures are employed. This effect is called surface enhanced Raman spectroscopy (SERS). For preparing metallic nanoparticles different colloids or bottom up strategies [2, 3] as well as top down approaches [4- 6] can be utilized.

Within this contribution solid substrates fabricated by means of EBL are implemented in a partly automated microfluidic setup to generate stable conditions for the detection of the antibiotic sulfamethoxazole. In this context a low-cost, fast and effective alternative to commonly used HPLC detection methods is introduced.

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9537-32, Session 6

Fiber enhanced Raman spectroscopy of biogenic gases

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Fiber enhanced Raman sensing is presented for versatile and extremely sensitive analysis of biogenic gases. Elaborated micro-structured optical fibers guide the light with very low losses within their hollow core and provide at the same time a miniaturized sample container for the analytes. Thus, fiber enhanced Raman spectroscopy (FERS) allows for chemically selective detection of minimal sample amounts with high sensitivity.

Conventional Raman spectroscopy provides excellent chemical sensitivity for sensing applications, but is a notoriously weak process. In order to enhance the Stokes Raman intensity, the number of analyte molecules that are involved in the Raman scattering process can be increased. In conventional Raman spectroscopy, the scattering volume in the focus spot of the optical setup from which the signal is efficiently provided is very small. This limitation can be overcome with help optical fibers. Novel micro-structured optical fibers provide the capability of light guiding within their hollow-core [1]. Thus a miniaturized sample container is provided for the flow of gaseous and aqueous analytes, leading to strong light-analyte interactions and enhanced analytical sensitivity at low sample amounts. The challenge of guiding light in gaseous media is the refractive index which is lower than the refractive index of the surrounding silica quartz material. Thus elaborated microstructures are needed for efficient light confinement within the fiber core. In Raman sensing, the sample is excited e.g. in the visible range at 532 nm and the Stokes-Raman spectrum extents 100... 150 nm towards longer wavelengths.

For quantitative Raman spectroscopy, first a fiber-adapter assembly was designed that provides a stable and reproducible optical coupling geometry of the fiber [2]. The laser excitation light is reflected with a dichroic mirror and focused into the fiber with help of a microscope objective. The fiber-adapter assembly (A) provides an optical window for coupling the laser light into the fiber and two side-ports for analyte flow and cleaning procedure. The optical transmission is always measured at the transmission window at the fiber end-face. The Raman signal that is generated along the fiber is collected with the same objective lens in backscattering geometry. The Rayleigh light is blocked at the dichroic mirror and the Raman signal is analyzed with help of a Raman spectrometer.

Here we present an enhancement in fiber enhanced Raman spectroscopy for ultrasensitive detection of biogenic gases. The Stokes Raman scattering intensity can either be enhanced by application of stronger excitation intensity (I0), higher excitation frequencies (e.g. UV excitation),

by resonantly matching the excitation frequency with an electronic transition of the molecule or by increasing the number (N) of target molecules that are involved in the Raman scattering process. Enhanced Raman multi-gas spectrometry is an extremely capable technique for non-consumptive and rapid investigation of environmental processes and for early-stage disease diagnostic of breath [3-6]. One important environmental example is the characterization of respirational activities of important soil microbes, since gas fluxes between the biosphere and atmosphere play a key role for global environmental changes. Continuous fiber enhanced Raman gas sensing is superior in order to elucidate intermediate process details, which might be overlooked by conventional gas sampling techniques. The advantage of fiber enhanced Raman gas spectroscopy is the possibility to measure all gases (except noble gases) simultaneously with no cross-sensitivities. Due to the sharp bandshape of the Raman signatures it is possible to trace minor components in the ppm range alongside major gas components in the high percentage range [4]. The fiber enhanced ro-vibrational Raman spectra of two major atmospheric components N2 and O2 are presented. Both diatomic homo-atomaric gases are difficult to measure with other gas sensing techniques and to do not have a permanent dipole moment for infrared absorption spectroscopy. However FERS easily allows the sensing of N2, O2, CO2, CH4, and N2O with just one single measurement. Elaborate novel micro-structured fibers in combination with new developments in Raman instrumentation provide excellent possibilities for the miniaturization of FERS setups. Thus fiber enhanced Raman spectroscopy bears high potential for point-of-care investigation of monitoring of disease markers in exhaled breath.

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9537-33, Session 6

Raman spectroscopy of stored red blood cells: evaluating clinically-relevant biochemical markers in donated blood

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Abstract (online display, 250 words): Countless lives have been saved by blood transfusions since the advent blood banking and the introduction of effective storage conditions. However, managing the supplies of stored blood products continues to be an administrative challenge. Presently, stored red blood cells (RBCs) are considered to be viable for 42 days, but there is strong evidence that blood from different donors degrades at different rates and observational studies have suggested that these variations could be responsible for a variety of post-transfusion illnesses. It would therefore be advantageous to assess the condition of stored blood rapidly and accurately without needing to sample the contents of the bag.

When stored in a plastic blood bag, RBCs are known to undergo a multitude of chemical, physiological, and morphological changes over time. Many of these age-related changes (e.g., loss of hemoglobin or a decrease in metabolic regulators) produce chemical species that can be characterized using Raman spectroscopy. This study clearly demonstrates that the Raman spectrum of stored RBCs changes as a function of storage age, most notably with specific bands related to the oxygenation state of hemoglobin and production of lactate. There is clear evidence that certain features are donor-dependent, as lifestyle choices, donor-age, and donor-gender factor into the analysis. Standard chemometric methods confirm the variations of these biochemical components are associated with cell breakdown. The presented data will show that Raman spectroscopy has promise as a clinical diagnostic tool for monitoring the viability of RBCs during blood bank storage.

9537-34, Session 6

A method to create a universal calibration data set for Raman reconstruction based on Wiener estimation

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Raman spectroscopy has been intensively explored in biomedical applications. However, Raman data acquisition is generally slow due to inherently weak Raman signals. Narrow-band Raman measurements can compensate for the weak Raman signal at each wavenumber by performing integration in the wavenumber dimension, in which the Raman spectrum with high spectral resolution needs to be reconstructed from the narrow-band measurements. However, this method is limited in the requirement of a calibration data set, in which the calibration samples are similar to test samples in Raman features. Therefore, a new calibration data set is often needed for every type of samples. We propose a method to create a universal calibration dataset for Raman reconstruction to overcome this limitation. In our method, the Raman spectra measured from each basic biochemical component in samples instead of actual samples are used in the calibration dataset. Because samples share the same set of basic biochemical components, the calibration dataset based on these biochemical components is applicable to all and only a handful number of Raman measurements are needed to create such a universal calibration dataset. In this study, the universal calibration dataset was test on 27 liquid phantoms in which three basic biochemical components were mixed. The Raman spectra reconstructed from the synthesized narrow-band measurements of phantoms were compared to those measured from the same phantoms. The results demonstrated the excellent performance of Raman reconstruction using the universal calibration dataset compared to the measured Raman spectra.

9537-35, Session 6

Detection of propofol concentrations in blood by Raman spectroscopy

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Anesthetic drugs, such as propofol, are commonly used during surgeries, Magnetic Resonance Imaging of children, and other medical procedures. Drugs are administered on the basis of patient's weight[1], but without the in-situ measurements of its concentration in patient's blood. Currently, there is a lack of fast and reliable methods for measuring the concentration of intravenously administered general anesthesia drugs in blood. Measurements are usually performed on blood sampled after the anesthesia wears off and the metabolites of pharmaceutical compound degradation are studied.[2,3] Therefore, reliable methods of measuring blood concentration of these drugs would be greatly useful in the operation rooms during surgical procedure.

In this paper we present a proof-of-concept of a Raman spectroscopy-based approach for measuring the content of anesthesia drugs in human blood, intended for use during clinical procedures. This method utilizes the Raman spectroscopy as a chemically–sensitive method for qualitative detection of the presence of a drug and a quantitative determination of its concentration. Good quality spectra were achieved with standard chemometric pre-processing methods. A set of samples from a number of patients with different concentrations were measured. Subsequent analysis of a set of spectra was carried out to extract qualitative and quantitative information.

SET-UP

Measurement setup was based on a pre-commercial Raman spectrometer Ramstas developed by the VTT – Technical Research Centre of Finland [4-6]. To reduce the influence of background fluorescence signal, the measurements were carried out with the use of 830 nm excitation

wavelength. Diode laser was coupled with a fiber optic probe with working distance equal to 2.5 cm and provided continuous wave power of 100 mW on the sample. A laser line filter was mounted in excitation part and the dielectric low-pass filter was mounted in collection part of the fiber optic probe. A detector, thermoelectric cooled CCD array (250K), was attached to an L-shaped axial transmissive spectrograph with holographic transmission grating with high throughput. The spectral resolution of the setup equals about 8 cm 1. The schematic of the spectrometer set up is presented on Fig1.

Data obtained during Raman measurement of biological samples are usually complex and distorted by interfering signals, such as wideband optical background. Thus, chemometric pre-processing of the raw spectra was applied for de-spiking, de-noising, background removal and normalization of the spectra.

EXPERIMENT

Data were measured on a set of whole blood samples obtained from a number of healthy blood donors with various hematologic parameters, such as hematocrit, hemoglobin concentration, etc. And few anemic patients with very low hematocrit (volume of RBC's in whole blood). Blood was obtained from Gda?sk Blood Donor Centre and was transported and handled according to standard protocols. Blood samples were divided into a set of aliquots and specific concentrations of propofol intravascular solutions were introduced into them. This yielded a set of blood samples from the same patient with variable concentrations (2:100 and 10:100 volume-by-volume) of the IV solution. Blood plasma samples were obtained by centrifugation of whole blood samples, and the same procedure was performed on them to make plasma-propofol mixtures. Prepared samples were thoroughly mixed to avoid sedimentation of erythrocytes and equal distribution of drug in the sample. For the measurements, a 50 μl volume drops were deposited on aluminum substrate to avoid recording of substrate spectra.

RESULTS

For each patient, spectra with 2 different concentrations of propofol, and one of unchanged sample were recorded and analyzed, as well as reference spectrum of IV propofol solution. We have conducted preliminary processing and analysis of the spectra which showed, that the quantification of propofol presence in human blood is possible, basing on the changes in intensity of the spectra in several regions. The reference spectrum of propofol IV solution (Fig.2) exhibits very prominent lines, mainly at 1450 cm-1, 1250-1260 cm-1, 1050 cm-1, 875-910 cm-1 , and 640 cm-1.Very small effect of the drug on blood spectra is seen, without evident presence of new peaks in the spectra. Thus, we have left the background without subtraction, as we have concluded that it contains useful information. The influence of propofol on the Raman spectra of blood is exhibited by slightly elevated intensity of the bands in spectral ranges around 1400 cm-1, 1250-1350 cm-1, 750-900 cm-1, and below 500 cm-1. Such behavior is very similar to the behavior of glucose in blood, as reported by other groups[7-9]. It's also possible that components of propofol dissolves or metabolizes when added to whole blood, which makes Raman bands wider. It is noteworthy, that the influence of propofol on all samples always reduces the total intensity of the Raman signal, which may be explained by a high light scattering introduced by the IV solution. This causes the Raman photons to scatter in all directions and not be collected by the fiber optic probe. Plasma samples (Fig.4.) were used only for qualitative analysis. The most evident changes were seen in the lower spectral region, mostly lower than 500 cm-1 and from 750 cm-1 to 900 cm-1. Much smaller changes are seen at 1450 cm-1 and between 1000 – 1250 cm-1. A set of samples from patients with anemia, with very low values of hematocrit and hemoglobin were also used for qualitative analysis (Fig.5.). The spectra are very similar to that of a healthy blood, however the spectral range 350 cm-1 – 550 cm-1 has less intensity, which is associated with the amount of hematocrit in blood sample.

CONCLUSIONS

Due to the strong absorption of blood, high scattering, and high levels of fluorescence background it was necessary to use chemometric pre-processing methods to improve the quality of the spectra. We have utilized a fast Fourier filtering for spike and noise removal, along with Savitzky-Golay smoothing. The spectra were normalized using sample normal variate (SNV) algorithm. Prepared spectra were qualitatively analyzed, to locate and identify the changes induced by the introduction of propofol into the samples of blood and plasma. The analysis yielded information about the behavior of the spectra, which was exhibited by changes in the intensity of several spectral regions. This was mostly visible in the regions: 1450 cm-1, 1250-1260 cm-1, 1050 cm-1, 875-910 cm-1, and 640 cm-1, This is consistent with the regions of most prominent lines in the reference spectrum of propofol IV solution. Some of the same spectral regions were affected by introduction of propofol for whole blood, and from samples with extremely low hematocrit values from anemia patients, as well as for blood plasma. We conclude, that a quantitative analysis may be carried out with grater dataset, which will allow statistical approach by employing multivariate models and classification algorithms[10], such as principle component analysis (PCA), partial least-square (PLS) regression, or support vector machines (SVM). Such approach will enable automatic detection of propofol presence in blood and subsequent blood propofol concentration determination, which was carried out in vitro in this study, but may as well be translated to an in vivo situation.

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9537-36, Session 7

Registration of intracellular pH in cancer cells with genetically encoded ratiometric sensor *(Invited Paper)*

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Intracellular pH (pHi) is known to be an important characteristic of cancer cell physiology. It is generally accepted that cancer cells and cancer-associated fibroblasts can change their metabolism to maintain tumor progression and adapt cancer cells to unfavourable conditions. However, very little known about changes in pHi, because evaluation of pHi is a challenge.

The purpose of the study was to develop a method for pHi registration in living cancer cells in vitro and tumor xenogtafts in vivo using a novel genetically encoded sensor SypHer2 and investigation of tumor-stroma interaction.

The experiments were performed on monolayer cell cultures, tumor spheroids and HeLa tumors xenogtafts stably transducted with SypHer2 gene. For investigation of pHi in conditions of interaction of cancer cells with fibroblasts, experiments on co-cultures were performed.

Registration pHi with genetically encoded sensor SypHer2 is based on obtaining fluorescence images at the wavelengths of 420 nm (I420) and 500 nm (I500) and calculating ratiometric signal I500/I420. Calibration of the ratiometric signal was made using buffer solutions, containing nigericin, and a value of pHi in cancer cells in vitro was determined. It was found to be 7.35±0.1. The data about pHi changes in conditions of co-culturing with fibroblasts were obtained. It was also demonstrated that spheroids and tumor in vivo are heterogeneous and have zones with different pHi.

9537-37, Session 7

Aqueous glucose measurement using differential absorption based frequency domain optical coherence tomography at wavelengths of 1310 nm and 1625 nm

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Enhancement of selectivity in measuring glucose by means of a dual wavelength approach is presented in this work. It involves a combination of differential absorption technique and frequency domain optical coherence tomography (DAOCT) so as to improve selectivity of glucose in the presence of interfering species especially water and to obtain faster acquisition of depth resolved information. Two wavelengths, namely 1310 nm and 1625 nm are selected since the absorption of water is relatively low and equal, while the difference of glucose absorption is relatively high at 1625 nm. Two broadband super-luminescent diode (SLED) sources with centre wavelengths 1586 nm and 1310 nm and a spectral width of ~ 60 nm (FWHM) are used. The overall spectral range of the SLED with 1586 nm was ranging from 1540 to 1640 nm. Absorption spectroscopy using various concentrations of glucose gave promising results to distinguish the absorption characteristics at these two wavelengths. Using OCT technique, interfering spectra were obtained using an optical spectrum analyzer with a resolution of 0.5 nm. Further processing of the interference spectra provided depth resolved information for the two wavelengths. Due to the absorption of glucose, with increase in concentration of glucose the intensity of the back reflected light was reduced in 1625 nm range as compared to 1310 nm. Difference in the spectral characteristics obtained from OCT interference pattern with and without glucose absorption is also simulated.

9537-38, Session 7

Towards real-time medical diagnostics using hyperspectral imaging technology

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Hyperspectral imaging provides the means to do non-contact high resolution spectral imaging of human tissue with a high spatial and spectral resolution. Real-time visualization of results is essential for the use of hyperspectral imaging in clinical diagnostics. The imaging technique itself is relatively fast, but processing can be extensive and time-consuming. Noise removal is of high importance to any spectral classification results. Noise removal without loss of spatial or spectral resolution usually requires the full image to be available for processing.

In this study a real-time system for hyperspectral analysis is proposed. A modification of the Minimum Noise Fraction-algorithm is used to obtain noise-free image data line by line. Each line is then processed by the subsequent processing algorithms. A combination of GPU processing and threaded CPU processing completes processing of the current line of data within the arrival of the next. Visualization of the results can be synced to the scanning speed.

Blood oxygenation, blood volume fraction and vessel enhanced images are obtained from the denoised data.

The results of this study show the potential for real-time processing of clinical hyperspectral data. The presented approach allows for determination of tissue properties in real-time.

9537-39, Session 7

Mid-infrared spectroscopic characterisation of an ultra-broadband tunable EC-QCL system intended for biomedical applications

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Mid-infrared spectroscopy has been successfully applied for reagent-free clinical chemistry applications. Our aim is to design a portable bed-side system for ICU patient monitoring, based on mid-infrared absorption spectra of continuously sampled body-fluids. Robust and miniature bed-side systems can be achieved with tunable external cavity quantum cascade lasers (EC-QCL). Previously, single EC-QCL modules covering a wavenumber interval up to 250 cm-1 have been utilized. However, for broader applicability in biomedical research an extended interval around the mid-infrared fingerprint region should be accessible, which is possible with at least three or four EC-QCL modules. For such purpose, a tunable ultra-broadband system (780 - 1900 cm-1, from Block Engineering) has been studied with regard to its transient emission characteristics in ns time resolution during different laser pulse widths using a VERTEX 80v FTIR spectrometer with step-scan option. Furthermore, laser emission line profiles of all four incorporated EC-QCL modules have been analysed at high spectral resolution (0.08 cm-1) and beam profiles with few deviations from the TEM 00 spatial mode have been manifested. Emission line reproducibility has been tested for various wavenumbers in step tune mode, and the overall accuracy has been found between ± 3 cm-1 compared to the FTIR spectrometer scale. With regard to an application in clinical chemistry, theoretically achievable concentration accuracies for different blood substrates based on blood plasma and dialysate spectra previously recorded by FTIR-spectrometers have been estimated taking into account the now accessible extended wavenumber interval.

9537-40, Session 7

Spectroscopic imaging of blood vessels only near the skin surface for non-invasive blood glucose measurement

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To realize the non-invasive blood glucose measurement, it will be effective to acquire the spectroscopic imaging of blood vessels only near the skin surface for eliminating other biological-component's disturbances. Our proposed imaging-type 2-dimensional Fourier spectroscopic imaging can limit the measuring depth into focal plane with high light detection sensitivity. Thus, the proposed method will be suitable for measuring only near the skin surface with detecting weak reflected light from inner biomembrane. Measuring specific blood-vessel area near the skin surface is effective to avoid disturbances optically. To specify the spectroscopic measurement area for spatial axis, spectroscopic imaging is valid and feasible ways. But for depth direction, obtained spectroscopic characters utilized transmitted light illumination include every components among optical path. So, the reflected illumination is essentially required for measuring near the skin surface. To obtain spectroscopic data of blood vessel near the skin surface, reflected lights from skin surface should be eliminated. Based on Fresnel equations, the reflection ratio is 1000 times larger than inside ratio of biological membrane. Weak reflected lights

from vessels should be detected without surface reflection. To eliminate the surface reflected lights, we introduced p-polarized light illumination from Brewster's angle. And also, weak reflected lights from vessel area were detected with crossed-Nicol dark field optics. We successfully confirmed spectroscopic characters of vein's hemoglobin as same as rat's blood without pulsebeat. In the future work, we will evaluate the accuracy of glucose concentration using near infrared light whose wavelength region 900nm to 1700nm.

9537-41, Session 7

In-situ monitoring of blood glucose level for dialysis machine by AAA-battery-size ATR Fourier spectroscopy

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For blood glucose level measurement of dialysis machines, we proposed AAA-battery-size ATR (Attenuated total reflection) Fourier spectroscopy in middle infrared light region. The extremely compact sensor is expected to be applied to blood glucose level measurement for dialysis machines. Patients of diabetic renal disease could not control blood-sugar level well. Because our proposed method is based on the spatial-phase-shift interferometer, interferograms can be obtained with the simple optical configuration. So, the proposed one-shot Fourier spectroscopic imaging is a near-common path and spatial phase-shift interferometer with high time resolution. Because numerous number of spectral data that is 60 (= camera frame rare e.g. 60[Hz]) multiplied by pixel number could be obtained in 1[sec.], statistical-averaging improvement realize high-accurate spectral measurement. We evaluated the quantitative accuracy of our proposed method for measuring glucose concentration in near-infrared light region with liquid cells. We confirmed that absorbance at 1600[nm] had high correlations with glucose concentrations (correlation coefficient: 0.92). But to measure whole bloods, complex light phenomenon caused from red blood cells, that is scattering and multiple reflection or so, deteriorate spectral data. Thus, we also proposed the ultrasound-assisted spectroscopic imaging that traps particles at standing-wave node. Thus, if ATR prism is oscillated mechanically, anti-node area is generated around evanescent light field on prism surface. By elimination complex light phenomenon of red blood cells, glucose concentration in whole bloods will be quantify with high accuracy. In this report, we successfully trapped red blood cells in normal saline solution with ultrasonic standing wave (frequency: 2[MHz]).

9537-42, Session 8

3D imaging of apoptosis by FRET, light sheet fluorescence and scattering microsopy *(Invited Paper)*

Herbert Schneckenburger, Petra Weber, Sarah Schickinger, Verena Richter, Thomas Bruns, Michael Wagner, Hochschule Aalen (Germany)

Non-radiative cell membrane associated Förster Resonance Energy Transfer (FRET) from a cyan (ECFP) to a yellow (EYFP) fluorescent protein is used for detection of apoptosis in 3-dimensional cell cultures. FRET is visualized in multi-cellular tumour spheroids by light sheet based fluorescence microscopy in combination with microspectrometry and fluorescence lifetime imaging (FLIM). Upon application of toxins (e.g. staurosporine) as well as chemotherapeutic agents or modulators (e.g. phorbol-12-myristate-13-acetate; PMA) the caspase-3 sensitive peptide linker DEVD is cleaved, resulting in a reduction of acceptor (EYFP) fluorescence as well as a prolongation of the fluorescence lifetime of the donor (ECFP). Fluorescence spectra and lifetimes may, therefore, be used for monitoring of apoptosis in a realistic 3-dimensional system, and light sheet based microscopy appears appropriate for 3D imaging at low light exposure.

9537-43, Session 8

Imaging the spectral reflectance properties of bipolar radiofrequency-fused bowel tissue

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Delivery of radiofrequency (RF) electrical energy is used during surgery to heat and seal tissue, such as vessels, allowing resection without blood loss. Recent work has suggested that this approach may be extended to allow surgical attachment of larger tissue segments for applications such as bowel anastomosis.

In a large series of porcine surgical procedures bipolar RF energy was used to resect and re-seal the small bowel in vivo with a commercial tissue fusion device (Ligasure; Covidien PLC, USA). The tissue was then imaged with a multispectral laparoscope to obtain a spectral datacube comprising both fused and healthy tissue. Maps of blood volume, oxygen saturation and scattering power were derived from the measured reflectance spectra using an optimised light-tissue interaction model.

A 60% increase in reflectance of visible light (460-700 nm) was observed after fusion, with the tissue taking on a white appearance. Despite this the distinctive shape of the haemoglobin absorption spectrum was still noticeable in the 460-600 nm wavelength range. Scattering power increased in the fused region in comparison to normal serosa, while blood volume and oxygen saturation decreased.

Observed fusion-induced changes in the reflectance spectrum are consistent with the biophysical changes induced through tissue denaturation and increased collagen cross-linking. The multispectral imager allows mapping of the spatial extent of these changes and classification of the zone of damaged tissue. Further analysis of the spectral data in parallel with histopathological examination of excised specimens will allow correlation of the optical property changes with microscopic alterations in tissue structure.

9537-44, Session 8

Angular and spectrally resolved investigations of yeast cells by light scattering microscopy and goniometric measurements

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Light scattering of yeast cells was investigated spectrally and angular resolved with a light scattering microscope and a goniometer. Elastic light scattering microscopy provides information about morphology and optical properties of individual cells with sub-wavelength resolution. The goniometric setup allows to directly measure the scattering phase function of suspended cells and is thus sensitive to collective changes in the cellular microstructures.

Interpretation of the obtained scattering patterns and phase functions relies on physically motivated theoretical models. Our results demonstrate that the standard Mie model of homogeneous spheres does not suffice to describe the measurements appropriately.

For individual yeast cells, the angular light scattering patterns exhibit additional modulations. These are attributed to light scattering from the nucleus by performing scattering calculations for a spherical cell model with a spherical, non-concentric inclusion. Comparison between measured and calculated scattering patterns yields size and refractive index of both cell and nucleus. The spectrally resolved patterns prove challenging to interpret though exhibiting pronounced oscillations.

With the goniometer, the scattering phase function of suspended yeast cells was measured spectrally resolved and with high sensitivity over more than three orders of magnitude. The single scattering data reveal pronounced scattering in forward direction. From theoretical calculations based on Mie theory for light scattering of suspended particles, we infer that a quantitative analysis of the goniometric measurements requires a more sophisticated cell model which takes into account internal cellular structures and the morphological diversity of the suspended cells due to cellular budding.

9537-45, Session 8

Light scattering microscopy: an appropriate tool for probing apoptosis and tumor cell recognition?

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Light scattering experiments with angular or spectral resolution have been used for characterization of various types of cells or for measurement of morphological changes of cells undergoing necrosis or apoptosis. This paper describes a conventional wide-field microscope which has been modified for backscattering experiments of living cells with high angular resolution, permitting simultaneous visual control of cells and their sub-structures.

The method is based on focusing a laser beam at various positions of the aperture plane within the microscope objective lens, which results in a parallel beam hitting the sample under variable inclination. Backscattered light collected by the same objective lens is focused to an exit pupil, where an angle of 180° is selected by a pinhole, while the illumination angle can be varied from 190° - 257° with a precision of ? 0.6°.

In comparison with control cells, 3T3 human fibroblasts cultivated as 2D monolayers show pronounced changes after initiation of apoptosis (by staurosporine) including shrinking and almost spherical shape. Concomitantly scattering intensity increases and exhibits pronounced oscillations, which are typical for Mie scattering.

When light scattering microscopy is applied to more realistic 3-dimensional multi-cellular spheroids, scattering profiles are more complex, but similar to those of 2D cell cultures. In first experiments for differentiation of tumorigenic and non-tumorigenic cells the scattering behaviour of 3D spheroids of 3T3 fibroblasts changes upon infiltration of HeLa cervix carcinoma cells, thus indicating a potential of elastic light scattering experiments for tumour cell recognition.

9537-46, Session 8

5-aminolevulinic acid for quantitative seek-and-treat of high-grade dysplasia in Barrett's esophagus cellular models

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High-grade dysplasia (HGD) in Barrett's esophagus poses increased risk in developing esophageal adenocarcinoma. To date, early detection and treatment of HGD regions is still challenging due to the sampling error from tissue biopsy and relocation error during the treatment after histopathological analysis. In this study, CP-A (metaplasia) and CP-B (high-grade dysplasia) cell lines were used to investigate the "seek and treat" potential using 5-ALA induced PpIX. The PDT photosensitizer then provides both phototoxic effect and additional image contrast for automatic detection and real-time laser treatment. In addition to automatic classification of HGD cells, we characterized subcellular irradiation and the potential phototoxicity on both metaplasia and HGD. Support Vector Machines (SVM) was trained using the aforementioned extracted features to obtain an automatic and robust detection of HGD. Our results showed 95% sensitivity and 87% specificity using the optimal feature combination, which pave the way for a further extension to a 3D cellular model. The treatment results showed that the HGD cells are less viable than metaplastic cells due to more PpIX production at earlier times. Also, due to mitochondrial localization of PpIX, better killing effect was achieved by involving mitochondria or whole cells compared to just nucleus irradiation in the detected region.

9537-47, Session 8

Gold nanorods as photothermal agents and autofluorescence enhancer to track cell death during plasmonic photothermal therapy

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Photothermal therapy procedure with temperature monitoring alone fails to indicate the treatment outcome. The redox ratio based on cell autofluorescence measurements directly measures the respiratory status of cells, which is a effective indicator of cell death. However, weak tissue autofluorescence limits the versatility of this approach. The dual peaks of gold nanorods enables conducting plasmonic photothermal treatment and enhancing weak cellular autofluorescence simultaneously, which thus can play an important role in therapy and monitoring of the treatment outcome. In this study, human renal cell carcinoma cells taking up PEGylated gold nanorods via endocytosis were illuminated by a 785-nm laser with a power density of 5.14 W/cm2. The cells were treated for different time durations and its autofluorescence was measured before and after therapy. Due to the plasmonic effect of gold nanorods, two-fold enhancement in the autofluorescence emission intensity was observed. Moreover, the time profiles of the traditional cell viability test result and autofluorescence intensity are highly correlated with each other, both indicating the progress of cell death over time. Hence, if cell autofluorescence is measured continuously and quickly during therapy, one can determine the progress of the treatment outcome online by just monitoring the autofluorescence intensity and thus eliminating the need of performing the time consuming and tedious cell viability test. The continuous monitoring of autofluorescence can help optimize the treatment outcome in real time by providing instant feedback for the fine adjustment of laser illumination.

9538-53, Session PD

Whole-head functional brain imaging of neonates at cot-side using time-resolved diffuse optical tomography

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We present a method for acquiring whole-head images of tissue optical properties and haemodynamics from the infant brain at cot-side using time-resolved diffuse optical tomography (TR-DOT). At University College London, we have built a portable TR-DOT device, known as MONSTIR II, which is capable of obtaining a whole-head (1024 channels) image sequence in 75 seconds. Datatypes extracted from the temporal point spread functions acquired by the system allow us to determine changes in absorption and reduced scattering coefficients within the interrogated tissue. This information can then be used to define clinically relevant measures, such as oxygen saturation, as well as to reconstruct images of relative changes in tissue chromophore concentration, notably those of oxy- and deoxyhaemoglobin. Additionally, the effective temporal resolution of our system is improved with spatio-temporal regularisation implemented through a Kalman filtering approach, allowing us to image transient haemodynamic changes. By using this filtering technique with intensity and mean time-of-flight datatypes, we have reconstructed images of changes in absorption and reduced scattering coefficients in dynamic 2D phantoms. These results demonstrate that MONSTIR II is capable of resolving slow changes in tissue optical properties within volumes that are comparable to the preterm head. Following this verification study, we are progressing to imaging a 3D dynamic phantom as well as the neonatal brain at cot-side. Our current study involves scanning healthy babies to demonstrate the quality of recordings we are able to achieve in this challenging patient population, with the eventual goal of imaging functional activation and seizures.

9538-1, Session 1

Non-contact scanning time-domain functional imaging of the adult human brain *(Invited Paper)*

Heidrun Wabnitz, Physikalisch-Technische Bundesanstalt (Germany); Mikhail Mazurenka, Physikalisch-Technische Bundesanstalt (Germany) and Hannoversches Zentrum für Optische Technologien (Germany); Katja Fuchs, Physikalisch-Technische Bundesanstalt (Germany); Laura Di Sieno, Gianluca Boso, Davide Contini, Alberto Dalla Mora, Alberto Tosi, Politecnico di Milano (Italy); Yoko Hoshi M.D., The Tokyo Metropolitan Institute of Medical Science (Japan); Antonio Pifferi, Politecnico di Milano (Italy); Rainer Macdonald, Physikalisch-Technische Bundesanstalt (Germany)

We present a novel non-contact scanning system for time-domain functional near-infrared spectroscopy of tissues, its characterization by phantom measurements and first in-vivo tests. Employing a supercontinuum laser with an acousto-optic tunable filter as light source, the tissue was scanned by a galvanometer scanner from a distance of more than 10 cm. The scan was performed with a fixed, small (4 mm) separation between the illumination spot and the detection spot. A fast-gated single-photon avalanche diode was employed to eliminate the intense early part of the diffusely remitted signal and to detect late photons only that carry information on absorption changes in the brain. A second parallel detection channel was equipped with a non-gated detector to record superficial absorption changes. Arrays of gated and non-gated time-of-flight distributions of photons were recorded by imaging time-correlated single-photon counting. A tissue area of typically 4?4 cm2 was scanned with 32?32 pixels within a frame time of 1 s. The wavelength was switched line by line between two bands around 760 nm and 860 nm. Absorption changes were derived from changes in photon counts in selected time windows of the distributions. The system was characterized by a set of phantom measurements to assess the responsivity of the detection system, the lateral spatial resolution, penetration depth and depth selectivity. First in vivo applications included the imaging of hemodynamic changes in the brain during various cognitive and motor activation tasks. These tests demonstrated the successful non-contact imaging of hemoglobin concentration changes in deep tissues.

9538-2, Session 1

Imaging brain function in children with autism spectrum disorder with diffuse optical tomography

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Autism Spectrum Disorder (ASD) is a common and currently incurable neurodevelopmental disorder defined by impaired social interactions, altered language function, and repetitive behaviors. ASD affects an estimated 1% of children, and engenders enormous personal, social, and economic costs. Investigating the neuroscience of ASD in childhood is vital because early behavioral and educational interventions starting at 18-24 months of age have been shown to improve outcomes. Neuroimaging studies using functional magnetic resonance imaging (fMRI) have identified specific brain regions whose responses to biological motion perception stimuli are correlated with behavioral metrics of ASD; these responses are potential interventional outcome measures. However, current neuroimaging methods (e.g., fMRI) are limited in ASD due to the constrained imaging environment. Our lab has been developing diffuse optical tomography (DOT) methods that overcome ergonomic limitations of fMRI and image brain function with a wearable cap. The wearability of DOT will allow a fuller assessment of brain function in severely affected children with ASD, exceedingly challenging to study with MRI methods. We present here a feasibility study imaging with our high density DOT system school-aged typically developing children (TDC) and sex/age/IQ-matched children with autism (ASD). Both groups of children are able to tolerate imaging for over 30 minutes, and exhibit acceptable raw data quality, and maps of functional brain activity in response to simple language tasks like hearing words and verb generation. Group-matched brain responses of biological motion perception and resting state networks will also be presented.

9538-3, Session 1

Efficient method for near real-time diffuse optical tomography of the human brain

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Previous studies have showed only regions with a sensitivity higher that 1% of the maximum value can affect the recovery result for diffuse optical tomography (DOT). Two methods of efficient sensitivity map generation based on Finite Element Models (FEM) are developed based on (1) reduced sensitivity matrix and (2) parallelization process. Time and memory efficiency of these processes are evaluated and compared with conventional methods. It is shown that the computational time for a full head model containing 200k nodes is reduced from 3 hours to 48 minutes and the required memory is reduced from 5.5 GB to 0.5 GB. For a range of mesh densities upto 320k nodes, the required memory is improved by ~1000% and computational time by ~400% to allow near real-time image recovery.

9538-4, Session 1

Cerebral autoregulation during pediatric extracorporeal membrane oxygenation therapy

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Extracorporeal membrane oxygenation (ECMO) therapy provides a critical bridge to recovery, transplant or long term mechanical support for children and adults with serious, but treatable, afflictions of the heart and/or lungs. In essence, it functions to mechanically circulate externally oxygenated blood into and through the body. Blood pressure optimization is critical to preserve end-organ function during ECMO therapy. However, intrinsic autoregulatory mechanisms for certain end-organs such as the kidney and brain may lead to significant flow discrepancies between systemic and cerebral or renal flow.

Neurological injury remains a debilitating and relatively common complication among ECMO survivors, and current limitations in diagnostic modalities often lead to late diagnosis. Thus, early detection of periods of high risk prior to injury is vital to improve outcomes following ECMO therapy. Currently, clinicians have no tool to directly monitor cerebral oxygen metabolism and instead rely on systemic measurements. A bedside cerebral blood flow monitor could be used, for example, to set appropriate mechanical pump rates to optimize cerebral perfusion. In this contribution we describe our first attempts to utilize diffuse optical and correlation spectroscopies to measure cerebral blood flow and oxygenation during ECMO therapy. We are thus able to explore the effects of variation in ECMO pump rates and assess cerebral blood flow compensatory mechanisms in the extreme pathological state created by extracorporeal circulation through manipulation of ECMO flow rates in a pediatric population on a patient-by-patient basis and as a function of time.

9538-5, Session 1

Time Resolved optical detection for white mater lesion detection: preclinical tests on macaque brains and MRI co-registration

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We designed a bedside noninvasive optical-based instrument to address new-born infant brain imaging. The system is based on a Time-Resolved instrumentation coupled with a method based on Mellin-Laplace Transform to reconstruct 3D optical characteristics deeply buried in diffusive tissues. This instrument has the potentiality to reconstruct the optical properties of the biological tissues in depth. The work we present in this paper is the very first experiments we performed to address the detection and identification of white matter lesion in young macaques. We present the protocol we have designed to conduct the preclinical assessment aimed at detecting brain white matter lesions by our system through the scalp of the animal; we correlated our optical measurements with a reference MRI acquisition and provide a 3D reconstructed absorption map co-registered with MRI data for the 4 macaques we tested.

9538-6, Session 2

Non-contact, scanning hyperspectral diffuse optical spectroscopy and diffuse correlation spectroscopy system

Miguel A. Mireles, Johannes D. Johansson, Parisa Farzam, Turgut Durduran, ICFO - Institut de Ciències Fotòniques (Spain)

We present a new, non-contact, scanning broadband (or hyperspectral) diffuse optical spectroscopy (DOS) and diffuse correlation spectroscopy system for small animal imaging. DOS and DCS have been used for monitoring murine cancerous tumors by using various contact and non-contact probes. They have either suffered from probe pressure induced variability in case of the contact systems or from limited number of wavelengths in the non-contact systems.

Our DOS system combines a large number of source detector pairs with a broad wavelength range by imaging the tissue directly to a 2D spectrophotometer. This allows us to recover accurately chromophore concentrations simultaneously with blood flow measurements. It is readily adapted for scanning and therefore is suitable for the investigation of spatial variability of the hemodynamics.

The system performance was validated by scanning on liquid phantoms and by doing a dynamic study on a subject's palm during arterial cuff occlusion. The system is meant to be applied for longitudinal studies on both healthy and cancerous tumors in mice, before and after therapy. We will present extensive characterization and validation results.

9538-7, Session 2

Single shot multi-exposure laser speckle contrast imaging with a novel single photon avalanche diode (SPAD) array

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Laser speckle contrast imaging is an optical technique for imaging blood flow. Recently, multi-exposure speckle imaging (MESI) was proposed to exploit the dependence of speckle contrast over exposure time in order to fit absolute values. In MESI, many frames are acquired at each exposure time and utilized in a model based fitting. One disadvantage of that method is the long acquisition time, due to such sequential measurements at different exposure times, and the limited signal-to-noise ratio at low exposure times. Herewith, we present a novel approach for MESI, through an advanced 62x32 pixels single-photon avalanche diode

(SPAD) array. Its single-photon sensitivity, low noise, and high frame-rates provide a breakthrough in enabling just a single and continuous measurement to acquire all the exposure times. We demonstrate the method both in phantoms and in vivo measurements of cerebral blood flow in a mouse during hyperoxia and hypercapnia. The current SPAD array provides 2048 pixels, but can be designed for megapixel resolutions and even faster speed, soon enabling even more complex speckle-based measurements, such as parallel diffuse correlation spectroscopy or speckle-contrast optical tomography (SCOT).

9538-8, Session 2

Can we measure blood flow and optical properties of tissue by diffuse correlation spectroscopy?

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Diffuse correlation spectroscopy (DCS) is a relatively new technique to measure blood flow in the microvasculature noninvasively. One of the main limitations of DCS is the influence of the assumed optical properties of the medium on the calculated blood flow index (BFI). Many DCS experiments were carried out with hybrid DCS and diffuse optical spectroscopy (DOS) devices but in general it is difficult with these systems to measure the absolute absorption and scattering coefficients. In this work we present an algorithm that utilizes multi-distance DCS (MD-DCS) and enables us to estimate the absolute values of static (reduced scattering and absorption coefficient) and dynamic (blood flow) properties of the tissue simultaneously, hence minimizing the errors in the BFI estimation. We have investigated the robustness of the proposed algorithm on noise-added simulated data as well as tissue simulating phantoms. To validate the MD-DCS technique in a more realistic scenario we have applied the method on a mouse tumor which is a highly heterogeneous medium. The results suggest that MD-DCS can be used to decouple the absorption and scattering coefficients from BFI in small source-detector separations to allow simpler, DCS-only approaches for the blood flow measurements.

9538-9, Session 2

Time-domain diffuse optics: towards next generation devices

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We present a compact Time-Domain (TD) probe hosting both pulsed laser source and single photon detectors. We developed two versions of this device: one based on time gated Single Photon Avalanche Diodes (SPADs) and the second based on Silicon Photo Multipliers (SiPMs). While the first exploits the possibility to perform null-distance measurements, the second exploits the large area of SiPMs. We tested the single device both on phantoms and in vivo measurements, showing performances comparable with state-of-the-art TD systems. With simulations we demonstrate that a dense null-distance arrangement of these novel TD devices yields 1 decade higher contrast and 3-fold increase in the maximum achievable depth in diffuse optics measurement as compared to continuous wave approaches. These devices represent a step towards compact and wearable TD instruments with a tremendous reduction in size and costs, and with the possibility to overcome the actual limits in terms of achievable depth and contrast of optical techniques. We believe that this novel technology will enlarge the field of applications of Diffuse Optics in a way nowadays unthinkable. First, this approach can increase sensitivity, quantitation and localisation capabilities in already existing clinical applications. Secondly, it can permit to non-invasively investigate organs too deep to be monitored with traditional optical instruments, such as the lung or the heart. In principle, TD Diffuse Optics could evolve to a new general purpose imaging technique with a depth of view comparable to ultrasonography, with lower spatial resolution but with a good functional and chemical specificity.

9538-10, Session 2

Novel information extraction methods and high-speed instrumentation in spatial frequency domain imaging

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Spatial frequency domain imaging (SFDI) is a spectral imaging modality enabling quantification of in vivo tissue chromophores. SFDI characterizes the attenuation of diffusely reflected light in the spatial frequency domain by employing sinusoidal illumination patterns. Conventional SFDI employs three projected patterns per wavelength per spatial frequency, limiting data acquisition speed. The requirement for multiple patterns per spatial frequency can be obviated by increasing the complexity of the pattern. We have devised a multi-frequency synthesis and extraction approach (MSE), employing custom patterns having multiple spatial frequency components. To maximize speed, we apply binary, square wave patterns to MSE. Since these patterns have only two unique values (off and on), they are projected more rapidly than sinusoids, which require grayscale values. We have adapted this approach to a real-time, multi-spectral SFDI platform using a high-speed digital micromirror device (DMD) to generate binary patterns, having a refresh rate faster than the camera exposure time. Our results demonstrate the ability of MSE using square wave patterns to obtain optical property values with accuracy to within 1% of those obtained using conventional SFDI. We have used our platform to image an in vivo arm pressure-cuff occlusion model, and obtained tissue oxygen saturation maps at near video rates (15 Hz), with frame rates 5 times faster than conventional SFDI. To further advance instrumentation, we are developing a single element detector imaging platform, based on frequency encoding of both spatial location and wavelength. We expect this technique will allow for hyperspectral (20 wavelengths) SFDI at 10-20 Hz.

9538-11, Session 2

In-depth quantification by using multispectral time-resolved diffuse optical tomography

Judy Zouaoui, CEA-LETI (France) and MINATEC (France); Lionel Hervé, MINATEC (France) and CEA-LETI (France); Laura Di Sieno, Politecnico di Milano (Italy); Anne Planat-Chrétien, Michel Berger, CEA-LETI (France) and MINATEC (France); Alberto Dalla Mora, Antonio Pifferi, Politecnico di Milano (Italy); Jacques Derouard, Univ. Grenoble Alpes (France) and Lab. Interdisciplinaire de Physique (France); Jean-Marc Dinten, CEA-LETI (France) and MINATEC (France)

Near-infrared diffuse optical tomography (DOT) is a medical imaging which gives the distribution of the optical properties of scattering biological tissues.

To obtain endogenous chromophore features in the depth of a scattering medium, a multiwavelength/time-resolved (MW/TR) DOT setup was used. Reconstructions of the three-dimensional maps of chromophore concentrations of probed media were obtained by using a data processing technique which manages Mellin-Laplace Transforms of their MW/TR optical signals and those of a known reference medium. The point was to put a constraint on the medium absorption coefficient by using a material basis composed of a given set of chromophores of known absorption spectra.

Experimental measurements were conducted by injecting the light of a picosecond near-infrared laser in the medium of interest and by collecting, for several wavelengths and multi positions, the backscattered light via a two fibers (with a source-detector separation of 15mm) connected to fast-gated single-photon avalanche diodes (SPAD) and coupled to a time-correlated single-photon counting (TCSPC) system.

Validations of the method were performed in simulation in the same configuration as the experiments for different combination of chromophores. Evaluation of the technique in real conditions was investigated on liquid phantoms composed of a homogenous background and a 10 mm depth inclusion formed of combination of intralipid and inks scanned at 30 positions and at three wavelengths.

Both numerical and preliminary phantom experiments confirm the potential of this method to determine chromophore concentrations in the depth of biological tissues.

Results will be presented at ECBO'15 conference.

9538-12, Session 3

Analytical solutions of the radiative transport equation for the reflectance and fluorescence from layered media illuminated by a finite beam

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We derived analytical solutions of the radiative transport equation for layered scattering media in the spatial frequency, the spatial, the temporal frequency, and the time domains. In all domains an excellent agreement (within the statistics of the simulations) compared to the Monte Carlo method was obtained. Besides the reflectance also the fluence and the radiance in the scattering medium can be calculated by the derived analytical formulae which also agree excellently with the Monte Carlo method. In addition, solutions of the radiative transport equation for the fluorescence were derived. We showed results for a semi-infinite medium but also solutions for layered media were derived. Both, the solutions for the reflectance and for the fluorescence are efficient and robust. For example, the P_3-solution cite{P3} for the spatially resolved reflectance from a two-layered medium at 500 distances between source and detector can be calculated in about one millisecond, in the time domain for the same number of distances in about 30 millisecond. Thus, it is many orders of magnitude faster than Monte Carlo simulations with GPU-acceleration and, in addition, without statistical noise. Therefore, the analytical solutions are predestinated for the fast and exact solution of the inverse problem.

9538-13, Session 3

Near infrared topography using low-resolution image reconstruction

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One of the serious problems of NIR topography is that the spatial resolution is not sufficient for the measurements of localized brain functions. Dense probe arrangements and image reconstruction using the spatial sensitivity profiles can improve the spatial resolution of the NIR topography. Although high-density diffuse optical tomography achieved the spatial resolution equivalent to functional magnetic resonance imaging, the double-density probe arrangement and simple mapping algorithm were adopted in the currently available commercial instrumentation for NIR topography.

In this study, a low-resolution image reconstruction is proposed to improve the topographic image measured by the double-density probe arrangement. In this imaging method, coarse image, which consisted of 7.5 mm size pixel, was firstly reconstructed using the spatial sensitivity profile. The value of each pixel in the coarse image obtained by the reconstruction was plotted for the pixel in the final image corresponding to the center of each pixel in the coarse image. The values of the other pixels were interpolated by a spline function. The topographic image obtained by this algorithm was compared with the image obtained by two algorithms, the mapping method and conventional image reconstruction.

The low-resolution image reconstruction can improve the topographic image compared to the mapping method and the artifacts, which tend to occur when the number of pixel in the reconstructed image is much greater than that of measurement points, were reduced compared to the image obtained by the conventional image reconstruction algorithm.

9538-14, Session 3

Determining optical properties of epithelial tissues with an obliquely incident beam

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We present a technique for determining the scattering coefficient of epithelial tissue from diffuse reflectance measurements.

Specifically due to an obliquely incident Gaussian beam, and applying the convolution form of the diffuse reflectance determined by the corrected diffusion approximation.

9538-15, Session 3

Light propagation through weakly scattering media. A study of Monte Carlo vs. Diffusion theory with application to neuroimaging

Daniele Ancora, Athanasios Zacharopoulos, Foundation for Research and Technology-Hellas (Greece); Jorge Ripoll, Univ. Carlos III de Madrid (Spain); Giannis Zacharakis, Foundation for Research and Technology-Hellas (Greece)

Introduction

During the past years Optical Imaging has been revolutionized by combining several advanced techniques1 that cover the whole range of sizes and optical properties of biological tissue. An area of particular interest that could benefit from this evolution is neuroimaging especially with applications of in-vivo preclinical optical imaging. The need for high resolution and quantitative images have increased the demand for accurate modelling of light propagation through biological media of increased optical complexity. The commonly used tool for calculating light transmission though biological turbid media is the Diffusion Equation (DE), an approximation based on the Radiative Transfer Equation (RTE), valid for high scattering regimes, but inaccurate for regions of low scattering such as the region between the brain and the skull which is filled with Cerebral Spinal Fluid (CSF). To overcome this limitation the imaging field can take advantage from modern General-Purpose Graphics Processing Units (GPGPU) computing, enabling the simulation of photon propagation through both highly and low scattering media2 in real-time using parallelized Monte Carlo (MC) approach also based on the RTE. Using Monte Carlo methods low scattering regions can now be accurately simulated in details enabling us to set a realistic comparison between the models, and draw conclusions on the importance of modelling the clear layer regions for brain imaging applications in mice and thus improve real biological in vivo experiments. Here we present our results of MC modelling and its comparison with diffusion theory, applied to imaging a mouse brain simulating phantom.

Materials & Methods:

MC algorithm consists of the repetition of a huge number of statistical sampling in order to recover the probability distribution for the property we want to measure. This requires high computational power which has been recently become available thanks to the increasing complexity in the GPU architecture and the number of cores, which opened the possibility to run multi-thread processes in real-time on truly low cost hardware. In our study we have used a CPU Intel i7-4930K 6 cores 3.40GHz with RAM

32GB and a GPU nVidia GeForce GTX 780 Ti with 2880 CUDA cores. In this work we have simulated a mouse head and brain with a multilayer cylindrical phantom, each layer representing one region of the head with the optical properties given in Table 1. With the inclusion of a thin non-scattering layer representing the CSF we have defined a 7 layer phantom with different thicknesses according to a realistic model (Fig.1).

We have implemented both photon propagation models, DE and MC, in an integrated Matlab environment (we have used the Finite Elements Method (FEM) Toast package6 to solve DE and MC eXtreme2, while a beam with a Gaussian profile was used in both cases as a source.

We performed ?30?^2 simulations at different ?? (?_a?[0,0.05]mm^(-1)) and ?'s (??'?_s?[0,0.1]mm^(-1)) collecting the light distribution profiles on the output side of the phantom. The profile is then integrated in the range of ??[0,ϖ]rad in polar coordinates along the diameter mostly to increase the resolution for MC simulations taking advantage of the symmetry in the experiment. This method enable us to make a direct comparison between the two models by choosing as output observables the peak intensity and the FWHM to analyze the shape properties and the Euclidean Distance between the profiles to have a more general indication about the difference between the models.

Then we performed ?10?^2 TOAST and MC simulation at different optical properties in the range of ?_a?[0,0.015]mm^(-1) and ?_s?[0,0.015]mm^(-1) for the CSF regions and we compared the profiles obtained at the output side of the structure. Despite CSF region being only 6.7% of the entire volume, DE as expected is not producing meaningful results.

Results

To depict a better scenario we first need to compare DE solution with MC photon propagation in order to find the range of optical properties (?a, ?'s) where only the statistical approach will produce the correct answer, in other words we want to investigate the limits where DE approximation ?_a ? ?'_s is no longer valid. Gaussian beam source and cylindrical synthetic phantoms, characterized by a set of optical properties easily reproducible in the lab, are a good choice in order to compare the simulations with experimental results.

As expected TOAST's solution starts to diverge from the MC solution approximately when? ??_a <10 ?'_s. In those optical properties DE fails and cannot be used to compute photon propagation in low scattering biological tissues such as CSF. Laboratory validation of the MC model will be implemented to further explore this behavior.

This behavior make DE a good choice to simulate efficiently photon propagation through biological media in most of the tissues, except for those with optical properties violating the assumption ?_a ? ?'_s. In clear regions surrounding the brain, such as the CSF, this approximation fails and the tissue cannot be included in the model while solving the Diffusive Equation.

Assuming the MC simulation with the 7 layer geometry (Fig. 2) as the ground truth solution for the RTE2, we compared it with the simulation on a 5 layer phantom where the CSF region was replaced by either brain or bone tissue. In this case as we can see in Fig. 2, we get similar output profiles for all simulated cases.

In this case it seems a good choice to replace CSF with its neighbours, and moreover optimize the value of the ??'?_s of the replacement layer in order to further minimize the distance between the profiles. Assuming ?_a of the new CSF region according to Table 1 and changing ??'?_s we found that the difference can be minimized.

Conclusion

Thanks to the MC approach for photon propagation based on GPU2 we managed to perform an interesting comparison between DE and MC models providing some initial results about the importance of the inclusion of low scattering regions in a model of the mouse head. After this study it is evident that the DE is not the right tool to include such a regime and despite the thickness of the layer being only about 6% of the total, the simulated light transmission was corrupted. MC had no problem to simulate the propagation through this structure which make it the ideal choice whenever such kind of tissue is present with the speed and accuracy of both models in the less than one minute range on a normal desktop. Moreover we understood that DE6 is still usable for the case of mouse brain imaging since the results showed that it is possible to neglect clear layers up to 0.5mm in thickness and still keep the solution coherent with MC. A complete study on a more realistic mouse-like phantom7 geometry is going to be implemented in our future work, and further experimental comparisons will be used to validate the finds of this study. Furthermore, it could be interesting to study such behaviour where clear layers are big enough and cannot be ignored.

9538-16, Session 3

Analytical solution of the simplified spherical harmonics equations for spherical media

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We present for the first time the analytical solution for the simplified spherical harmonics equations, a reliable approximation to the radiative transfer equation for describing light transport inside turbid media. This solution is calculated for a steady-state point source inside a spherical homogeneous scattering medium. To arrive at this solution, a modification is applied on the coupled partial differential equations of the simplified spherical harmonics equations based on the eigen method resulting in a set of decoupled partial differential equations. The equations are solved along with the partially reflective boundary conditions due to the difference in refractive indices between the turbid medium and its environment (air) in practical cases. This computationally inexpensive and fast solution is compared with the analytical solution of the diffusion equation approximation and benchmarked with the gold standard of Monte Carlo. The SP3 analytical solution proves to be to be in good accurate agreement with the Monte Carlo results.

9538-17, Session 3

Simulation of light propagation in biological tissue using a modified finite volume method applied to three-dimensional radiative transport equation

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An important issue in tissue optics and Optical Tomography is to have an efficient forward solver. In this work, a new numerical algorithm was developed for solving light propagation with the radiative transport equation within a three-dimensional absorbing and a highly forward-scattering medium such as a biological tissue subjected to an incident beam. Both elastically scattered light and fluorescence light were studied. Two problems used to assess the performance and accuracy of the proposed algorithm are presented.

9538-43, Session PWed

Methodology of manufacturing of solid tissue-like phantoms with absorption spectra of oxy- and deoxy-hemoglobin

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Near Infrared Spectroscopy (NIRS) techniques are noninvasive diagnostic methods which are promising in terms of bedside brain functions diagnostics. Most of the NIRS methods are based on the assessment of changes in hemoglobin concentration which are related to the changes in the absorbing properties of the examined tissue. In order to assess the accuracy of NIRS devices and to evaluate the results of measurements, one has to perform a series of tests on tissue phantoms with known optical properties. Most of the phantoms presented in the literature use a black dye to mimic the absorption properties of the tissue. Those materials tend to have a flat absorption spectrum which does not reflect the spectral properties of oxy- and deoxy-hemoglobin. The goal of our study is to manufacture a tissue phantom with absorption spectrum reflecting the specific properties of oxy- and deoxy-hemoglobin. We have examined a set of dyes in terms of their absorption spectrum.

An optimization problem of minimizing the error between the given hemoglobin spectrum and the mixture of dyes was formulated. The optimal set of concentrations was obtained using the interior point method with logarithmic barriers. In the end we hope to be able to produce a solid phantom would mimic the human tissue of specified saturation in range of 650 to 900nm.

9538-44, Session PWed

Surface layering effect of diluted Intralipid

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Many groups in the field of biomedical optics apply diluted Intralipid for characterization and comparison of their instruments In many publications the optical properties and the stability of Intralipid are studied and the usefulness of the Intralipid is doubtless Nevertheless extensively comparison of measurements of the spatially resolved reflectance at the surface of an Intralipid dilution with the best solution of the radiative transport equation (optimized scattering phase function, ...) leads to remaining errors especially for short distances to the source. As the same comparison with a spatially frequency domain setup SFD showed also errors for high frequencies, a further source of error had to be identified. Having a closer look to the surface of an Intralipid (Fresenius Kabi AG, Germany) dilution with added absorber 15 minutes after standstill and gently stirring a surface layer in form of flow marks at the surface can be visualized

In this study the influence of this surface layer on the measurements itself and further on the determination of the optical properties was investigated. Therefore the results of the measurements with the above mentioned methods were compared to a layered solution of the radiative transport equation. Inversely the optical properties of a 2-layered simulation were fitted with a semi-infinite model to analyze the errors caused by this layer.

9538-45, Session PWed

A new volume scanner

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Optical imaging through complex biological media remains a very challenging task due to the extremely high scattering experienced. A new design scanner is proposed and modelled which images scatter spatio-temporally. Modeling confirms the performance of the design. The inversion algorithm to reconstruct the scattering object remains as future work.

9538-46, Session PWed

Monitoring the injured brain: registered, patient specific atlas models to improve accuracy of recovered brain saturation values

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The subject of superficial contamination and signal origins remains a widely debated topic in the field of Near Infrared Spectroscopy (NIRS), yet the concept of using the technology to monitor an injured brain, in a clinical setting, poses additional challenges concerning the quantitative accuracy of recovered parameters.

Using high density diffuse optical tomography probes, quantitatively accurate parameters from different layers (skin, bone and brain) can be recovered from subject specific reconstruction models. This study assesses the use of registered atlas models for situations where subject specific models are not available. The ICBM152 atlas model was registered to 24 subject specific models using a non-iterative point to point (nP2P) algorithm. Data simulated from subject specific models was reconstructed using the 24 registered atlas models implementing a regional (layered) parameter recovery in NIRFAST.

A 3-region recovery based on the atlas model yielded results with lower quantitative accuracy than the spatially resolved spectroscopy (SRS) technique based on a simple NIRS probe design. This highlighted differences in superficial (skin and bone) layer thickness between the subject and atlas models. This layer thickness mismatch was propagated through the reconstruction process decreasing the parameter accuracy. By combining the superficial layers into a single region and using a 2-region recovery process greatly increased quantitative accuracy, improving on that of the SRS method. Further improvements to the reconstruction routine require more accurate segmentation of the skin and bone layers in the atlas model.

9538-47, Session PWed

Cortical model including the structure of the blood vessels for optical intrinsic signal imaging

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Optical intrinsic signal imaging is a key technology for understanding neurovascular coupling. In the previous studies, it was revealed that the hemoglobin concentration changes evoked by the brain activation were different among the different types of the blood vessels in the cortical tissue. In the case of optical imaging, light in the cortical tissue is strongly scattered and the detected light travels a large volume of the tissue. The contribution of the hemoglobin concentration change in the large blood vessels and surrounding capillary bed to the reflectance change on the cortical surface has not been quantitatively examined.

In this study, we constructed a realistic model of the exposed cortex of mice including the structure of the blood vessels obtained by two-photon microscopy. The model consisted of the arteriole, venule, capillary and parenchymatous tissue. Light propagation in the realistic cortical model was predicted by a Monte Carlo simulation to estimate the contribution of the hemoglobin concentration changes in the three types of the blood vessels to the reflectance change in optical intrinsic signal imaging. The light detected above the large arteriole and venule reflects the hemoglobin concentration change in those blood vessels, however, the detected light also travels with in the surrounding capillary bed. The contribution of the capillary is about 20% even if the reflectance change is measured above the arteriole of 100 ?m in diameter.

9538-48, Session PWed

CW fluorescence imaging of tissue like media in reflectance geometry

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We present experimental results and simulations of CW imaging of turbid media phantoms with inclusions emulating lesions containing different concentrations of absorber and ICG. The aim is to localize the inclusion and give an assessment of its optical properties and ICG concentration, by analysis of the absorption and fluorescence data.The diffuse reflectance geometry is used for the experiments. The light source is a CW NIR laser. The light is detected by an EMCCD camera that images the surface of the medium. An interferometric filter is used in front of the camera to

selectively choose the excitation or the emission light emerging from the tissue. Measurements were carried on liquid phantoms consisting of a mixture of water, milk, India ink and ICG. The inclusion was a hollow capsule also filled with a liquid solution with increased absorption and flourescence. It was embedded inside the liquid at different depths and at different horizontal distances from the illumination point.

Due to the exponential reduction of the light intensity with increasing distance from the source typical of this geometry, subtle variations of intensity introduced by the inclusion (if present) are difficult to detect. To overcome this problem, we introduced a normalization procedure by which the image of the medium with inclusion is divided by another one "without" inclusion. We showed, using realistic fluorophore and absorber concentrations, in both the inhomogeneity and in the bulk, that inclusions can be detected with good modulation.

9538-49, Session PWed

Assessment of contrast-to-noise for the statistical moments of distributions of times of flight of photons obtained at different source-detector separations: a Monte Carlo study

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Contrast-to-noise for statistical moments during detection of absorption change located at different depths was assessed by Monte Carlo simulations for different source-detector separations.

9538-50, Session PWed

Determination of the optical properties of multilayered phantoms by time-resolved reflectance measurements

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In Optics of Turbid Media the light propagation in substances which present strong scattering characteristics is studied. This is of particular interest for applications in Medical Diagnostics, because many biological tissues behave, from an optical point of view, like turbid media. The simplest approach for describing light propagation in tissue is the homogeneous model, i. e., the tissue is assumed to be a medium with constant absorption (?a) and reduced scattering (?'s) coefficients. However, in most cases, biological tissues have inhomogeneities, or they are intrinsically heterogeneous. In particular, systems like the human head can be thought as formed by several layers (scalp, skull, meninges, cerebrospinal fluid (CSF), gray matter, white matter) of different optical properties and, therefore, clearly differ from being a homogeneous medium.

In this work we present results of experiments performed on phantoms (turbid media made in the lab, which emulate the optical behaviour of biological tissues) of two and three layers; in all cases the goal is to retrieve the optical properties of the deepest (liquid) layer through time-resolved reflectance measurements. This is of interest for developing applications such as recording of functional activation in the brain, measurement of tissue oxygenation, monitoring of glucose concentration in blood, etc. In these applications the characterization of deeper tissues (cortex) is of high relevance.

9538-51, Session PWed

Time-resolved transmittance: a comparison of the diffusion model approach with Monte Carlo simulations

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We have analyzed distributions of times of flight of photons obtained from measurements of time-resolved transmittance by a Monte Carlo model to estimate the optical properties of phantoms with a thickness of up to 6 cm. The results have been compared to those obtained by employing the diffusion model whereby the effect of a temporal shift between diffusion model and measurement was investigated, too. For liquid phantoms covered by glass plates, the absorption is underestimated by the diffusion model with respect to the Monte Carlo model used as gold standard by about 10%. The optional temporal shift parameter reduces this underestimation to about 5%. However, the scattering coefficient is then overestimated by about 10% compared to 5% for discarding such a shift. For solid phantoms (without covering layers) both the absorption and the reduced scattering coefficients estimated by the diffusion model are overestimated by typically 5%. If a temporal shift is allowed as additional fit parameter, the absorption coefficients agree well with the results obtained by the Monte Carlo model. The reduced scattering coefficients are then underestimated by about 5%. The experimental results are confirmed by a theoretical analysis in which distributions of times of flight obtained by the Monte Carlo simulations were fitted by the diffusion model with and without the temporal shift. This analysis shows the accuracy which can be obtained with the diffusion model over a large range of optical properties.

9538-52, Session PWed

Assessment of tissue optical parameters in a spherical geometry using three different optical spectroscopy methods: comparison based on a theoretical approach

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The non-invasive research of information inside the biological tissues can be made by means of continuous, impulsive or modulated light source, emitting in the visible or infrared range. We are interested to the case of homogeneous and spherical shape to be closer to biological structures as brain, breast or fruits. The aim of this paper is to consider the assessment of tissue optical parameters in a spherical geometry by means of fitting with an analytical solution, but in using three different optical spetroscopy methods. Through a simulation based on Monte Carlo code, a homogeneous sphere is considered here. Firstly the data are compared with the solution of the semi-infinite model according to the frequency level; and so we observe that the similarity between planar and spherical geometry solutions depends on the value of the frequency. Then we use algorithms of optimization to find the optical coefficients from the usual semi-infinite function for both a given frequency and the zero frequency, i.e the continuous case. The steady-state considered as the case of zero frequency seems to have the ability to support more correctly the effect of the boundary curvature. In temporal mode, we use again a similar algorithm in order to compare the fitting between the analytical solution of the semi-infinite system with the one of the sphere. We observe that the retrieval of the coefficients is improved when the sphere geometry is taken into account in the time domain.

9538-54, Session PWed

Fiber-based hybrid probe for non-invasive cerebral monitoring in neonatology

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Improved cerebral monitoring systems are needed to prevent preterm infants from long-term cognitive and motor restrictions. Combining advanced near-infrared diffuse spectroscopy measurement technologies, time-resolved spectroscopy (TRS) and diffuse correlation spectroscopy (DCS) will introduce novel indicators of cerebral oxygen metabolism and blood flow for neonatology. For non-invasive sensing a fiber-optical probe is used to send and receive light from the infant head. In this study we introduce a new fiber-based hybrid probe that is designed for volume production. The sensor supports TRS and DCS measurements in a cross geometry, thus both technologies gain information on the same region inside the tissue. The probe is highly miniaturized to perform cerebral measurements on heads of extreme preterm infants down to head diameters of 6cm. Considerations concerning probe production focus on a reproducible accuracy in shape and precise optical alignment. In this way deviations in measurement data within a series of probes should be minimized. In addition to that, requirements for clinical use like robustness and hygiene are considered. An additional soft-touching sleeve made of FDA compatible silicone allows for a flexible attachment with respect to the individual anatomy of each patient. We present the technical concept of the hybrid probe and corresponding manufacturing methods. A prototype of the probe is shown and tested on tissue phantoms as well as in-vivo to verify its operational reliability.

9538-55, Session PWed

Enhancing fluorescence diffuse optical tomography (fDOT) system by creating prior information by means of elastically deformed generic mouse atlases

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Fluorescence Diffuse Optical Tomography (fDOT) has been proven a reliable technique for tracing biomedical information expressing fluorescence targets inside live small animals. Recently, the combination of fDOT with a modality like XCT or MRI has increased in popularity since the information content and the quality of the resulting tomographic images of fluorescence distributions can be enhanced by including crucial structural prior information to the inverse problem of the image reconstruction. In this work we propose a method to include structural information to the reconstructions of brain imaging in mice, by the use of generic deformable mice atlases. We present results showing a good approximation of the real mouse head structure, by simply fitting the generic atlas to the whitelight photographs of the mouse specimen, which are routinely acquired from most fDOT systems available in research labs. Therefore we reduce the need for a second structural modality available next to the fDOT system, reducing cost (MRI) and safety requirements (XCT).

9538-18, Session 4

Accounting for systematic errors in bioluminescence imaging to improve quantitative accuracy

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Bioluminescence imaging (BLI) is a widely used pre-clinical imaging technique with limitations to its quantitative accuracy. Substantial limitations are demonstrated together with methods and algorithms, which are used to overcome these issues. Position of the imaging subject and unknown source depth are both shown to affect the measured luminescence intensity. While Free Space Modelling can be used to eliminate the systematic error due to the camera/subject geometry, bioluminescence tomography (BLT) can provide additional information about the depth and intensity of the source. Finally, a substantial limitation in apparent number of sources identified using BLI is presented. It is shown that when a given source is at a significant depth, it can appear as multiple sources when imaged using BLI, while the use of BLT is shown to overcome this issue.

9538-19, Session 4

Deep tissue blood flow imaging with speckle contrast optical tomography *(Invited Paper)*

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We present speckle contrast optical tomography (SCOT), a novel method to image deep tissue blood flow. SCOT can be envisioned as the merging of the concept of laser speckle flowmetry (LSF) and diffuse correlation spectroscopy (DCS). Similar to DCS, SCOT uses the photon diffusion model to employ point sources and detectors at a distance as diffuse optical tomography. Analogous to LSF, SCOT use fast and relatively inexpensive data acquisition from a wide field geometry (with cameras) which allows measuring thousands of speckles simultaneously resulting in an improved signal-to-noise-ratio. This helps to achieve dense source-detector sampling allows a three dimensional tomographic reconstruction of blood flow. We develop the theoretical background of SCOT, demonstrate its utility in phantoms and in vivo for obtaining bulk blood flow then proceed to carry out three-dimensional reconstructions in phantoms and in vivo small animal models. We will discuss the advantages and disadvantages of the method, compare and contrast it to other blood flow imaging methods and speculate about its future.

9538-20, Session 4

Monitoring hemodynamics and oxygenation of the kidney in rats by a combined near-infrared spectroscopy and invasive probe approach

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We have performed a pre-clinical study on 13 rats to investigate the potential of a hybrid approach for kidney characterization combining near-infrared spectroscopy with established invasive techniques. A fiber optic probe was used to record spatially-resolved diffuse reflectance at three near-infrared wavelengths from the surface of the exposed kidney. Tissue hemoglobin concentration and oxygen saturation of hemoglobin were derived by a Monte Carlo model of photon propagation. The NIRS measurements were combined with laser-Doppler fluxmetry and a fluorescence quenching technique for quantification of tissue oxygen tension by employing a fiber optic probe inserted into the renal cortex. Arterial blood pressure and total renal blood flow were measured by a catheter and an ultrasonic probe, respectively. We applied our methods to investigate temporal changes during selected test interventions. For short occlusions of the renal artery or vein the decrease or increase in the cortical hemoglobin concentration per tissue volume could be monitored. Furthermore, the temporal behavior of oxygen saturation of hemoglobin and tissue oxygen tension in the kidney was found to be different. In a second group of interventions the mixture of the inspired gas was changed to induce hyperoxic, hypoxic and hypercapnic conditions which resulted in an increase, a decrease, or unaltered oxygen saturation. As expected, changes in hemoglobin concentration were much smaller during these interventions compared to the occlusions. Our results show the benefit of combining NIRS and invasive methods to gain advanced insight into renal hemodynamics and oxygenation.

9538-21, Session 4

Optical investigation of antiangiogenic therapy in renal cell carcinoma

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Antiangiogenic therapy is a relatively novel treatment that reduces tumour growth by preventing them from sprouting new blood vessels, which are needed for a tumour to grow beyond a very limited size. Diffuse optical spectroscopy and diffuse correlation spectroscopy was used to monitor antiangiogenic therapy in renal cell carcinoma tumors xenoimplanted in athymic mice (7 treated, 7 controls). The therapy has slowed down the tumor growth, reduced the vascular density and altered the progression of the blood flow index and the total haemoglobin concentration compared to the control group. There were several correlations with the therapy outcome. Of particular interest, the initial blood flow index at the start of the therapy correlated with the final vessel density measured by histopathology and inversely correlated with the extracted tumor weight. The latter correlation may possibly be due to those mice staying healthier longer and being sacrificed later. In conclusion, diffuse optical techniques reveal important hemodynamic biomarkers of antiangiogenic therapy outcome with potential applications in drug development and individualized therapy planning.

9538-22, Session 4

Time-resolved diffuse optical tomography for non-invasive flap viability assessment: pre-clinical tests on rats

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Flap surgery is a well-known technique in reconstructive surgery. The main complication of this technique is the thrombosis which is caused by a low permeability of reconnected vessels. In this case, an early diagnosis is crucial to intervene as soon as possible, thus saving the flap. However, clinical signs of thrombosis can occur late and an instrument able to non-invasively monitor perfusion in depth is needed. To meet this problem we conceive a time-resolved diffuse optical system based on high dynamic range acquisitions.

In this work, we realize a fully automatized setup for fast time-gated optical tomography based on two injection and collection couples. High-dynamic range acquisitions are analyzed using the "Mellin-Laplace transform" in order to obtained 3D maps of concentration of oxy- and deoxygenated hemoglobin.

We use this setup for pre-clinical in-vivo measurements on abdominal fascio-cutaneous flap done on rats. We perform venous occlusion, since it is a relevant model to simulate complications that can arise after a flap surgery. From computed 3D maps of concentration of oxy- and de-oxygenated hemoglobin, we can clearly detect changes in concentrations caused by a venous occlusion. Indeed, after a venous clamp an increase in deoxygenated hemoglobin and the raise of a thrombus occurred.

With this work we demonstrate the suitability of fast gated tomographic approach for non-invasively assessment of flap viability thus opening the way to the application of this technique in animal model with higher flap thickness where high-dynamic range acquisitions are fundamental to increase depth sensitivity.

9538-23, Session 5

Hybridization of Hamamatsu TRS20 time resolved near infrared spectroscopy and HemoPhotonics HemoFloMo diffuse correlation spectroscopy

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We present the integration of two devices: a commercial diffuse correlation spectroscopy (DCS) device (HemoFloMo) from HemoPhotonics and a Time Resolved Near-Infrared Spectroscopy (TR-NIRS) prototype device (TRS20) from Hamamatsu. Time resolved near infrared spectroscopy (TR-NIRS) is capable of measuring oxy- and deoxy-haemoglobin concentration and diffuse correlation spectroscopy (DCS) can assess microvascular blood flow. Advantages of an hybrid device which implements the two mentioned techniques are already known and exploited. DCS analysis needs optical properties as input variables; measuring simultaneously the two parameters with TR-NIRS makes DCS evaluation more complete and accurate. In addition, knowing both blood flow and oxygenation parameters from the same measurement allows to have the complete picture about provision and consumption of oxygen by the tissue. We will present how to integrate the two afore mentioned devices in order to develop an hybrid DCS/TR-NIRS device. A trigger protocol allows the communication between the two, which measure alternately. The hybrid device is validated in tissue simulating liquid

phantoms to evaluate its accuracy, its repeatability and its stability. In addition we will present in-vivo measurements on skeletal muscles during static exercise and measurements from the human brain.

9538-24, Session 5

Multi-wavelength time-resolved measurements of diffuse reflectance: depth-resolved estimation of absorption changes during inflow of Indocyanine Green

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In this paper methodology of time-resolved detection of diffusely reflected light is presented. This methodology enables depth-resolved estimation of changes in absorption in the brain cortex and in the extracerebral tissue using the moments of distributions of times of flight of photons measured at multiple wavelengths in the near-infrared region. Results of the tests on physical phantoms during the injection of ICG are presented. Measurements on the phantom were carried out to simulate the dynamic inflow of ICG at different depths of human head. Results of depth-resolved estimation of absorption coefficient changes obtained using multi-wavelength measurements of time-resolved diffuse reflectance signal during ICG injection are presented. Considering different light penetration depths at different wavelengths the time-resolved multi-wavelength technique may provide information on blood supply to brain cortex of the human adults.

9538-25, Session 5

Design and construction of a solid switchable phantom for diffuse optical imaging

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We propose a simple and reliable solid phantom for mimicking localized absorption changes within a diffusive medium. The phantom is based on a solid matrix holding a movable rod embedding a black inclusion. Translating the rod parallel to the phantom surface, the inhomogeneity can be positioned beneath the source-detector pair (perturbed case) or far from it (unperturbed case). The phantom is based on the Equivalence Relation stating that any realistic absorption change can be mimicked by a totally absorbing sphere of a proper volume. Four black objects were used (volumes = 20, 50, 100, 270 mm3), corresponding to four equivalent absorption changes over a 1000 mm3 volume (0.05, 0.1, 0.17, 0.4 cm-1).

The phantom was characterised using both a time-resolved optical mammograph and a time-resolved brain imager, to derive transmittance and reflectance measurements, respectively. Although the rod itself – nominally identical to the phantom matrix – creates an optical perturbation, nevertheless, the effects of the small black objects are clearly detectable. Both X-scans and Z-scans can be produced by translating the rod, and taking the homogeneous region of the rod far from the inclusion as reference state. Examples of time-resolved reflectance measurements are provided.

The great value of the proposed phantom is that it is easily operated in a clinical environment, it is stable, rugged, and fairly reproducible, and can yield dynamic changes with no need to detach the fibres. Thus, it could be used for routine quality tests both of clinical instruments and of laboratory settings, and even adopted in standards.

9538-26, Session 5

Broadband time-resolved diffuse optical spectrometer for clinical diagnostics: characterization and in-vivo measurements in the 600-1350 nm spectral range *(Invited Paper)*

Sanathana Konugolu Venkata Sekar, Andrea Farina, Edoardo Martinenghi, Alberto Dalla Mora, Paola Taroni, Antonio Pifferi, Politecnico di Milano (Italy); Turgut Durduran, Marco Pagliazzi, Claus Lindner, ICFO - Institut de Ciències Fotòniques (Spain); M. Mora, Institut d'Investigacions Biomèdiques Agustí Pi Sunyer (Spain); Mattia Squarcia, Alvaro Urbano-Ispizua, Univ. de Barcelona (Spain)

We report on the design, performance assessment, and first in vivo validation of a Time-Resolved Diffuse Optical system for broadband (600-1350 nm) nm measurements of absorption and scattering spectra of biological tissues for non-invasive clinical diagnostics. The system is based on a supercontinuum laser, and an electronic chain for Time-Correlated Single-Photon counting.

Two strategies to reduce drift and enhance responsivity are adopted. Continuous acquisition of IRF along with signal in a temporally shared window proves the novelty of the system's adaptability to undesirable temporal drift and pulse shape distortion in real time clinical environment. Two detectors, a silicon photomultiplier SiPM (600-940 nm) and InGaAs PMT (940-1350 nm) were chosen so to efficiently cover the spectral range with highest responsivity.

The system was characterized and optimized with MEDPHOT protocol in a view of routine use in a clinical environment with the aim to improve the spectral coverage, and to bestow reliable results under suboptimal and quick operating conditions. The system was finally enrolled in a first in vivo test phase measurement on healthy volunteers, carrying out non-invasive, in vivo quantification of key tissue constituents (oxy- and deoxy-hemoglobin, water, lipids, collagen) and tissue micro-structure (scatterer size and density) of human manubrium.

9538-27, Session 5

Characterization of turbid homogeneous phantoms for performance tests in diffuse optical imaging and spectroscopy

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Performance characterization and validation of instruments for biomedical optical imaging and spectroscopy necessitate phantoms that mimic relevant aspects of the tissue under investigation. Basic requirements for phantoms for diffuse optical imaging and spectroscopy

of organs like brain and breast are a realistic attenuation as well as a nearly Lambertian angular characteristic of the outgoing radiation. Both requirements can be met with solid homogeneous turbid phantoms of suitable dimensions and optical properties.

To characterize the diffuse attenuation, we propose a simple method to estimate the total power diffusely transmitted through an aperture on the surface of a phantom by means of a power meter. The influence of the finite distance of the sensitive area of the power meter from the phantom surface can be accounted for by a radiometric correction factor. Alternatively, if the reduced scattering and absorption coefficients of the phantom material are known the diffuse transmittance or reflectance can be calculated based on the diffusion model for light propagation. We tested the consistency of both approaches on slab phantoms in transmission and found good agreement.

Slab phantoms with known diffuse transmittance can be used to measure the responsivity of the detection system of diffuse optical instruments. It depends on the detector sensitivity as well as on effective detection area and acceptance angle of fiberoptic components. This approach was discussed previously [H. Wabnitz et al., J. Biomed. Opt. 19, 086010 (2014)] in the context of single photon counting. In the present work we extend this approach to power-related quantities.

9538-28, Session 5

Adjusting subdiffusive parameters in optical phantoms

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In the literature some work on solid phantoms is described (see e.g. Moffitt et al. or Pogue and Patterson), however, an extensive analysis of matrix material, scatterers and absorbers cannot be found. We show measurements of an epoxy resin based optical phantom system. Measurements of the matrix material's optical properties such as scattering and absorption coefficient, scattering phase function, the refractive index dispersion and the intrinsic fluorescence were performed. Furthermore, the scattering as well as the reduced scattering coefficients and the phase function of titanium dioxide were examined. The same optical properties were measured for an aluminium oxide powder. With the knowledge of these optical properties, we show how to adjust the anisotropy g or the phase function related parameter ? in a solid optical phantom. For a detailed description of ?, see Bevilacqua and Depeursinge and Chamot et al..

9538-29, Session 5

Non-contact quantitative diffuse reflectance spectroscopy

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In this work, we validate a non-contact Diffuse Reflectance Spectroscopy (DRS) system as a first stage towards the development of a quantitative multi-spectral imaging technique. The non-contact DRS system, with separated illumination and detection paths, was established with different progressive set-ups which were all compared to a well-founded contact DRS system. The system is based on a probe that measures spatially-resolved reflectance between 470 - 880 nm and consists of a central illumination fibre and concentric detection fibres at six different distances.

Using the contact DRS system, the computation of the optical parameters of an unknown phantom relies on the reflectance comparison with a reference phantom for which the optical properties are known. The reflectance signals are simulated by a Monte Carlo approach which solves the radiative transfer equation. Optimal absorption quantitation is well achieved with this method. However, the relevance of the scattering coefficient estimation decreases as it grows apart from the reference phantom's coefficient, and deteriorates even more when using non-contact system measurements. Therefore, we propose a new method to achieve a consistent estimation of both absorption and diffusion coefficients, consisting in a reference-based algorithm which is compliant with any set-up geometry. The algorithm takes into account the optical aberrations induced by the system such as parasite reflections, optical transfer function and illumination beam profile.

We test and validate the method on phantoms featuring optical parameters μa = [0.2 , 2] cm-1 and μ's = [14 , 40] cm-1 at 600 nm.

9538-30, Session 5

Handheld NIR camera for fluorescence-guided sentinel lymph node mapping in gynecological malignancies

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We present a novel, compact fluorescence imaging system developed for intraoperative sentinel lymph node (SLN) mapping applying indocyanine green (ICG) as a contrast agent. The device uses two near-infrared wavelengths (high-power light emitting diodes as a light source) to record fluorescence and anatomical images in real-time with a single CCD camera. To retrieve attenuation-corrected fluorescence images with our device, the ratio technique (or F/R) was employed, i.e. the raw local fluorescence signal (F) of each pixel is divided by the corresponding local reflectance (R) at 830 nm. Experiments on lymph node phantoms confirmed that the amount of dye in superficial lymph nodes can be better estimated due to the absorption correction procedure integrated in our device. Also the contrast, defined as the ratio of the lymph node fluorescence signal to the signal of surrounding tissue, was substantially higher in the ratio images than in the raw fluorescence images. Correction for the re-absorbed photons facilitates the detection of the sentinel lymph node(s) and, as a consequence, can improve the medical procedure by increasing its sensitivity in cases where high concentrations (μM range) of the dye molecule are expected in lymph nodes after the peritumoral injections.

9538-31, Session 6

Vascular optical tomographic imaging of peripheral artery disease in diabetic and non-diabetic patients

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Vascular optical tomographic imaging (VOTI) is a novel imaging modality that is capable of detecting hemoglobin concentrations in tissue. VOTI is non-invasive, non-ionizing and does not require contrast injection. This technology was applied to the diagnosis of peripheral arterial disease (PAD) within lower extremities of diabetic patients with calcified arteries. This could be of substantial benefit as these patients suffer from comorbidities such as arterial incompressibility, which complicates diagnosis and monitoring. Forty individuals (10 non-diabetic patients with PAD, 10 diabetic patients with PAD, and 20 healthy volunteers) were enrolled in a diagnostic pilot study using the VOTI system. The patients were imaged during a thigh pressure cuff occlusion. The VOTI system was capable of quantifying the blood volume changes within the foot

during the thigh cuff occlusion and outputting diagnostic parameters, such as change in hemoglobin concentration, enabling the assessment of foot perfusion. This study resulted in a statistically significant difference between the healthy cohort and both the non-diabetic and the diabetic PAD cohorts (p=0.006, p=0.006). Receiver operating characteristic (ROC) curve analysis showed that PAD diagnosis could be made with over 80% sensitivity or specificity depending on the characteristic cutoff point. In addition, VOTI was capable of providing the locations of under-perfused regions within the foot and evaluating the severity of arterial disease, even within diabetic patients with calcified arteries, who are traditionally difficult to diagnose. Overall we have shown that VOTI can effectively diagnose PAD independently of arterial compressibility, making it a promising new tool for assessing vascular disease in diabetic patients.

9538-32, Session 6

Time-resolved diffused optical characterization of key tissue constituents of human bony prominence locations

Sanathana Konugolu Venkata Sekar, Andrea Farina, Edoardo Martinenghi, Alberto Dalla Mora, Paola Taroni, Antonio Pifferi, Politecnico di Milano (Italy); Eugènia Negredo, Jordi Puig, Roser Escrig, Fundació Lluita contra la Sida (Spain); Quim Rosales, Digest Ctr. Mèdic (Spain); Claus Lindner, ICFO - Institut de Ciències Fotòniques (Spain); Marco Pagliazzi, ICFO-Institut de Ciències Fotòniques (Spain); Turgut Durduran, ICFO - Institut de Ciències Fotòniques (Spain)

We report broadband time-resolved characterization of selected bony prominence locations of the human body. A portable broadband time-resolved system equipped with pulse drift and distortion compensation strategy was used for absorption and scattering measurements. Detection strategy where two detectors complimenting their efficient wavelength range was deployed to bestow system with higher efficiency over entire measurement range, particularly (900-1000 nm) range where lipid, water, collagen peaks arise sequentially.

Clinical studies were performed at six different bony prominence locations namely radius distal, radius proximal, ulnadistal, ulna proximal, trochanter, calcaneus of 53 subjects. Measurement at all locations except calcaneus was performed in reflection geometry with 2.5 cm as source detector separation.

Key tissue constituents were quantified by fitting the measured spectrum with linear combination of tissue constituent spectra(oxy-hemoglobin, deoxy-hemoglobin, lipid, water, collagen) as a pilot step towards non-invasive optical assessment of bone pathologies.

9538-33, Session 6

Validation of time domain near infrared spectroscopy in muscle measurements: effect of a superficial layer

Rebecca Re, Davide Contini, Lucia M. G. Zucchelli, Alessandro Torricelli, Politecnico di Milano (Italy); Lorenzo Spinelli, Consiglio Nazionale delle Ricerche (Italy)

In reflectance spectroscopy a major concern is the possibility to discriminate signals coming from different layers of the investigated medium. In the time domain (TD) approach to diffuse optics, by exploiting the information encoded in the photon time-of-flight, signals coming from different depths of the tissue can be discriminated. However, the accurate quantification of the hemodynamic changes in the different compartments of tissues is still an open issue. In this work we applied a recent analysis method based on refined computation of the pathlength traveled by photons within each layer the tissue is composed of, by taking into account the non-idealities of the system set-up and the heterogeneous structure of the tissue. In this work we validated the applications of this method in TD diffuse optics measurements of the muscle and surrounding tissues where the thickness variability of the adipose layer can be a problem (as we demonstrate: thinner the superficial layer less accurate the estimation of its chromophores concentration). In the first part of the work we simulate a realistic situation of concentration of oxy- and deoxy-hemoglobin during venous and arterial occlusions with different conditions in terms of geometry (layer thickness, interfiber distance), instrument set-up (different instrument response functions are considered) and possible data analysis (homogenous or layered) in order to estimate possible source of error. While in the second part, we validate the results of the previous section with in vivo data.

9538-34, Session 6

Modeling of the blood flow in the lower extremities for dynamic diffuse optical tomography of Peripheral Artery Disease

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Peripheral Arterial Disease (PAD) is caused by a reduction of the internal diameters of the arteries in the upper or lower extremities mainly due to atherosclerosis. If not treated, its worsening may led to a complete occlusion, causing the death of the cells lacking proper blood supply, followed by gangrene that may require chirurgical amputation. We have recently performed a clinical study in which good sensitivities and specificities were achieved with dynamic diffuse optical tomography. To gain a better understanding of the physiological foundations of many of the observed effects we started to develop a mathematical model for PAD. The model presented in this work is based on a multi-compartment Windkessel model, where the vasculature in the leg and foot is represented by resistors and capacitors, the blood pressure with a voltage drop and the blood flow with a current. Unlike existing models, the dynamics induced by a thigh-pressure-cuff inflation and deflation during the measurements are taken into consideration. This is achieved by dynamically varying the resistances of the large veins and arteries. By including the effects of the thigh-pressure cuff, we were able to explain many of the effects observed during our dynamic DOT measurements, including the hemodynamics of oxy- and deoxy-hemoglobin concentration changes. The model has been implemented in MATLAB and the simulations have been normalized and compared with the blood perfusion obtained from healthy, PAD and diabetic patients. Our preliminary results show that, in unhealthy patients, the total system resistance is sensibly higher than in healthy patients.

9538-35, Session 6

Estimation of path length in blood vessels from two-dimensional image of exposed cortex

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In optical intrinsic signal imaging, the partial optical path length in the blood vessel is necessary to accurately estimate the change in the hemoglobin concentration using the modified Lambert-Beer law. Analysis of light propagation in the realistic cortical model of which the blood vessel structure is based upon the three-dimensional image of the cortical tissue acquired by two-photon microscopy enables us to estimate the accurate partial optical path length, however, the data acquisition of two-photon microscopy and the model construction are time consuming. In

this study, the relationship between the spatial distribution of the partial optical path length in the blood vessel and the blood vessel diameter was obtained from a simple semi-infinite slab model with a cylindrical blood vessel. The partial optical path length in the blood vessel increases with an increase in the blood vessel diameter and the Gaussian fit reasonably agrees with the path length distribution. The distribution of the partial optical path length estimated from the blood vessel diameter in the two-dimensional cortical image agrees with that predicted by the three-dimensional realistic model obtained by two-photon microscopy. The hemoglobin concentration changes in the artery and venule calculated using the partial optical path length estimated by the proposed method are smaller than those using the optical path length estimated by the homogeneous model.

9538-36, Session 7

Combined dynamic and static optical tomography for prediction of treatment outcome in breast cancer patients *(Invited Paper)*

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Breast cancer patients who respond well to 5-month-long neoadjuvant chemotherapy (NACT) have a high rate of 5-year survival. However, only 10-30% of patients receiving NACT show a pathological complete response (pCR), while 70-90% do not respond well and 5-year survival drops drastically. Therefore it is highly desirable to identify biomarkers that can predict treatment outcome early on, which would allow adapting therapy accordingly. Optical tomographic imaging appears to be a promising technology for providing such biomarkers, as initial pilot studies have shown its ability to observe physiological changes that occur early within NACT. Here we present the latest results on an ongoing longitudinal clinical study, in which we follow more than 25 patients over 5 months of treatment. We image subjects with invasive breast cancer using a dynamic dual-breast optical tomography system to collect both static and dynamic information that is used to observe changes in tumor vascularity over the course of treatment. Subjects are asked to hold their breath, which causes an increase of blood in the chest that returns to normal after they resume breathing. This is called the recovery time where the washout rate of blood can be observed. In addition to several case studies, we show cumulative results for the week 2 time point of 23 patients. We observe that the washout rate is significantly higher in subjects that show a pCR after 5 months ($p<0.01$). In addition we observe that patients with a pCR show a significant reduction in total hemoglobin and deoxy-hemoglobin in their tumor bearing breast after 2 weeks of treatment. Therefore, it appears that utilizing both static and dynamic information of the tumor progression can be used to in determining and response to treatment be predicted.

9538-37, Session 7

Optical study on the dependence of breast tissue composition and structure on subject anamnesis *(Invited Paper)*

Paola Taroni, Giovanna Quarto, Antonio Pifferi, Politecnico di Milano (Italy); Francesca Abbate, Nicola Balestreri, Simona Menna, Enrico Cassano, Istituto Europeo di Oncologia (Italy); Rinaldo Cubeddu, Politecnico di Milano (Italy)

Breast tissue composition is responsible for breast density, a strong and independent risk factor for breast cancer. Breast density is quantified by x-ray mammography that is essentially sensitive only to the overall tissue attenuation, and cannot effectively discriminate among individual contributions. Time domain multi-wavelength (635 to 1060 nm) optical mammography was performed on 200 subjects to estimate their average breast tissue composition (in terms of oxy- and deoxy-hemoglobin, water, lipid and collagen content) and structural information (as provided by scattering amplitude and power). Significant (and often marked) dependence was demonstrated on several risk factors: age, menopausal status, body mass index (BMI), and use of oral contraceptives (OCs). Briefly, older age, higher BMI, and post-menopausal status correspond to an increased weight of the adipose fraction of breast tissue (higher lipids, lower water and collagen) and a decrease in the average tissue vascularization, while the use of OCs has opposite effects. The study shows that optical spectroscopy is an effective non-invasive means for the characterization of breast tissue composition and allows one to investigate and monitor over time the dependence on parameters that may affect breast density and the related risk to develop breast cancer.

9538-38, Session 7

Collagen content as a risk factor in breast cancer: a pilot clinical study

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The identification of risk factors for breast cancer could permit personalized diagnostics – not sustainable in population-wide screening programs. Recently, optical techniques have been proposed for the non-invasive assessment of breast density, a strong risk factor for breast cancer usually derived from X-ray mammography. In this paper we explore an even more ambitious goal, which is to investigate whether collagen – quantified by time-resolved diffuse optical spectroscopy – can directly assess breast cancer risk factors and not just indirectly by correlation with breast density.

A retrospective pilot clinical study on time domain multi-wavelength (635 to 1060 nm) optical mammography was exploited, including a total of 109 subjects (53 healthy and 56 with malignant lesions). Selecting the 15% subset of subjects with the higher age-matched collagen content, an increased cancer occurrence is observed, similarly to what obtained when the X-ray age-matched percentage breast density is used for clustering. Quite noticeably the two high-risk group, selected by collagen and density, are not overlapped, possibly indicating that collagen could address a somewhat different or complementary risk factor as compared to X-ray density.

If confirmed statistically, and on larger populations, these results would have a huge impact. First, optical techniques could be used for screening programs aiming at personalized diagnostics on high-risk subgroups. Further, the different clustering of collagen and density could be exploited to refine identification of subjects at high risk. Finally, the non-invasive assessment of collagen is interesting for studying the relation between collagen and cancer, and the possible differentiation in malignancies and treatment response.

9538-39, Session 7

Optical discrimination between malignant and benign breast lesions?

Giovanna Quarto, Antonio Pifferi, Rinaldo Cubeddu, Politecnico di Milano (Italy); Francesca Ieva, Univ. degli Studi di Milano (Italy); Anna Maria Paganoni, Politecnico di Milano (Italy); Francesca Abbate, Enrico Cassano, Istituto Europeo di Oncologia (Italy); Paola Taroni, Politecnico di Milano (Italy)

Time domain multi-wavelength (635 to 1060 nm) optical mammography was performed on 82 subjects with breast lesions (45 malignant and 38 benign lesions). A perturbative approach based on the high-order calculation of the pathlength of photons inside the lesion was applied to estimate differences between lesion and average healthy breast tissue in terms of: i) absorption properties, and ii) concentration of the major tissue constituents (oxy- and deoxy-hemoglobin, water, lipid and collagen). The absorption difference between lesion and healthy tissue is significantly different for malignant vs. benign lesions at all wavelengths. Logistic regression fitted to the absorption data identifies 975 nm as the key wavelength to discriminate malignant from benign lesions. When the difference in tissue composition between lesion and healthy tissue is considered, malignant lesions are characterized by significantly higher collagen content than benign lesions. Also the best model for the discrimination of malignant lesions obtained applying regression logistic to tissue composition turned out to depend only on collagen.

9538-40, Session 8

Hemodynamic response patterns during sleep: a concurrent time-domain fNIRS/EEG study in adults *(Invited Paper)*

Stefan P. Koch, Charité Universitätsmedizin Berlin (Germany); Alexander Jelzow, Physikalisch-Technische Bundesanstalt (Germany) and Becker & Hickl GmbH (Germany); Sophie K. Piper, Charité Universitätsmedizin Berlin (Germany); Hellmuth Obrig M.D., Max-Planck-Institut für Kognitions- und Neurowissenschaften (Germany); Renate Wehrle, Max-Planck-Institut für Psychiatrie (Germany); Heidrun Wabnitz, Physikalisch-Technische Bundesanstalt (Germany); Michael Czisch, Max-Planck-Institut für Psychiatrie (Germany); Jens M. Steinbrink, Charité Universitätsmedizin Berlin (Germany)

In this study, we investigated the nocturnal sleep during an entire night (~11pm to 8 am) and the influence of visual stimulation during different stages of sleep by combined time-domain functional near-infrared spectroscopy (td-fNIRS) and electroencephalography (EEG) in 7 healthy adult subjects. The use of td-fNIRS enabled the selective detection of cerebral hemodynamic changes.

Flicker light stimulation during wakefulness induced the typical stimulus-related hemodynamic response in the visual cortex, characterized by an increase in oxyhemoglobin concentration (ΔHbO) and a decrease in deoxyhemoglobin concentration (ΔHbR) compared to baseline. The average response over all 7 subjects during S1 sleep showed a pattern similar to the wake state. However, the magnitude of the average hemodynamic response gradually decreased with the depth of the sleep stages and showed a reversed response pattern, possibly indicating a 'deactivation' for sleep stages 2 and 3/4. During REM sleep, the hemodynamic response was diminished compared to the wake state or sleep stage S1. The stimulus-locked 8Hz EEG response gradually increased with the depth of the non-REM sleep stage and significantly (anti-) correlated with the hemodynamic changes (ΔHbO, ΔHbR).

To the best of our knowledge, this is the first concurrent fNIRS-EEG study exploring the stimulus-related hemodynamic response during different stages of sleep. These results suggest that primary cortical areas are deactivated during deep sleep.

9538-41, Session 8

Depressed cerebral blood flow response to hypercapnia in children with obstructive sleep apnea syndrome

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We utilize non-invasive diffuse optical tools to measure blood flow in pediatric subjects during induced hypercapnia, including subjects with obstructive sleep apnea syndrome (OSAS), snorers, and normal controls. Specifically, we utilize diffuse correlation spectroscopy to continuously monitor microvascular cerebral blood flow throughout a rebreathing task that elevates end-tidal CO2 from ~45 mmHg to ~65 mmHg; multi-distance frequency-domain diffuse optical spectroscopy is also employed to measure baseline total hemoglobin concentration and blood oxygen saturation.

Subjects in each group had similar age, normalized body mass index, hemoglobin concentration and blood oxygen saturation.

We found that children with sleep apnea have depressed reactivity to elevated levels of end-tidal CO2 (7 [6.2 8] %/mmHg CO2, median [25th 75th] percentile) compared to healthy controls (8.3 [8.1-10] %/mmHg CO2). The difference in reactivity between snorers (6.2 [5.2 8.7] %/mmHg CO2) and controls was not significant.

These findings suggest that diffuse optical measurements of hypercapnic reactivity may provide significant insight into the pathophysiology of OSAS in children.

9538-42, Session 8

Comparison of neurological NIRS signals during standing Valsalva manoeuvers, pre and post vasoconstrictor injection

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Near infrared spectroscopy (NIRS) has potential to offer a fast and non-invasive method of probing cerebral saturation in a clinical setting. However, there are concerns about contamination from superficial layers (i.e., skin and skull) and whether this contamination could confound our ability to see neurological NIRS measures. This study addressed the following questions 1) Is NIRS observing brain haemodynamic changes? 2) How much of an effect does superficial contamination have? 3) Is NIRS capable of quantitatively accurate measurements of brain haemodynamics (i.e., saturation, oxygenated, deoxygenated and total haemoglobin)?

The Valsalva manoeuver (VM) was used to differentiate between superficial (from somatic tissue) and neurological NIRS measures. A VM produces notable changes in both skin and brain haemodynamics. However, given the marked difference in their metabolic activity, it is ideal to distinguish the haemodynamic characteristics between neurological and superficial tissues. To assess the impact of superficial contamination a potent vasoconstrictor was injected to decrease the concentration of haemoglobin in the skin.

Frequency domain NIRS measurements during the VM pre and post vasoconstrictor injection, combined with simulation data conclusively show that NIRS can detect neurological changes, in both haemoglobin content and saturation, when positioned on the forehead. The effect of superficial contamination in this instance appeared to be insignificant, even with a drop in superficial haemoglobin concentration due to the vasoconstrictor. Simulations suggest that the absolute values of the recovered NIRS parameters are not quantitatively accurate however a direct comparison with invasive measures is needed to confirm this.

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9539-1, Session 1

Transmission matrix approach to photoacoustic imaging *(Invited Paper)*

Sylvain Gigan, Lab. Kastler Brossel (France)

Recently, wavefront shaping method have revolutionized imaging by allowing optical focusing in scattering media, at depth where ballistic light is absent. I will review our recent work on measuring and exploiting the photoacoustic transmission matrix, allowing better photoacoustic imaging, and focusing on acoustic absorbers.

9539-2, Session 1

Common Path Optical-Resolution Photoacoustic Remote Sensing Microscopy

Parsin Haji Reza, Roger J. Zemp, Univ. of Alberta (Canada)

Photoacoustic imaging has been shown to provide exquisite images of microvessels, and is capable of imaging blood oxygen saturation, gene expression, and contrast agents such as optical dyes and nanoparticles, among other uses. In most photoacoustic and ultrasound imaging systems, an ultrasound coupling medium such as water or ultrasound gel is required. However for many clinical applications such as burn diagnostics, surgery, wound healing and many endoscopic procedures physical contact, coupling, or immersion is undesirable or impractical. Optical means of detecting ultrasound and photoacoustic signals has been widely investigated [1-3], however most such techniques require direct contact or fluid coupling. We demonstrate a new method of acquiring optical-resolution photoacoustic images using non-contact optical interferometric sensing. We excite a sample with a focused pulsed laser spot and interrogate the acoustic signatures using a long-coherence length probe beam co-focused with the excitation spot. In this method we record the large initial pressures from chromophores locally and in a non-contact manner without appreciable acoustic loses. Detected signal-to-noise is shown to be significantly higher than piezoelectric recordings. We call our technique photoacoustic remote sensing (PARS). Our PARS microscopy system offers optical lateral resolution but is depth-limited to a transport mean-free path similar to optical-resolution photoacoustic microscopy. We present implementations using common-path interferometry and demonstrate both phantom and in vivo imaging in reflection mode. The experimental setup of our optical-resolution photoacoustic remote sensing (OR-PARS) microscopy system is depicted in Figure 1. A multi-wavelength visible laser source using stimulated Raman scattering [4,5] has been implemented to generate photoacoustic signals. Briefly, a 1 ns pulse width, ytterbium-doped fiber laser (IPG Photonics Inc.) with a pulse repetition rate (PRR) of 40 kHz was coupled using a fiber launch system (MBT621D/M, Thorlabs Inc.) into a 3 m polarization-maintaining single-mode fiber (SMF) (HB-450, Fibercore Inc., UK) to generate SRS peaks at 543, 560, 590, and 600 nm and pulse energies between 300 and 500 nJ [4,5]. The reported system has been optimized in order to takes advantage of a multi-focus OR-PAM [4,5] for improving the depth of field of 2D and 3D OR-PAM imaging. During PARS imaging depth scanning by wavelength tuning has been performed. The chromatic aberration in the collimating/objective lens pair used to refocus light from a fiber into the object so that each wavelength is focused at a slightly different depth location as shown in Figure 1. Using these wavelengths simultaneously has been proved to improve the depth of field and signal to noise ratio for 2D maximum amplitude projection imaging [4,5].

The output of the PM-SMF was collimated (F280APC-A, Thorlabs Inc.) and combined using a beam combiner (BC) with the receiver arms of the system. For the receiver arms we utilized and tested two different configurations, common path and Michelson interferometry. In both configurations a tunable continuous wavelength (CW) C-band laser with 100-kHz linewidth (TLK-L1550R, Thorlabs Inc., New Jersey) was used. The light at the laser aperture was coupled to a single mode fiber and collimated. The collimated interrogation beam was passed through a polarized beam splitter (VBA05-1550, Thorlabs Inc., New Jersey) and ?/4 zero order wave plate (Thorlabs Inc., New Jersey) and a BC and then scanned across the samples via a 2D galvanometer scanning mirror system (GVS012/M, Thorlabs Inc.). The scanning mirrors were driven by a two-channel function generator. The scanning light was then focused tightly using an objective lens. The reflected light back through the wave-plate creating 90° polarization which then reflects at the polarizing beam-splitter in order to guide the maximum possible intensity of reflected light to a 150 MHz-bandwidth InGaAs photodiode (PDA10CF, Thorlabs Inc., New Jersey). The common path PARS does not have any external reference arm and the reference beams provided by reflection from surface of optical components. The reference beam power has been measured between 8-200 mW depends on the position of the beam corresponds to the optical axis of the components.

Figure 2(a) shows PARS imaging of carbon fiber networks. Figure 2(b) shows in vivo images of the Chorioallantoic Membrane (CAM) of 5-day chicken embryos. During imaging sessions the fast and slow speed mirror scanning rates are set at 60 Hz and 0.25 Hz respectively, with the laser repetition-rate set at 40 kHz. Using a knife edge experiment, the lateral resolution of the system has been measured as ~2.5±1. The signal to noise ratio of image 2(a) was measured as 45dB.

9539-3, Session 1

Holographic non-contact photoacoustic tomography

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We introduce an ultrafast non-contact photoacoustic tomography approach based on holographic measurement of the photoacoustic surface displacement. Using a pulsed laser for illumination and a high speed camera, phase differences in consecutive interferograms are analyzed in order to retrieve displacement data. Excitation and detection are performed from the same side. The field of view is variable. The resolution of the displacement measurement scales with the magnification and is in the range of 40 μm lateral and 1 nm axial in the presented setup. Employing components working in the kHz range in a repetitive triple pulse scheme, a full photoacoustic dataset with an effective sampling rate of up to 80 MHz can be acquired in a few hundred milliseconds. The required excitation radiant exposure is below the ANSI limit of about 20 mJ/cm?. Measurement data obtained from silicone phantoms and the corresponding 3D reconstructions based on the delay-and-sum method are shown. The photoacoustic sensitivity and tomographic resolution are discussed. The data prove the feasibility of the approach. Due to the non-contact mode, the high acquisition speed and the variable field of view, the approach seems prospectively clinically usable. Potentially, new medical applications not allowing acoustic contact can be served, like neurosurgical intervention monitoring or burnt skin investigation.

9539-4, Session 1

Forward-viewing multi-element photoacoustic probe for 3D endoscopy

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There is considerable interest in the development of photoacoustic endoscopy probes (PAE) for guiding interventional procedures in foetal medicine or the assessment of cancers in the gastrointestinal (GI) tract. However, most previous PAE probes integrate mechanical scanners and piezoelectric transducers at the distal end which can be technically complex, expensive and pose challenges in achieving the necessary level of miniaturisation. We present a novel all-optical forward-viewing endoscopic probe operating in tomography mode that has the potential to overcome these limitations. It comprises a transparent Fabry-Pérot ultrasound sensor positioned at the tip of a coherent fibre-optic bundle. The distal end of the probe is fitted with relay optics that deliver interrogation light to different locations on the Fabry-Pérot sensor. In this way, the sensor acts as a 2D array of ultrasound detectors at the tip of the bundle. The pulsed excitation laser beam is full-field coupled into the fibre bundle at the proximal end and uniformly illuminates the tissue at the tip. The proximal end of the fibre bundle is optically scanned in 2D with a CW wavelength-tunable interrogation laser beam thereby interrogating different spatial points on the sensor. A time-reversal image reconstruction algorithm was used to reconstruct a 3D image from the detected signals. 3D imaging capability of the developed probe is evaluated using tissue phantoms. This new approach to PAE offers several advantages over previous distal-end scanning probes such as high degree of miniaturisation, no moving parts at the distal end and simple and inexpensive fabrication.

9539-5, Session 1

Optical-resolution photoacoustic endoscopy through thick tissue with a fluid-filled capillary

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Optical-resolution photoacoustic endomicroscopy goes beyond the limited penetration depth and large footprint of conventional optical-resolution photoacoustic microscope (OR-PAM). In current OR-PAE devices, bundles of single-mode fibers are used to focus light and the generated photoacoustic waves are detected after propagation back through tissue (therefore with a limited imaging depth for high frequency photoacoustic waves). In this work, we introduce the use of a fluid-filled silica capillary as a minimal footprint device to both guide the illumination pulse into the tissue and guide the photoacoustic waves outside the tissue. As a proof-of-principle, we first demonstrate that high-frequency photoacoustic signals generated by raster-scanning a focused pulsed nanosecond beam through a microscope objective can be guided by the capillary to form an optical-resolution photoacoustic image through the tissue. Specifically, an optical-resolution photoacoustic image of a 30μm diameter absorbing nylon thread was obtained by guiding the acoustic waves in a 30 mm long capillary (150 μm inner diameter, 330 μm outer diameter) through a 3cm thick pork fat layer. Second, we demonstrate that both light and ultrasound can be respectively injected and detected through the same proximal tip of the capillary.

9539-6, Session 1

Polymer Optical Fibre Sensors for Endoscopic Opto-Acoustic Imaging

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Opto-acoustic imaging (OAI) shows particular promise for in-vivo biomedical diagnostics. Its applications include cardiovascular, gastrointestinal and urogenital systems imaging. Opto-acoustic endoscopy (OAE) allows the imaging of body parts through cavities permitting entry. The critical parameter is the physical size of the device, allowing compatibility with current technology, while governing flexibility of the distal end of the endoscope based on the needs of the sensor.

Polymer optical fibre (POF) presents a novel approach for endoscopic applications and has been positively discussed and compared in existing publications. A great advantage can be obtained for endoscopy due to a small size and array potential to provide discrete imaging speed improvements. Optical fibre exhibits numerous advantages over conventional piezo-electric transducers, such as reduced safety risks, immunity from electromagnetic interference, array potential and a higher resolution at small sizes. Furthermore, polymer optical fibres offer over 20 times the sensitivity of silica fibre through a lower Young's modulus and better impedance matching.

We present an optimised intrinsic polymer fibre Bragg grating based sensor with a core diameter of less than 200 microns, making it more suitable for endoscopic use than current piezo electric transducers. We discuss the opto-acoustic signals received and compare them to predictive models in order to improve the instrument, drawing conclusions on the comparative advantages of applying this technology in biomedical applications. Finally, we demonstrate image reconstruction from the given signals, using various sensor types to compare the efficiency of the polymers and structures.

9539-7, Session 1

Hybrid label-free multiphoton and optoacoustic microscopy (MPOM)

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A comprehensive understanding of complex biological properties requires simultaneous observation of different anatomical structures, a feature that is typically not offered by a single imaging modality. Over the past decades, a wide range of optical microscopy techniques utilizing different contrast mechanisms have been developed. However, apart from offering only limited and very specific visualization capabilities, most of the commonly employed microscopy methodologies rely on staining procedures, which can interfere with biological systems.

We present a hybrid microscope combining multiphoton microscopy incorporating second-harmonic generation contrast and optical-resolution optoacoustic (photoacoustic) microscopy. This integrated multiphoton and optoacoustic microscope (MPOM) offers visualization of a broad range of structures by employing different contrast mechanisms and at the same time imparts pure label-free imaging of biological systems. We study the relative performance of the two combined modalities and investigate their multi-contrast abilities by demonstrating the label-free imaging of a zebrafish larva ex vivo, simultaneously visualizing the fish musculature and pigments. Overall, this hybrid implementation can prove valuable in multi-parametric microscopy studies, enabling more comprehensive information to be obtained from biological specimens.

9539-8, Session 2

Intraplaque lipid photoacoustic imaging: acoustic and optical spectroscopy

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The identification of vulnerable atherosclerotic plaques in the coronary artery is emerging as an important tool for guiding percutaneous coronary interventions. The structure and composition of the plaque are significant determinants of its vulnerability. Spectroscopic photoacoustic (sPA) imaging can visualize the atherosclerotic plaque composition on the basis of the optical absorption contrast. It is an established fact that the frequency range of the PA signal is correlated with the structural tissue properties. However, the frequency content of the PA signal from intraplaque lipids is unknown. In this ex vivo study on human coronary arteries, we combined sPA imaging and analysis of frequency component of its PA signals. Utilizing a broadband PVDF transducer with a 1mm needle hydrophone in the setup, we covered a large frequency range (0.25-35 MHz) for receiving the PA signal. sPA imaging was performed at wavelengths ranging from 1125 to 1275 nm with a step of 2 nm, allowing discrimination between plaque lipids and adventitial tissue. Guided by the sPA imaging, frequency content of the PA signal from the plaque lipids was quantified. Using histological data we model the PA response of the intraplaque lipid morphology observed in histological slides in the k-wave simulation toolbox. Our simulation results confirm our experimental finding that more than 80% of the PA energy of the coronary plaque lipids lies in the frequency band below 15 MHz. This frequency information can guide the choice of the transducer element used for PA catheter fabrication.

9539-10, Session 2

A handheld raster scan optoacoustic mesoscopy system for skin imaging.

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It has recently been shown that broadband raster scan optoacoustic mesoscopy has the ability to image several key structures in human skin, having the potential to overcome the depth related limitations of the optical imaging techniques commonly used in the dermatology clinical routine. However, in its initial form, the proposed optoacoustic mesoscopy methods are not suitable for clinical imaging due to the unpractical large size of the systems. We have further developed the skin imaging optoacoustic mesoscopy technology building the first broadband, high frequency, raster scan handheld system. The apparatus is designed to provide cross sectional images that contain rich depth dependent information. A compact fixed illumination scheme fulfills the imaging and size demands of the scanner. The system capabilities haves been characterized in terms of stability and resolution, using experiments with phantoms. The ability of the system image human skin, revealing anatomical features with respect to depth, is showcased with experiments involving healthy subjects.

9539-11, Session 2

The potential of photoacoustic microscopy as a tool to characterize the in vivo degradation of surgical sutures

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In the development of new absorbable sutures or new degradable materials in the medical industry, a lack of relatively low-cost, non-invasive and high resolution technique able to perform longitudinal studies at the same subject is missing.

In this work, a custom dark-field photoacoustic microscopy (PAM) is developed in order to show the potential of the technique to characterise the degradation of a clinical suture (500 ?m diameter) up to 1 cm depth with 50 ?m resolution under in vivo conditions for mice. Moreover, PAM is non-invasive, which makes it suitable for long term measurements such as in vivo physiological studies without any animal sacrifice.

Experimentally, the diameter of degraded and non-degraded sutures in tissue mimicking phantoms (up to 1 cm depth) and the measurement of two different sutures inside euthanized mice (up to 3 mm depth) has been proved using PAM technology. Furthermore, we validated the results by combining theoretical simulations with K-wave package and a practical algorithm, and using ex vivo measurements with an optical microscope as a gold standard technique.

In the future, longitudinal measurements under in vivo conditions for different mice have to be performed in order to establish this technology and reduce animal and financial costs. PAM has potential to be introduced in these studies to develop new degradable structures in medical applications.

9539-12, Session 2

Photoacoustic CT in healthy and inflamed interphalangeal joints

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Photoacoustic (PA) or optoacoustic (OA) imaging combines the high (blood) contrast to light with the high-resolution possible with ultrasound. The method can visualize vascularization and angiogenesis deep inside tissue. Of late there is interest in PA imaging of synovial joints which are expected to be associated with dysfunctional vascularization in the event of rheumatoid arthritis (RA). We will describe here our approach in investigating the application of the PA technique in this disease area. We are developing a CT-geometry version PA finger imager, intended for early clinical assessment of the method. The imager uses two curved array ultrasound detectors with central frequencies 1.5 and 7.5 MHz respectively, stacked above each other. Both cover approximately 180 degrees of the circle. Illumination is provided with a multiple of optical fiber bundles coupled to an laser-OPO system. To start with we systematically investigated imaging of finger vasculature in healthy volunteers using an earlier laboratory prototype. The PA slice images at multiple wavelengths in the near-infrared were correlated with power Doppler ultrasound as a reference. Studies from healthy volunteers include PA responses to externally applied triggers such as application of a pressure cuff in the upper arm and temperature changes in the bath. The effect of skin color and presence of artefacts from underlying bone on the results will be discussed. Finally we will present finger imaging results using the new imager from a small cohort of rheumatoid arthritis patients and discuss these in the clinical context of imaging the inflamed synovium as a surrogate for the disease.

9539-13, Session 2

An interventional multispectral photoacoustic imaging platform for the guidance of minimally invasive procedures

Wenfeng Xia, Daniil I. Nikitichev, Jean Martial Mari, Simeon West, Sébastien Ourselin, Paul C. Beard, Adrien E. Desjardins, Univ. College London (United Kingdom)

Precise image guidance is of paramount importance for many minimally invasive procedures. These procedures include fetal interventions, tumor biopsies and treatments, central venous catheterisations and peripheral nerve blocks. Ultrasound imaging is commonly used for guidance, but it often provides insufficient contrast with which to identify soft tissue structures such as vessels, tumors, and nerves. In this study, a hybrid interventional imaging system that combines ultrasound imaging and multispectral photoacoustic imaging for guiding minimally invasive procedures was developed and characterized. The system provides both structural information from ultrasound imaging and molecular information from multispectral photoacoustic imaging. It uses a commercial linear-array ultrasound imaging probe as the ultrasound receiver, with a multimode optical fiber embedded in a needle to deliver pulsed excitation light to tissue. Co-registration of ultrasound and photoacoustic images is achieved with the use of the same ultrasound receiver for both modalities. The imaging depth and spatial resolution of photoacoustic images was measured using tissue-mimicking phantoms. The results indicate that blood vessels can be identified at distances from the needle tip of up to 15 mm. The system has a nearly constant axial resolution of 100 μm throughout the imaging plane, and a depth-depended lateral resolution that varies from 600 μm to 1000 μm. Using a phantom comprising ex vivo tissue, it was shown that photoacoustic spectra can be used to discriminate between blood and fat.

9539-14, Session 2

Virtual Intraoperative Surgical Photoacoustic Microscopy

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We developed a near-infrared virtual intraoperative surgical multimodal imaging system by combining photoacoustic microscopy (PAM) and conventional surgical microscopy to provide simultaneous complementary biological information, such as vascular networks, tumor boundaries, and magnified surface, to surgeons in real-time. By sharing the common optical path in the PAM and optical microscopy system, we can simultaneously acquire the co-registered PAM and microscope images. Moreover, by utilizing a small beam projector, 2D PAM images are back-projected onto the microscope view plane as augmented reality. Therefore, both the conventional microscopic and 2D cross-sectional PAM images are displayed on the plane via an ocular lens of the microscope. In this approach, additional image display monitor is not necessary to show additional images. Thus, it potentially provides significant convenience to surgeons without movement of their sights during surgeries. In order to demonstrate the performance of our system, first, we successfully monitored needle intervention in phantoms. Furthermore, we successfully guided needle insertion into mice skins in vivo by visualizing surrounding blood vessels from the PA images and the magnified skin surfaces from the conventional microscopic images simultaneously. In addition, we succesfully imaged the boundaries of subcutaneous tumors in mice in real time, and further we monitored local agent delivery into the mice skins.

9539-15, Session 2

Optoacoustic endoscopy using a miniaturized detector array

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White light optical endoscopy has been clinically established to visualize disease inside the human body by minimal invasive means. Typically applied as a photographic or video method, white light endoscopy allows visual inspection of interior structures for diseased tissue and abnormal morphological changes. Yet despite its widespread clinical use, white light endoscopy comes with limitations. The most relevant ones are the difficulty of visualizing under the tissue surface and that color-based contrast and architectural features are not always very specific. To improve on these limitations, optoacoustic imaging has been considered for endoscopy. By utilizing optical absorption based ultrasound, optoacoustic imaging can visualize subsurface tissue morphology at high resolution and when tissue is illuminated at different wavelengths, also spectral information. Herein, we present a novel endoscopy concept for intracavital real-time imaging. In contrast to the previously reported dominantly radial looking and single wavelength based approaches our novel design concept offers real-time 2D image formation based on parallelized tomographic detection and aims for spectrally enriched real-time on-the-spot examination of suspicious tissue regions and targets deeper lying structures. We will report on the implementation concept and showcase initial imaging results on phantoms, mice and tissue samples demonstrating the previously undocumented ability to resolve dynamically spectral features in endoscopic imaging mode. Overall the initial results leave great hope that the suggested implementation of endoscopic multispectral optoacoustic tomography may help benefiting clinical diagnosis, for instance by early stage tumor detection and determination of infiltration depth.

9539-16, Session 3

Directed Evolution of Chromoproteins for Photoacoutic FRET Imaging of Protein-Protein Interactions *(Invited Paper)*

Alexander Forbrich, Yan Li, Robert E. Campbell, Roger J. Zemp, Univ. of Alberta (Canada)

Forster Resonance Energy Transfer (FRET) is a widely used phenomenon for sensing protein-protein interactions, however, imaging of FRET in vivo has proved challenging using optical methods due to limitations associated with light scattering in tissues. Photoacoustic imaging is a promising method for imaging with optical absorption contrast in deep tissues with scalable ultrasonic spatial resolution, however, FRET constructs have yet to be optimized for photoacoustic imaging, where optical absorption, rather than fluorescence changes must be sensed. We report on a photoacoustic directed evolution approach to engineer chromoproteins with optimal photoacoustic and FRET characteristics. Ultramarine is evolved into a variant with high molar extinction coefficient and low quantum yield and multiwavelength imaging is demonstrated in vitro and in vivo. Multiwavelength photoacoustic imaging of Cathespin D-sensitive FRET constructs at the donor and acceptor absorption peak wavelengths demonstrate ratiometric changes associated with enzymatic degradation of the linker molecule as high as 9 times larger than blood. Photoacoustic signal changes of two-fold were observed with enzymatic cleavage. Fluorescence changes upon protease cleavage were as large as 6 times that of the quenched pre-cleaved state. Live-cell imaging is demonstrated for visualizing dynamic apoptosis induction. The constructs have demonstrated potential for imaging apoptosis with both fluorescence and photoacoustic imaging.

9539-17, Session 3

High spatiotemporal resolution 4D optoacoustic imaging of the live mouse heart and methods for determination of cardiac function and perfusion

Steven J. Ford, Xosé Luis Deán-Ben, Helmholtz Zentrum München GmbH (Germany); Daniel Razansky, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany)

Determination of cardiac function parameters from 3D, video-rate images remains an outstanding challenge for imaging mouse models of cardiovascular health and disease. Furthermore, the limited temporal resolution or 2D nature of existing imaging methods makes fast, truly volumetric estimations of the cardiac chambers difficult. Herein, we present a novel method to evaluate cardiac function, exploiting the advantages of fast 3D image acquisition by optoacoustic imaging. The mouse heart was illuminated with a laser triggered at 50 Hz, and the subsequent optoacoustic signals were measured using a semi-spherical ultrasound array, providing high resolution reconstructed images of the mouse heart at a rate of 7-8 volumes per cardiac cycle. The reconstructed images depicted prominent physiological features of the heart, including ventricles and atria. Based on the intrinsically-high contrast provided by blood in these chambers, we developed segmentation tools to extract important cardiac function parameters, such as cardiac volume and ejection fraction. Tracking of an intravenous bolus injection of 200 nmol indocyanine green (ICG) allowed for the distinction of right and left chambers of the heart, whereby ICG showed an early arrival into the right cardiac chambers, and late arrival into the left chambers of the heart. The kinetics of ICG distribution were further analyzed to provide pulmonary transit time, the amount of time needed for blood to travel through the pulmonary circulation. Such analysis also provided a 3D map of the blood flow dynamics in a healthy mouse heart and allowed for segmentation of left and right ventricles.

9539-18, Session 3

Photoacoustic imaging of subcutaneous tumor in mice and its vascular response to photodynamic effect using indocyanine green-labeled biodegradable nanocarrier

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Photoacoustic (PA) imaging enables not only detection of tumor but also visualization of vascular structure and angiogenesis in the tumor. The use of a contrast agent can further increase the sensitivity of tumor detection and realize imaging and evaluation of molecular and cellular characteristics of the tumor in vivo. Various inorganic agents, such as metal nanoparticles and carbon nanomaterials, have been shown to be effective to enhance the contrast in PA imaging, and many of these agents can also be used to induce photothermal effect for tumor treatment. However, the toxicity of inorganic agents limits clinical application. In this study, we used indocyanine green-labeled biodegradable nanocarrier, ICG-lactosome, as a theranostic agent for PA imaging and photodynamic therapy (PDT) of a tumor in mice. We performed PA imaging of blood vessels and the distribution of ICG-lactosome in the tumor before and after PDT. The results showed that PA signals originating from ICG-lactosome were increased considerably at 18 h after injection and rapidly decreased after PDT, indicating efficient accumulation of ICG-lactosome in the tumor and its photobleaching due to the photodynamic reaction. After PDT, most of PA signals originating from vasculatures disappeared and amplitudes of the remaining signals were decreased greatly, probably indicating vascular shutdown. These results show the usefulness of ICG-lactosome-mediated PA imaging for diagnosis and prediction of the therapeutic outcome.

9539-19, Session 3

Visualization of the microcirculatory network in skin by high frequency optoacoustic mesoscopy

Mathias Schwarz, Juan Aguirre Bueno, Andreas Buehler, Helmholtz Zentrum München GmbH (Germany); Murad Omar, Vasilis Ntziachristos, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany)

Optoacoustic (photoacoustic) imaging has a high potential for imaging melanin-rich structures in skin and the microvasculature of the dermis due to the natural chromophores melanin and hemoglobin. The vascular network in human dermis comprises a large network of arterioles, capillaries, and venules, ranging from 5 ?m to more than 100 ?m in diameter. Given the large difference in size, the frequency content of the microcirculatory network in human skin is intrinsically broadband. In our group we have developed raster scan optoacoustic mesoscopy (RSOM) that applies a 100 MHz transducer with ultra-wide bandwidth in raster scan mode achieving lateral resolution of up to 18 ?m. Herein, we report on the application of high frequency RSOM to imaging healthy human skin. We analyzed the frequency content of anatomical structures with respect to depth and show that frequencies above 60 MHz contain valuable information of structures in the epidermis and the microvasculature of the papillary dermis. We illustrate that RSOM is capable of visualizing the fine vascular network at and below the epidermal-dermal junction, revealing the vascular fingerprint of glabrous skin, as well as the larger venules deeper inside the dermis. We evaluate the performance of RSOM in measuring epidermal thickness in both hairy and glabrous skin. Finally, we showcase the capability of RSOM in visualizing benign nevi that will potentially help in imaging the infiltration depth of melanoma.

9539-20, Session 3

Fiber Optic Photoacoustic Probe with Ultrasonic Tracking for Guiding Minimally Invasive Procedures

Wenfeng Xia, Charles A. Mosse, Richard Colchester, Jean Martial Mari, Daniil I. Nikitichev, Simeon West, Sébastien Ourselin, Paul C. Beard, Adrien E. Desjardins, Univ. College London (United Kingdom)

In a wide range of clinical procedures, accurate placement of medical devices such as needles and catheters is critical to optimize patient outcomes. Ultrasound imaging is often used to guide minimally invasive procedures, as it can provide real-time visualization of patient anatomy and medical devices. However, this modality can provide low image contrast for soft tissues, and poor visualization of medical devices that are steeply angled with respect to the incoming ultrasound beams. Photoacoustic sensors can provide information about the spatial distributions of tissue chromophores that could be valuable for guiding minimally invasive procedures. In this study, a system for guiding minimally invasive procedures using photoacoustic sensing was developed. This system included a miniature photoacoustic probe with three optical fibers: one with a bare end for photoacoustic excitation of tissue, a second for photoacoustic excitation of an optically absorbing coating at the distal end to transmit ultrasound, and a third with a Fabry-Pérot cavity at the distal end for receiving ultrasound. The position of the photoacoustic probe was determined with ultrasonic tracking, which involved transmitting pulses from a linear-array ultrasound imaging probe at the tissue surface, and receiving them with the fiber-optic ultrasound receiver in the photoacoustic probe. The axial resolution of

photoacoustic sensing was better than 70 μm, and the tracking accuracy was better than 1 mm in both axial and lateral dimensions. By translating the photoacoustic probe, depth scans were obtained from different spatial positions, and two-dimensional images were reconstructed using a frequency-domain algorithm.

9539-21, Session 4

Fluence compensation for quantitative optoacoustic imaging using near-infrared imaging and Monte Carlo simulations

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The accurate determination of the oxygenation saturation in deep blood vessels is a challenge in optoacoustic imaging. Knowledge of the wavelength-dependent light attenuation and the local fluence distribution is required for the correct determination of the optoacoustic spectrum of blood. In this proof-of-principle study, we employ near-infrared imaging to determine the optical properties (absorption and reduced scattering coefficients) of a tissue phantom at two wavelengths (830 and 760 nm). Based on these optical properties a Monte Carlo simulation code estimates the internal fluence distribution which is then used to compensate for the fluence dependent optoacoustic spectrum of a dye. The results demonstrate that the combination of near-infrared imaging and Monte Carlo simulations can correct the optoacoustic spectrum of absorbers in highly scattering media.

9539-22, Session 4

Laser diode based photoacoustic setup to analyze Grüneisen relaxation-effect induced signal enhancement

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1. INTRODUCTION

In the past decades photoacoustic (PA) imaging has been under extensive development. It has been shown, that PA could bring its benefits, such as usage of nonionising electromagnetic waves and natural contrast agents both for microscopy and tomography systems [1]. Due to rapid progress in the field, different modalities of the PA setups are nowadays being more and more used as a functional tool, capable of providing information on anatomy, function or metabolism [2].

Despite the predominance of solid-state based photoacoustic setups, lots of efforts are put into development of more compact, low-cost and robust systems. Most often high power laser diodes (LD's) are used as a promising alternative, but the challenge remains the same: LD's typically cannot offer sufficient fluences, leading to a poor SNR of the acquired signal. To overcome this limitation, two major approaches have been suggested: one could either place the LD as close as possible to the object, focusing all emitted power on the sample [3] [4], or implement coding to compensate the low SNR by adequate increase of the repetition frequency [5].

During recent years PA imaging has been shown possible with various illumination sources including the above mentioned solid state lasers, semiconductor lasers, or even LEDs [6], the influence of variations of the pulsed excitation sequences on the PA signal were not studied well.

This situation has many reasons, some of them could be the already satisfying image quality with the most widely used Nd:YAG-based systems, the complexity of realization of sequences of multiple pulses using solid state lasers or the upper limit on the repetition rate of semiconductor lasers. However, a recent publication [7] introduces Grüneisen relaxation photoacoustic microscopy with a double pulse excitation, providing contrast by the alteration of the absorber's properties by the first pulse which remains until the second pulse comes and changes its absorption properties.

In the field of sophisticated illumination patterns, laser diodes have many obvious advantages over solid state lasers, offering, apart from low cost, high robustness and small sizes, the inherent possibility of usage of complex triggering, which could be easily varied to generate arbitrary excitation pulse sequences. In this paper we propose a multispectral laser diode based setup to study PA signal amplification by the Grüneisen thermal relaxation effect. By systematic variation of excitation parameters like excitation pulse width, pulse amplitude, pulse splitting of pulse sequences and excitation wavelengths, we analyze whether the proposed concept may increase the contrast of PA imaging setups with laser diodes.

2. EXPERIMENTAL SETUP

This work is performed with our multispectral photacoustic diode laser system shown in Fig.1 [8]. Each of the laser diodes with four different wavelengths (650 nm, FBH, Germany; 808 nm, Jenoptik, Germany; 850nm, LDI, USA; 905nm, Laser Components, Germany) is coupled into a 600 μm core fiber, which then forms a fiber bundle to provide light delivery to a blackened plastic foil used as a phantom sample, which is immersed in water. The photoacoustic signal is detected by a focused single element transducer (Olympus Panametrics-C380, focal length: 75 mm, center frequency: 3.5 MHz), amplified (Miteq AU-3A-0110, 58dB) and registered with an oscilloscope (LeCroy Wavesurfer 104MXs, 4 channels, 1 GHz bandwidth) together with a current monitor from the laser diode driver. Dual channel Arbitrary/Function Generator is used as a trigger source (Tektronix AFG3102).

Figure 1. Schematic of the experimental setup. Laser diodes of different wavelengths are coupled into a fiber bundle to illuminate the sample in the water tank. A focused single element transducer is used for detection. After amplification the signal is registered with the oscilloscope.

3. EXPERIMENTAL STRATEGY

As thermal relaxation processes are relatively slow and last up to microsecond time, longer individual excitation pulse width, which is usually seen as a drawback of LD based systems, is not under the question anymore. With the excitation pulse width range of 30-100 ns, pulse energies up to 20 μJ, and widely variable pulse-to-pulse delays, multispectral semiconductor based photoacoustic system offers great potential for systematic analysis of possibilities of PA signal amplification by the Grüneisen thermal relaxation effect.

The exemplary excitation sequence and respective photoacoustic response along with varied parameters are shown in Fig.2.

4. ACKNOWLEDGEMENT

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9539-23, Session 4

Absorption characterization of a nanoparticle solution by using a multi-wavelength high power laser diode excitation in an optoacoustic setup

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Numerous applications of nanoparticles in the field of biomedical imaging have been widely reported in recent years. In particular, several studies have been carried out to use Fe3O4 and gold nanoparticles as contrast agents for optoacoustic (OA) and magnetic resonance imaging, drug delivery systems, and cancer theranostics. For example, these nanoparticles can be used in OA imaging to reveal the presence of tumors inside biological tissues.

In this paper, we employ a compact and cost-effective diode laser set-up to stimulate the OA response of various nanoparticle solutions immersed in a phantom gel, mimicking the optical behavior of a turbid biological tissue. Since the nanoparticles need to be studied over a relatively wide spectrum, the light outputs from diode lasers emitting at 870 and 905 nm are combined together.

At these wavelengths, the phantom optical absorption is low and the detectability of nanoparticles is high. The multi-wavelength configuration of diode lasers facilitates the enhancement of both the signal-to-noise ratio and the quality of optoacoustic signals. The light beams are combined in a multimode optical fiber bundle to illuminate the phantom containing the nanoparticles. In order to minimize the power dispersion, both a primary calibration of the system and the proper choice of an optical fiber are necessary. The choice of the optical fiber depends on its numerical aperture that has to be greater or equal than the beam divergence of the diode lasers. Finally, the absorption peaks of the gold and Fe3O4 nanoparticle solutions are evaluated and compared with previous results.

9539-24, Session 4

Theoretical investigation of thermal-based nonlinear photoacoustic generation from silica-coated gold nanospheres

Florian Poisson, Amaury Prost, Emmanuel Bossy, Institut Langevin (France) and Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France) and INSERM (France)

Photoacoustic imaging provides a unique and high optical-absorption contrast in biological tissue. To selectively map anatomical or physiological feature, one alternative to endogenous absorption is to use contrast agents. Gold nanoparticles have emerged as excellent candidates because they are biocompatible, readily functionalizable, and have a particularly high effective absorption cross section. The photoacoustic signal generation from these particles is however not yet fully understood and can be affected by their surrounding environment. In particular several recent experimental studies have shown that adding a layer of silica on the particles tends to enhance the generated photoacoustic signal. To better understand the photoacoustic generation in the case of a gold nanoparticle, we model and solve both the thermal and thermo-elastic problems in the case of a gold nanosphere. Specifically, we study the influence of a silica coating of controlled thickness and of the interfacial thermal resistances between the different materials (gold, silica, water). The thermal problem was first solved analytically. The spatio-temporal temperature field was then used as a source term in a thermo-elastic model solved by a FDTD approach to compute the photoacoustic signals. We also studied the nonlinearities in the thermoelastic regime, which are due to the dependence of the coefficient of thermal expansion on temperature. We report quantitative estimates of how the temperature fields and the photoacoustic signals are affected by the interfacial thermal resistances and the silica coating.

9539-25, Session 4

Analytical calibration of linear transducer arrays for photoacoustic tomography

Milan Oeri, Wolfgang Bost, Marc Fournelle, Fraunhofer-Institut für Biomedizinische Technik (Germany)

Tomographic photoacoustic imaging (PAT) allows to overcome the anisotropic resolution in axial and lateral direction of conventional reflection mode imaging. In order to achieve high-resolution tomographic images, precise information on the position of each detector element is required. PAT systems that acquire signals from rotating linear transducer arrays come with inevitable transducer misalignments. Up to now, transducer orientation (x/y-tilt) and radial distance uncertainty were measured experimentally or have not been considered. Uncalibrated, these systems suffer from characteristic artifacts yielding misinterpretations of anatomic structures. Herein, we derive the artifact mathematically and investigate an analytical calibration method that enables the calculation and compensation of important transducer positioning parameters: the rotational radius and in-plane tilt. We studied the approach theoretically and evaluated the performance of the developed algorithm both on numerical and experimental data. A PAT system based on a 5-MHz linear transducer array, a multichannel electronics platform with channel data access, a NIR-emitting laser system and rotating samples is used to demonstrate the benefit of the transducer calibration method providing isotropic resolution of 160 μm.

9539-26, Session 4

PhotoAcoustic-guided Focused UltraSound imaging (PAFUSion) for reducing reflection artifacts in photoacoustic imaging

Mithun K. A. Singh, Wiendelt Steenbergen, Univ. Twente (Netherlands)

Influence of acoustic reflectors and consequent reflection artifacts is an important problem in reflection mode photoacoustic imaging. Absorption of light by skin and superficial optical absorbers may produce photoacoustic transients, which proliferate into the tissue and get reflected from structures having different acoustic impedance. These reflected photoacoustic signals, when reconstructed may appear in the region of interest, which causes difficulties in interpreting the images. We propose a novel method (called PAFUSion) using photoacoustic and ultrasound signals to identify and eliminate reflection artifacts in photoacoustic images. We present simulation and phantom measurement results to demonstrate the validity and impact of this method. Results show that PAFUSion technique can identify and differentiate reflection signals from the signals concerned and thus envisages good potential for improving photoacoustic imaging of deep tissue.

9539-27, Session 4

Photoacoustic-guided wavefront shaping: towards deep tissue photoacoustic imaging and light focusing

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In biological tissue, light scattering limits the penetration depth of most optical imaging techniques to a few hundred micrometers. In the last few years, wavefront shaping appeared as a powerful tool to compensate light scattering and focus light in deep tissue.

However it requires a feedback signal that monitors the light intensity on the target. In most practical in vivo scenarios, one cannot directly place a photodetector at the target position. Photoacoustic imaging has been investigated to provide such a feedback and to perform controlled focusing deep inside scattering media. We recently demonstrated light focusing using photoacoustic feedback and a transmission-matrix approach. We show here that the photoacoustic transmission matrix can also help to unveil structures that are usually hidden because of limited view ultrasonic detection. Furthermore, a major challenge to apply these techniques inside scattering samples is the mismatch between the acoustic resolution (tens of micrometers) and the speckle grain size inside tissue (fractions of micrometers): the modulation of the photoacoustic feedback signal vanishes when too many speckle grains are contained within one acoustic resolution cell. We report here on the use of spectrally filtered photoacoustic feedback to focus to tighter regions, thus improving the focusing process efficiency. These results pave the way towards deep focusing and imaging in scattering biological tissue.

9539-28, Session 5

Fast photoacoustic imaging with a line scanning optical-acoustical resolution photoacoustic microscope (LS-OAR-PAM)

Robert Nuster, Guenther Paltauf, Karl-Franzens-Univ. Graz (Austria)

We present the concept, the setup and a preliminary experiment using optical ultrasound detection with a CCD camera combined with focused line excitation for photoacoustic microscopy. The line scanning optical-acoustical resolution photoacoustic microscope (LS-OAR-PAM) with optical ultrasound detection is capable of real-time B-scan imaging providing acoustical resolution within the individual B-scans and optical out of plane resolution up to a depth limited by optical diffusion. A 3D image is composed of reconstructed B-scan images recorded while scanning the excitation line along the sample surface.

Proof of concept is shown by imaging a phantom containing black human hairs and carbon fibers. The obtained C-scan image clearly shows the different resolution in the two perpendicular directions, namely diffraction limited by optical focusing in scan direction and acoustically limited in direction parallel to line orientation by the properties of acoustic wave propagation.

9539-29, Session 5

Optoacoustic measurements of optical effective attenuation for multi-wavelength fluence compensation

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Spectral optoacoustic (OA) imaging enables spatially-resolved measurement of the local blood oxygenation level, due to the different optical absorption spectra of oxygenated and deoxygenated blood. Wavelength-dependent optical attenuation in the bulk tissue, however, distorts the spectral OA signal of the blood and thus renders absolute oxygenation measurements challenging. We show that correction for this spectral distortion is possible without requiring a-priori knowledge of the tissue optical properties. By scanning an irradiation spot over the tissue surface, the resulting relative signal amplitude of blood vessels as a function of light propagation distance enables the reconstruction of the effective optical attenuation coefficient ?effbulk(?). If performed at various irradiation wavelengths, the proposed method can help to retrieve accurate spectral information of embedded absorbers for quantitative OA imaging, by compensating for a simulated fluence distribution. To investigate the potential of this technique for clinical combined OA and ultrasound imaging, we have performed silicone phantom studies using a research system with a linear array probe and an OPO laser system in the wavelength range from 700 nm to 900 nm. The reconstructed wavelength-dependent ?effbulk(?) is in good agreement with reference measurements and allowed an accurate measurement of the OA absorption spectrum of Indocyanine-green dye perfusing embedded artificial vessels. Our results make this technique promising for quantitative functional OA imaging combined with handheld clinical ultrasound.

9539-30, Session 5

High-resolution epi-illumination raster-scan optoacoustic mesoscopy for imaging of model organisms and microvessels

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We have developed an epi-illumination raster-scan optoacoustic mesoscopy system (RSOM), the new system is capable of imaging model organisms, and vasculature. The newly developed system is based on a custom designed; spherically focused detector with a Characterization of the system shows an isotropic lateral resolution of 18 μm, and an axial resolution of 4 μm. The scan times are on the order of 8 minutes for a field of view of 10x10 mm2. The achieved resolution is slightly degraded up to a depth of 5 mm. After characterizing the system we showcase it's performance on a zebrafish ex vivo, and an excised mouse ear. Additionally, to improve the visibility of small structures we have reconstructed the high frequencies, and the low frequencies separately, and at the end overplayed the two reconstructions using different colors, this way the high frequencies are not masked by the low frequencies which have a higher signal to noise ratio.

9539-31, Session 5

Adding the third modality to simultaneous photoacosutic and optically-mediated ultrasound microscopy: co-registration of backscattered optical fluence by ultrasonic detector.

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We present the cost-effective upgrade of the acoustic-resolution photoacoustic microscope [1] to triple-modality imaging system. In papers [2, 3] we demonstrated the phantom and in vivo capabilities of "free" ultrasonic modality of the photoacoustic microscope, based on optical excitation of probing ultrasound pulses by the backscattered laser radiation. Our recent phantom experiments demonstrate that there is an opportunity to realize the additional "free" purely optical modality, since at the time of the laser excitation the photoacoustic detector (Figure 1) can act as a photodiode and measure the optical fluence backscattered from the investigated tissue (Figure 2).

We call the new "free" optical modality of our photoacoustic microscope Acoustical Diffuse Optical Reflectometry (ADOR). Some results of single-modality ADOR imaging are already presented at Figure 3. More phantom results obtained with the triple-modality imaging setup will be prepared for ECBO presentation.

To ensure the efficacy of fluence-compensation algorythms other optoacosutic groups use more complex imaging setups [4,5], as the independent measurements of optical fluence distribution are important for quantitative spectroscopic optoacoustic imaging [6]. Although the ADOR method does not measure the optical fluence inside the investigated tissue directly, the ADOR measurements of the backscattered optical fluence allow its indirect estimations [7,8].

Therefore, ADOR can potentially serve as a simple tool for some optoacoustic imaging systems devoted to the absolute measurements of tissue's chromophore concentrations and the optoacoustic community can be interested to listen to the oral talk on this matter.

9539-32, Session 5

Combined real-time ultrasound plane wave compounding and linear array optoacoustics

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1. Introduction

In optoacoustic imaging, the high optical contrast between different tissue types is combined with the high resolution and low scattering of ultrasound imaging. Signal generation results of subsequent conversion of light into heat and pressure due to light absorption processes in tissue.

Using adapted reconstruction algorithms, images of the distribution of light absorption in tissue can be obtained. This technique can be particularly valuable for imaging of subcutaneous blood vessels since haemoglobin is one of the best absorbing chromophores in biological tissue. Further, the intrinsic scalability of optoacoustic imaging allows the usage of this technique for imaging of microvasculature having diameters beyond the resolution limit of standard vascular imaging techniques such as Doppler ultrasound.

However, such as in any emerging modality, there is limited experience regarding the interpretation of optoacoustic images. For ease of understanding of optoacoustic images, the combination with an established modality such as ultrasound imaging is advantageous. For this reason, we developed a flexible hardware platform combining ultrasound imaging with optoacoustic techniques. The system is based on the software processing of channel data and different types of reconstruction algorithms are implemented. The system was characterized on phantoms and first in-vivo datasets from subcutaneous vasculature were acquired.

2. Materials and Methods

2.1 Hardware setup

The hardware platform is based on the latest generation of DiPhAS (Digital Phased Array System, Fraunhofer IBMT) combined with a Q-switched Nd:YAG system (Handy, Quanta System) operating at its fundamental wavelength of 1064 nm. The device can furthermore be combined with different laser sources such as OPOs by providing arbitrarily programmable trigger signals for laser flashlamp and Q-switch. For signal acquisition, a 7,5 MHz linear array transducer (Vermon SA, France) with a pitch of 300 μm has been used. The hardware platform DiPhAS has 128 independent transmit and receive channels and allows to digitize signals with 40 MSamples/s. The hardware concept relies on fast data transfer to an integrated PC via Gigabit Ethernet for subsequent software-processing of data. In the current setup, ultrasound or optoacoustic frames (having 128 lines) can be transferred and visualized with up to 80 Hz. However, the optoacoustic frame rate is limited to 20 Hz by the repetition rate of the currently used laser. For targeted light delivery, the laser pulse is coupled into a custom made two-arm fibre bundle (Fiberoptic, Spreitenbach, CH). The bundle design has been chosen as a result of light distribution simulations based on a Monte Carlo model. Since the fibre output is placed adjacent to the transducer array, an illumination of structure placed directly in front of the aperture is not possible. Accordingly, no optoacoustic signals can be acquired from structures right in front of the aperture. For this reason, a custom made coupling pad with a thickness of 15 mm is connected to the transducer surface. The pad is made of a thermoplastic elastomer certified for clinical use which guarantees its safety when used in skin contact. Furthermore, it is optically transparent and has a low acoustic damping coefficient of approximately 0,2 dB/MHz?cm, a sound velocity of 1471 m/s and a density of 0,78 g/cm?.

For the sake of ergonomy, the transducer, the coupling pad as well as the fibre outputs are integrated in a custom made holder. With all components assembled, the size of the probe approximately corresponds to that of a standard matrix array. The probe design as well foresees a slit for attachment of the tracker of an electromagnetic tracking device that can potentially integrated for acquisition of 3D data.

2.2 Algorithms

In both imaging modes, channel data are acquired and transferred to a PC where a beamforming algorithm is implemented in a GPU (graphics processing unit). Massive parallelization in GPUs allows to perform channel data reconstruction in real time with frame rates higher than 20 Hz. For the optoacoustic data, beamforming is performed with a conventional delay-and-sum algorithm with optional coherence, apodization and statistical filtering. In ultrasound mode, images are generated by plane wave compounding in order to take full profit of the channel data capabilities of the system. Different presets with optimal number of angles and angle increment have been defined according to the results of preliminary simulations. Reconstruction of the individual data sets (one for each angle) is then as well performed based on conventional delay-and-sum beamforming and the compound image is generated by summation of the individual data sets.

3 Results

3.1 Phantom experiments

For assessment of the system´s performance, wire phantoms were imaged in both modalities. For this purpose, an optically absorbing wire was moved in axial direction and optoacoustic channel data were acquired in each step. The wire phantom was immersed in a 6% milk in water mixture in order to mimick optical scattering properties of biological tissues. At each step (corresponding to different depths), the optoacoustic data were reconstructed with conventional beamforming with optional filtering. The signal to noise ratio and the full-width-half-maximum (FWHM) of the point-spread-function (PSF) were assessed for each depth and the influence of additional filter algorithms was assessed.

The results depicted in figure 2 show that absorbing structures can be detected with adequate SNR up to depths of approximately 45 mm in highly scattering media. Furthermore, the results show the impact of additional filter algorithms during sum-and-delay beamforming. While the FWHM of the PSF is in the range of 330 μm for the first 25 mm in depth in the case of conventional beamforming, coherence and statistical filtering allow to decrease the FWHM down to ~ 290 μm and ~210 μm respectively.

3.2 In-vivo experiments

In a next step, combined imaging of subcutaneous vasculature was performed in the hand of a proband. In all optoacoustic measurements, optical fluency far below the safety threshold of 100 mJ/cm? at 1064 nm was used. The measurements were performed with the above mentioned acoustic coupling pad and were repeated with the hand immersed in a water tank in order to assess the influence of the pad. The comparison shows that the contour of the finger can – obviously – not be reconstructed accurately when using the pad since acoustic coupling is limited to the top side of the finger. Furthermore, the upper part of the acoustic images (first 15 mm) is affected with artefacts which are characteristic for plane wave compounding when an offset is present between the transducer surface and the investigated object. Ultrasound images presented in figure 3 were all generated with plane wave compounding using 7 angles.

Since the acoustic velocity in the pad is comparable to that in water, there is no image deterioration induced by the usage of the pad and vessels can accurately be reconstructed (see figure 3b). A direct assessment of the effect of the pad on the SNR cannot be performed since distinct vessels with unknown radius have been imaged. However, the analysis of signal amplitudes shows that the influence of the pad is limited since SNRs of 58 and 63 dB have been measured respectively with and without coupling pad.

4 Conclusion

A system for combined imaging based on plane wave compounding and optoacoustic imaging using linear array transducers has been developed and characterized using wire phantoms and first in-vivo measurements on probands. The system includes a custom made integrated probe based on a linear array transducer, fibre guides for targeted light delivery and a coupling pad. The results show the limitations and benefits of the approach involving an acoustic coupling pad. Especially when irregular contours need to be imaged, an approach involving a water tank is preferred since acoustic coupling can only partially be guaranteed in such cases using a pad. The results further show that a loss in SNR in the range of 2 to 5 dB can be expected as a result of using an acoustic coupling pad. However, in applications where a high SNR is given and structures placed directly under the skin need to be visualized (e.g. subcutaneous vasculature), a setup with an integrated coupling pad such as proposed allows convenient freehand scanning without the need for water immersion. The system is furthermore suitable for freehand 3D imaging since both the used data format (binary format .grb, Fraunhofer IBMT) and the probe design foresee the integration of electromagnetic trackers. The calibration of the system and the acquisition of first freehand 3D optoacoustic data sets are work in progress.

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9539-33, Session 5

Nanodroplets as a platform for multi-modality imaging and drug delivery

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Porphysomes were recently introduced as organic liposome-variants with each lipid loaded with a porphyrin molecule. These agents have considerable promise as contrast agents for photoacoustic and

fluorescence imaging as well as for photodynamic therapy. Nanodroplets are shelled nanoscale agents with a liquid perfluorocarbon core. We introduce porphysome nanodroplets, which are lipid-porphyrin-shelled with super-critical liquid perfluorocarbon core. These all-organic agents can be triggered with external ultrasound, heat, or optical energy to trigger a phase-change of the perfluorocarbon core to produce microbubbles. Such microbubble contrast agents have significant potential for ultrasonic molecular imaging as single microbubbles can be detected deep in tissue using ultrasonic nonlinear contrast imaging schemes. While microbubbles are too large to access extravascular targets their nanodroplet counterparts can be made as small as 100nm and are thus capable of extravasation. We demonstrate our porphysome nanodroplets can be imaged with fluorescence, photoacoustic imaging, and ultrasound imaging in vitro and in vivo. Tumor xenografts in the CAM membranes of chick embryos are used as model systems to study accumulation via targeting and the enhanced permeability and retention effect. Multi-wavelength photoacoustic imaging is performed with a Vevo LAZR photoacoustic imaging platform and spectral de-mixing reveals accurate profiling of the agents and oxy- and deoxy-hemoglobin. Ultrasound imaging before and after high-mechanical-index bursts reveals distributions of phase-changed nanodroplets in vitro and in vivo. Folate-targeted agents demonstrate high affinity for HeLa cells (which over-express folate receptor) but not ZR-75-1 cells (which have negligible folate-receptor expression). Agents can be loaded with hydrophobic drugs such as doxyrubicin.

9539-34, Session 5

Scattering and Absorption Characterization of Nanoparticles Using Optoacoustic Spectroscopy in the Near-IR Regime

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The hemoglobin is the main iron-containing and oxygen-transport protein in the red blood cells and exhibits a high absorption in the near infrared spectrum [1]. To improve the optoacoustic imaging (OA) in tissues, introduction of exogenous contrast agents are highly desired [2-4]. The use of proper size contrast agents with absorption peaks in this range yields a longer circulation of nanoparticle in the human body and allows their accumulation in the target area for an amount of time sufficient to carry out the imaging procedure [5].

The previous studies have demonstrated the potential use in OA of silica- and gold-coated Fe3O4 and NIR- Fe3O4 nanoparticles at different concentrations and with a core size of 3-50 nm [5-7]. For example, R. Alwi et al [5], used a single NIR wavelength at 1064 nm, nevertheless, Pei-Hsien et al. [7], explored the OA imaging at 715 nm with 10-Hz pulse repetition rate. These wavelengths corresponds to the absorption peak at specific nanoparticles sizes, where the endogenous blood-pool contrast might not be sufficient enough to differentiate a malignant tumor from normal tissues.

Our group has recently studied the absorption properties of gold nanoparticles obtained for an incident light energy (? 20 mJ/cm2) in the range of 410-2400 nm with a repetition rate of 10 Hz [8, 9]

The first drawback of the system in figure 1 is the use of water tank to improve the coupling between the transducer and the ultraviolet cuvette containing the gold nanoparticle. Another drawback is the spectroscopy is only possible within the range of 410-100 nm.

In this conference, we will use an advanced OA experimental setup , where the water tank is replaced by a four channel ultraviolet cuvette holder, for simultaneous measures of absorption and scattering measurements. The spectral range covered by the system is from 410 nm to 1700 nm, which represents an extension further into near infrared with higher sensitivity than our previous experimental setup. The wavelength extension allows us to investigate the nanoparticle properties further towards infrared, which is of high interest for OA imaging and diagnostic applications.

Conclusion and Further Work:

We will start with the measurements of the reflected light, the collimated light transmission, a fraction of the scattering light and the optoacoustic signal. This system configuration takes into account any error due to fluctuation in the optical parametric oscillator (OPO) output energy for simultaneous accurate measurements of the collimated light transmission, scattering and afterwards by evaluating the optoacoustic signals through a multispectral spectroscopy at 715-1653 nm. In order to improve the signal to noise ratio, we will perform the signal averaging in the linear regime, over several measurements for each data point. Using Beer–Lambert, we extract the absorption coefficients and the scattering ones over the targeted range. The absorption spectra are used to verify the photothermal stability of the Fe2O3 after the exposure to 1000 laser pulses when applying different fluencies in the range up to 20 mJ/cm2. This paper presents for the first time simultaneous absorption and scattering measurements of iron oxide nanoparticles used in OA imaging by performing integrated measurements in a single experimental setup.

9539-35, Session 5

Light fluence correction for quantitative determination of tissue absorption coefficient using multi-spectral optoacoustic tomography

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MultiSpectral Optoacoustic Tomography (MSOT) is a fast developing imaging modality, combining the high resolution and penetration depth of ultrasound with the excellent contrast from optical imaging of tissue. Absorption and scattering of the near infrared excitation light modulates the spectral profile of light as it propagates deep into biological tissue, meaning the images obtained provide only qualitative insight into the distribution of tissue chromophores. The goal of this work is to accurately recover the spectral profile of excitation light by modelling light fluence in the data reconstruction, to enable quantitative imaging.

We worked with a commercial small animal MSOT scanner and developed our light fluence correction for its cylindrical geometry. Optoacoustic image reconstruction pinpoints the sources of acoustic waves detected by the transducers and returns the initial pressure amplitude at these points. This pressure is the product of the dimensionless Grüneisen parameter, the absorption coefficient and the light fluence. Under the condition of constant Grüneisen parameter and well modelled light fluence, there is a linear relationship between the initial pressure amplitude measured in the optoacoustic image and the absorption coefficient. We were able to reproduce this linear relationship in different physical regions of an agarose gel phantom containing targets of known optical absorption coefficient, demonstrating that our light fluence model was working. In preliminary tests, we were also able to observe promising results of light fluence correction effects onin vivo data.

9539-36, Session PWed

Portable, high-speed photoacoustic microscopy scanner for in vivo deep imaging of skin

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There have been developed two schemes for high-resolution photoacoustic (PA) imaging: optical resolution (OR) and acoustic resolution (AR) PA microscopy (PAM). In OR-PAM, a resolution higher than 1 ?m can be obtained, but the imaging depth is limited to < 1 mm. Deeper imaging can be obtained by AR-PAM, but imaging quality and accuracy in the defocused regions may be decreased. In this study, we developed a portable PA microscopy scanner for in vivo deep imaging

of tissue, in which a new image reconstruction method is used. In the imaging head, four transducer elements are arranged around the optical fiber for PA excitation. PA signals originating from a target optical absorber are detected by each transducer element, and the signals are used for imaging only when their propagation times are the same, enabling lensless high-resolution visualization of optical absorbers in tissue. The imaging head, which is held by a flexible arm, can be scanned over a 4 mm x 4 mm area in ~16 seconds. With this scanner, blood vessels in the human skin were visualized with a horizontal resolution of 40 ?m in the depth range up to ~3 mm in vivo.

9539-37, Session PWed

Characterisation of a PVCP based tissue-mimicking phantom for Quantitative Photoacoustic Imaging

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Well-defined, versatile and stable phantom materials are essential for assessing the accuracy and robustness of quantitative, multispectral photoacoustic imaging (qPAI) algorithms. The fabrication of a polyvinyl chloride plastisol (PVCP) phantom will be described, along with a thorough acoustic, optical and thermalisation efficiency characterisation. In contrast to previous work (Spirou 2005, Bohndiek 2013), we have considered multiwavelength optical excitation, multiwavelength optical characterisation and broadband acoustic property characterisation for different levels of PVCP hardness. These parameters might then eventually be controlled, tuned or isolated to assess accuracy, robustness and margin of validity of qPAI algorithms in phantom studies.

The influence of increasing hardness was studied, in terms of acoustic dispersion c(f) and acoustic attenuation ?(f), up to 15 MHz. Both acoustic properties decreased with increasing levels of softener: c={1407.9±0.8,1 406.4±0.7,1401.7±0.8} ms-1 @5MHz, ?={0.67±0.02,0.62±0.03,0.55±0.05} [dB/cm/MHz] @1MHz, respectively for 5, 10 and 20 %v/v softener.

Multiple wavelength optical absorption and photobleaching information was also obtained for PVCP with 3 added absorbers (red, blue and black) from the same manufacturer, showing their potential as oxy and deoxy-hemoglobin analogues for multispectral phantom studies. Finally, a significant decrease in the Grüneisen parameter was found with increasing absorber concentration.

The results show promise for the use of PVCP as a tissue-mimicking phantom in a qPAI context.

9539-38, Session PWed

Improved synthetic aperture focusing technique in optoacoustic imaging

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Synthetic aperture focusing (SAFT) is a well established method for focusing signals from a detector array, which has been applied extensively in radar. This technique was then implemented in ultrasound imaging, again for arrays. The essential scheme of the algorithm is to apply time delays to signals from the individual array elements and then sum them based on a spatial criterion i.e. an artificial focus can be steered through the field of view of an array. Both in radar and in pulse-echo ultrasound imaging the signals detected by the array are a scattered portion of a signal sent by said array. Consequently, the strength of signals returned by a scatterer depends on its position within the field of view of the array. In optoacoustics, however, the strength of the acoustic waves generated is dependent only on the light fluence and the optical absorption properties of the optacoustic source (such as a microsphere). This is a significant problem, especially when scanning a focused single-element detector in place of a stationary multi-element array, such as in optoacoustic microscopy (OAM). Although variations on SAFT have been presented for application in OAM [1], they suffer the caveat of inaccurate signal rectification, and are not applicable near the physical focus of the transducer.

Here we present SIR-SAFT, a method for achieving accurate signal rectification and restoration of lateral resolution at all depths within the field of view of a focused transducer. This method uses the spatial impulse response (SIR) of the transducer to weight the contributions at each scanning position, thus compensating the biased gains applied by conventional SAFT.

The algorithm is shown to outperform conventional SAFT on both simulated data and experimental OAM data, without compromising or impeding further processing often applied to SAFT results, namely the coherence factor.

[1] “Improved in vivo photoacoustic microscopy based on a virtual-detector concept”, Wang, Optics Letters 2006

9539-39, Session PWed

Geometrical super-resolution for planer optoacoustic imaging

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The resolution of optoacoustic systems is limited by the properties of the ultrasound transducers, the detection geometry and acoustic attenuation. Approaches to improve the image quality and resolution typically use advanced model-based reconstruction algorithms to account for specific detection geometries and transducer shapes. However, post-reconstruction image processing methods also offer the possibility to improve image quality and resolution. Herein, we demonstrate the application of a geometrical super-resolution technique to enhance the resolution and contrast in optoacoustic images. The proposed method acquires several low resolution images from the same object located at different positions inside the imaging plane. Thereafter it applies an iterative registration algorithm to integrate the information in the acquired set of images to generate a single high resolution image. We present the method and evaluate its performance in simulation, on phantoms and using tissue samples, showing that super-resolution techniques can be a promising alternative to enhance resolution in optoacoustic imaging.

9539-40, Session PWed

Sensitivity analysis of a non-contact holographic photoacoustic tomography approach

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Photoacoustic imaging has become very popular in recent years. The combination of optical- and ultrasound aspects gives some particular advantages: Biomedical imaging in a depth of up to several cm with a contrast much higher than in pure ultrasound becomes possible.

Generally, a great number of systems found in literature require an acoustic contact because of the used individually detection techniques, which is limiting the application field, especially for in vivo imaging without the use of anaesthesia.

The aim of the project was to develop and evaluate a novel high speed

non-contact holographic PAT system, which overcomes these limitations to bring the PAT a step closer towards non-anesthetized in vivo imaging of e.g. sub-dermal vessel structures and new medical applications not allowing acoustic contact.

In the characterization sensitivity is a significant attribute; in particular the signal to noise ratio (SNR) and the noise equivalent pressure (NEP). The knowledge of these properties is essential to find on the one hand new application fields of medical imaging and on the other hand to compare with existing well-established systems.

It is important to take into account, that the developed PAT setup measures surface displacements and not the pressure transient. The relationship between the pressure and surface modification is to be considered in the analysis as well as for the comparison.

9539-41, Session PWed

A system analysis and image reconstruction tool for optoacoustic imaging with finite-aperture detectors

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In optoacoustic tomography, detectors with relatively large areas are often employed to achieve high detection sensitivity. However, spatial averaging effects over large detector areas may lead to attenuation of high acoustic frequencies and, subsequently, of fine features in the reconstructed image. Model-based reconstruction algorithms improve image resolution in such cases by correcting for the effect of the detector's aperture on the detected signals. However, the incorporation of the detector's geometry in the optoacoustic model leads to a significant increase of the model matrix memory cost, which hinders the application of inversion and analysis tools such as singular value decomposition. In this paper we demonstrate the use of the wavelet-packet framework for optoacoustic systems with finite-aperture detectors. The decomposition of the model matrix in the wavelet-packet domain leads to sufficiently smaller model matrices on which SVD may be applied. Using this methodology, over an order of magnitude reduction in inversion time is demonstrated for experimental data. Additionally, our framework is demonstrated for the analysis of inversion stability and reveals a new, non-monotonic dependency of the system condition number on the detector size. Thus, the proposed framework may assist in choosing the optimal detector size in future optoacoustic systems.

9539-42, Session PWed

Real-time monitoring of indocyanine green perfusion in human finger vasculature by means of optoacoustic tomography

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Dynamic contrast enhanced imaging and extraction of a set of parameters from its kinetic profile has proven a valuable diagnostics tool in established clinical modalities like MRI. In optical imaging modalities the high temporal capabilities can be combined with a great variety of optical contrast agents while optoacoustic tomography is capable of additionally providing high ultrasonic spatial resolution. In this study, optoacoustic tomography was employed to monitor the bio-distribution of injected fluorescent agent in a human finger. Using a parallel detection array, we were able to render cross-sectional images of a human index finger in real-time at 10 Hz rate before and during the injection of Indocyanine Green (ICG). Optoacoustic tomography was able to characterize the spatio-temporal kinetics of ICG at clinically relevant concentrations. Anatomical images were further validated with MRI. Our results pave the way for optoacoustically diagnosing diseases based on dynamic kinetic profiles in peripheral vasculature, such as artery occlusion, liver functionality, cardiac output or rheumatoid arthritis.

9539-43, Session PWed

Speed-of-sound correction for photoacoustic and laser-ultrasound imaging with an integrating cylindrical detector

Guenther Paltauf, Gerhild Wurzinger, Robert Nuster, Karl-Franzens-Univ. Graz (Austria)

Photoacoustic imaging (PAI) provides good contrast for light-absorbing structures such as blood vessels. In order to visualize non-absorbing structures, often a second technique is combined with PAI, such as pulse-echo ultrasound. In laser ultrasound imaging, the acoustic waves are generated photoacoustically, by irradiating an external absorbing target. For photoacoustic and laser-ultrasound image reconstruction a correct relation is required between the ultrasound time of flight (TOF) from the various locations in the object to the detector and their distance. Usually this is the speed of sound (SOS) of water or some average speed of sound of water and object. For exact reconstruction, variations of the sound speed have to be taken into account in the reconstruction.

In this study, we use ultrasound transmission data for improving the reconstruction of photoacoustic and laser ultrasound pulse-echo images in the presence of SOS heterogeneity. Signals for all three modalities are measured with an optical line detector formed by a continuous laser beam in an optical interferometer. Ultrasound focusing is achieved with a cylindrical reflector, which at the same time acts as a target for laser-ultrasound generation. The reconstruction uses the standard inverse Radon transform in the case of constant SOS and a modified inverse Radon transform for heterogenous SOS. The performance of the modified reconstruction is demonstrated in a simulation, where it is shown to remove more artifacts than a reconstruction assuming a mean SOS. Finally, an experiment with a phantom that contains various structures with optical and ultrasound contrast shows that the SOS correction also works for real data.

9539-44, Session PWed

Error estimates for universal backprojection based photoacoustic tomography

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Photoacoustic tomography is a hybrid imaging technique, that combines the optical absorption based contrast with high penetration depth and resolution of ultrasound techniques. Accuracy of universal backprojection based photoacoustic reconstruction depends on the Fourier filters chosen for the filtering of forward data (pressure signals) measured by small aperture detectors, which are part of a detection surface, which partially or completely encloses our sample. Low pass filters are used for filtering purposes in order to avoid aliasing artifacts and attenuate the high frequencies, when the forward data is noisy. However, for phantoms having finite support, the photoacoustic signals cannot be band-limited, hence we have developed an error estimate formula with respect to the bandlimited photoacoustic reconstruction, that provides the "best" image for ideal, noise-free forward data. We then computationally validate the developed error estimates with respect to synthetic phantoms. Validations have also been carried out for noisy pressure signals, in order to study the effect of noise on the error estimates derived in our work. Although here we will discuss the error estimates for a planar detection geometry, estimates for spherical and cylindrical geometries can also be developed in a similar way.

9539-45, Session PWed

Image reconstruction in cross-sectional optoacoustic tomography based on non-negative constrained model-based inversion

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Optoacoustic tomographic images represent the light absorption distribution obtained by means of a reconstruction algorithm from detected acoustic pressure waves. The images obtained with most reconstruction algorithms are affected by negative values due to modelling inaccuracies and imperfect measurement conditions. Negative optical absorption values have no physical meaning and their presence hinders image quantification and interpretation of biological information. The performance of optimization methods imposing non-negative constraints in model-based optoacoustic inversion is investigated herein for cross-sectional tomographic imaging.

Specifically, we analyze the effects of the non-negative restrictions in three aspects, namely image quality and accuracy, quantitativeness of the image values and computational speed and efficiency.

Additionally, an improved non-negative constrained minimization method based on the projected conjugate gradient method is proposed.

9539-46, Session PWed

Light excitation methods for five dimensional optoacoustic imaging

Xosé Luis Deán-Ben, Thomas Felix Fehm, Sven Gottschalk, Erwin Bay, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

Multispectral optoacoustic tomography (MSOT) offers unprecedented capabilities in biological research and newly-developed systems prompt the clinical translation of this modality. By exciting tissues at multiple optical wavelengths, the distribution of spectrally-distinctive absorbers can be resolved with high resolution in deep tissues, thus enabling reading important biological parameters such as blood oxygenation or the biodistribution of photo-absorbing agents. The feasibility of three-dimensional imaging in real-time (four dimensional imaging) has been recently showcased. More importantly, two different approaches have been suggested to provided five dimensional optoacoustic imaging, namely multispectral three-dimensional imaging in real time. The first one is based on using a fast tuning laser allowing changing the wavelength on a per pulse basis. The second one consists in exciting the tissue with two lasers having different wavelengths and delayed a few microseconds. Herein, the capabilities of these two methods for functional imaging of moving targets are compared and the perspectives of clinical translation of five dimensional optoacoustic imaging with a hand-held approach are discussed.

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9540-33, Session PD

Interventional nerve visualization via the intrinsic anisotropic optical properties of the nerves

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We present an optical concept to visualize nerves during surgical interventions. The concept relies on the anisotropic optical properties of the nerves which allows for specific switching of the optical reflection by the nervous tissue. Using a low magnification polarized imaging system we are able to visualize the on and off switching of the optical reflection of the nervous tissue, enabling a non-invasive nerve specific real-time nerve visualization in a surgical setting.

9540-23, Session PWed

Optical properties of the chemotherapy drugs used in the central nervous system lymphoma therapy: monitoring drug delivery

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Our aim is to optically monitor the delivery of the chemotherapy drugs for brain tumours, particularly used in the central nervous system (CNS) lymphoma therapy. In vivo monitoring would help to optimize the treatment and avoiding unnecessary medications. Moreover, it would be beneficial to be able to measure which of the multi-regimen drugs actually do penetrate and how well into the brain tissue.

There exist several potential optical measurement techniques to be utilised for the purpose. The most desired method would allow the detection of the drugs without using optical biomarkers as a contrast agent. In this case, for non-invasive sensing of the drug in the brain cortex, the drug should have a reasonably strong optical absorption band somewhere in the range between 600 nm and 1700 nm, and not directly coincident with the strong bands of haemoglobin or water. Alternatively, mid-infrared (MIR) range has the potential for invasive drug monitoring techniques.

In this paper, we report the optical properties of several chemotherapy drugs used in CNS lymphoma therapy, such as rituximabi, cyclophosphamide and etoposide. We measured their transmittance and reflectance spectra in near-infrared (NIR) and MIR range, particularly 400 nm – 1100 nm and 1.8 um – 1800 um, to be considered when choosing the in vivo monitoring method to be developed. The absorption and scattering coefficients were retrieved from the measurements and applying Beer's law. For the measurement of the sum of total transmission and reflection in NIR range we used integrating sphere with spektralo to enable calculation of the scattering coefficient.

9540-28, Session PWed

Effect LED extracorporeal blood irradiation on the dynamics of the immune status of primary inoperable lung cancer patients

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Background. At present the problem of increasing the effectiveness of cancer treatment includes specific therapy along with new approaches and biophysical ef-fects on the body and the tumor. Among the new technologies have proliferated physical factors wave electromagnetic nature, especially the optical range. As an al-ternative to the use of medicinal immunomodulators in treatment of inoperable primary lung cancer patients may be the use of autologous blood irradiated ex vivo incoherent LED radiation optical range. The aim of this work is to study the dy-namics of immunological and clinical efficacy in lung cancer patients when used fotohromotherapy (FHT) in their complex treatment.

Methods. The results of examination of 30 patients with initially unresectable locally advanced lung cancer IIIA-IIIB were used. Performed in vitro exposure in-coherent LED radiation in the red spectrum from the LED device "Pulse 2", a dose of 3.06 J/cm2 300 ml of autologous blood followed by incubation with cytotoxic drugs for 40 minutes at T = 37?C and reinfusion to the patient. Cytostatic dose was: cisplatin-200mg, doxorubicin- 100mg, cyclophosphamide-2000mg. The course included 4 treatments with an interval of two days.We also observed a con-trol group of 30 patients The immune status of all patients before treatment and 10-14 days after the end of the course were evaluated. In heparinized blood samples the number of T (CD2 +) - and B (CD20 +) cells, as well as a number of lympho-cyte subpopulations CD4 +, CD8 +, CD16 + by indirect immunofluorescence test were determined, the functional activity of lymphocytes with PHA and RBTL LPS and neutrophils in HCT-test were carried out.

Results. We found positive changes in the content of some quantitive and qualitive characteristics of cellular immunity (CD4+, CD8+, NK, B-cells) after ad-ministration of blood photomodified ex vivo while in the control group of patients decrease of the parameters was observed.

Conclusions FHT is able to cause immunoprotective effect in lung cancer undergoing chemotherapy and partly to improve its` result.

9540-29, Session PWed

Tip enhanced Raman scattering of bacillus subtilis spores

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Understanding of the complex interactions of molecules at biological interfaces is a fundamental issue in biochemistry, bio-technology as well as biomedicine. A plethora of biological processes are ruled by the molecular texture of cellular membrane: cellular communications, drug transportations and cellular recognition are just a few examples of such chemically-mediated processes. Tip-Enhanced Raman Scattering (TERS) is a novel, Raman-based technique which is ideally suited for this purpose. TERS relies on the combination of scanning probe microscopy and Raman spectroscopy. The basic idea is the use of a metalled tip as a sort of optical nano-antenna, which gives place to SERS effect close to the tip end.

Herein, we present the application of TERS to analyze the surface of

Bacillus Subtilis spores. The choice of this biological systems is related to the fact that a number of reasons support the use of spores as a mucosal delivery system. The remarkable and well-documented resistance of spores to various environmental and toxic effects make them clear potentials as a novel, surface display system. As a matter of facts, being non-recombinant and able to stabilize the heterologous proteins displayed on their surface, spores appear particularly well suited for the delivery of bio-therapeutic molecules to animal and human mucosal surfaces.

Our experimental outcomes demonstrate that TERS is able to provide a nano-scale chemical imaging of spore surface. Moreover, we demonstrate that TERS allows differentiation between wilde-type spore and genetically modified strains. These results hold promise for the characterization and optimization of spore surface for drug-delivery applications.

9540-30, Session PWed

Visible optical radiation generates bactericidal effect applicable for inactivation of health care associated germs demonstrated by inactivation of E. coli and B. subtilis using 405 nm and 460 nm light emitting diodes

Katharina Hoenes, Felix Stangl, Michael Sift, Martin Hessling, Hochschule Ulm (Germany)

Clean water is the most relevant resource of our planet and all mankind is depending on it. Assured access to safe drinking water represents a considerable aspect in quality of human life. But especially in developing countries about 50 % of the population is forced to rely on water from contaminated sources, increasing the risk of serious diseases [1].

But this is not the only scope of application. Even in medical health institutions of industrialized countries sterile water supply is not certainly assumable. Studies found that hospital taps are contaminated in a double-digit percentage range with pathogens such as Pseudomonas aeruginosa. They also found a correlation between the contamination rate of hospital taps and the incidence of hospital-acquired infections in intensive care patients [2]. Likewise the rinsing water in dental surgeries often is even highly contaminated and has to be sterilized before usage. Contaminated pipework and long-term disuse in water tanks are the reasons for this grievance.

Due to this principal problem research was done for several years in different water treatment techniques which provided a simple to handle and cost-efficient application for water disinfection issues. Within the acquired approaches, the exposure of contaminated water to optical radiation represented a well-established method. In most cases wavelengths in the range of UV-C light (254-280 nm) were applied. Inactivation of bacteria is reached by creating damages in nucleic acids through generation of dimers in thymine nucleic bases which in turn affect the replication capability.

Unfortunately ultraviolet radiation is injurious to health so that safety precautions are necessary to be implemented. In most cases mercury arc lamps with a high voltage supply are integrated as a light source. Therefore present overall systems achieve solely slight efficiency and lifespan. Light emitting diodes (LEDs) which operate under more favorable conditions could be an alternative. But they are currently still extremely expensive within the required wavelength range and offer just a very limited life time. A further problem concerning ultraviolet disinfection is the deficient inactivation of specific microbial strains, like Bacillus subtilis.

Light in the visible spectrum range can also have a disinfectant effect of germs which was identified in principal. But so far that fact is often disregarded and to date it is not examined in detail. Light of specific wavelengths (e.g. 405 nm) causes damage to bacteria taking advantage of porphyrins. Porphyrins are dye pigments which are produced in bacterial metabolism and act as converter for the germicidal effect. Stimulated with wavelengths contained in their absorption spectra they produce reactive oxygen species (ROS) which internally affect microorganisms and destroy cell integrity. By this means inactivation of bacteria is independent of DNA repair mechanisms and can be established towards germs which inherit resistance against UV-C irradiation.

An advantage of this application is the possibility to use commercially available LEDs in the visible wavelength range. Instead of mercury arc lamps or UV-C LEDs, which are used in UV-C disinfection, they are cost-efficient and have a considerable longer life span. Above all they are absolutely non-hazardous. Furthermore resistances of bacterial strains towards the visible part of the optical spectrum are not reported to date.

This paper investigates the question whether the exposure to radiation of 405 nm wavelength is suitable for inactivation of health-hazardous bacteria in water and if resistances for the UV-C light range also exist at radiation of 405 nm. Corresponding results would be rewarding for drinking water applications as well as water sterilization in medical health care institutions.

Further subject is the appraisal whether commercially available LEDs in the blue wavelength range (450-480 nm) could be used, as it is suggested by the work of Enwemeka et al. [3]. Using a 470 nm LED they realized to provide a disinfection rate concerning MRSA with likewise effective results as a comparative study irradiating with 405 nm. Existing resistances in the UV irradiation range, which also could be at least suspected in the near-UV wavelength range at 405 nm, are not expected.

Methods

Bacteria

In the experiments two bacterial strains are used. E. coli as a common bacteria for laboratory experiments, which is also responsible for some diarrhea disease and B. subtilis because of its UV-C resistance.

Light source

Two different LEDs were used as light sources for radiation with 405 nm and 460 nm. Both LEDs were bonded to a heat sink and fan to minimize the heat production by the diode. By this proceeding it should be ascertained that the device would not reach a problematic temperature.

For the wavelength of 405 nm the LED LZ4-00UA00 is used, with an maximum electrical power of 10 W. The power density resulted from the overall setting of 33.5 mW/cm2 at 29 cm distance of the sample. For all experiments with 405 nm the light source was operated at 620 ± 1 mA.

For the wavelength of 460 nm the LED LZ4-40B208-0000 is used, with an maximum electrical power of 10 W. The power density resulted from the overall setting of 33.5 mW/cm2 at 29 cm distance of the sample. For all experiments with 460 nm the light source was operated at 540 ± 1 mA.

To ensure homogenous radiation both LEDs are used with a self-developed optical setup in the overall experimental setting (Fig. 2).

Optical setup

The optical setting was designed according to the criteria of maximum homogeneity of illumination over the total radiation area. An optical simulation was performed in ZEMAX® to evaluate the best geometrical appearance. Constructional alternatives were crosswise rated after technical feasibility and optical effectiveness. A truncated pyramid provided best outcome data. The aperture at the exposure to light was adapted to the dimensions of the LED (8x8 mm).

Results of the real measured distribution of irradiation generated by the structural element are shown in Fig. 1. The optical cone is made of polished aluminum with an excellent spectral reflectance. The result was a luminous efficiency with a variance of 10 % (Fig. 1).

Using this method, achieved homogeneity of irradiation leads to uniform illumination of the whole sample area, and skirts the need of a magnetic follower or other stirring techniques which in turn could cause optical shadowing effects.

Experimental setup

The specific LED was driven with the power supply Toellner TOE 8733 and positioned at the smaller aperture of the truncated aluminum pyramid. From a height of 29 cm (measured at the middle of sample dish) and an hourly sample drawing, the energy fluence increases by 120 J/cm2.

Inside a cell culture flask with an effective irradiation area of 32 cm2, 40 ml of bacterial test suspension was inserted to the experimental setup.

Potential temperature rise was measured directly by inserting a measuring head inside the culture flask during hourly probe drawing, which could be held at a constant level of 27 ± 1 °C.

Quantification of bacterial colonies

Duration of the experiment was set to 5 hours. In order to be able to examine the inactivation process quantitatively, triplicate samples of 100 µl each were drawn every hour and brought into serial dilution to receive

proper densities. Respectively 500 µl of different degrees of dilution were filtered using a vacuum pump together with standard nutrient pads. After an incubation period of 16 hours at 37 °C placed on specific nutrition disks, the colony counts were manually detected and subsequently determined in CFU/ml.

Preliminary Results

First results show disinfection effect for E. coli for radiation with 460 nm and 405 nm. The radiation with 405 nm has a much higher effect to the E. coli population. After 5 hours of radiation with 405 nm a reduction of four logarithmic steps could be achieved, with a nearly logarithmic progression. With 460 nm radiation a reduction of one logarithmic step could be achieved.

For B. subtilis similar results for both wavelengths are expected and will be presented at the conference.

9540-31, Session PWed

Quantitatively index imaging of tissue slices with scanning focused refractive index microscopy

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The reflection-type scanning focused refractive-index microscopy (SFRIM) technique is used in the field of quantitatively study of biotissues. The de-coherence method is used to diminish the effect of the interference within the thin slices. SFRIM is a promising technique in the field of optical waveguides, photosensitive materials, photochromic materials, and biological index imaging.

9540-32, Session PWed

Epithelial cancers and photon migration: Monte Carlo simulations and diffuse reflectance measurements

Jerome Tubiana, Ecole Normale Supérieure (France) and MobileOCT (Israel); David Levitz, MobileOCT (Israel)

Detecting pre-cancer in epithelial tissues such as the cervix is still a challenging task in low-resources settings. In an effort to achieve low cost cervical cancer screening and diagnostic method for use in low resource settings, mobile colposcopes that use a smartphone as their engine have been developed. Designing image analysis software suited for this task requires proper modeling of light propagation from the abnormalities inside tissues to the camera of the smartphones. Different simulation methods have been developed in the past, by solving light diffusion equations, or running Monte Carlo simulations. Several algorithms exist for the latter, including MCML, MCXYZ and the recently developed MCX.

For imaging purpose, the observable parameter of interest is the reflectance profile of a tissue under some specific pattern of illumination and optical setup. Extensions of the MCX algorithm to simulate this observable under these conditions were developed. Although MCX is much faster than MCXYZ and MCML thanks to its GPU implementation, the reflectance profiles varied from one simulation to another with the computer hardware used, and that there are discrepancies between the 2 methods at large distances.

To validate this model, tissue phantom were imaged with the mobile colposcope, measuring the reflectance profiles under several illumination and optical settings for various homogeneous and heterogeneous tissues. The measured reflectance profiles were than compared with the simulated reflectance profiles. The results of this analysis will be presented.

9540-34, Session PWed

Diagnosis of uterine cervix cancer using Müller polarimetry: a comparison with histopathology

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Today around 275000 women a year in the world keep dying from the cancer of uterine cervix due to the difficulty to meet the logistic requirements of an organized screening in the developing world. Polarimetric imaging is a new promising technique with a tremendous potential for applications in biomedical diagnostics: it is sensitive to slight morphological changes in tissues, can provide wide field images for the screening and requires light sources such as a LED for example. This work intends to characterize the polarimetric response of the uterine cervix in its healthy and pathological states. An extensive series of ex vivo measurements is in progress in three different hospitals of Paris (Institut Gustave Roussy, Kremlin Bicêtre and Institut Mutualiste Montsouris) using an imaging Mueller polarimeter in backscattering configuration. The goal of this study is to evaluate the performances of polarimetric imaging technique in terms of sensitivity and specificity, while using the diagnosis provided by pathologists from histology slides as the "golden standard". We show that performances as high as 70% sensitivity and 60% specificity are achieved by optimizing a simple threshold on the scalar retardance values. These results confirm the very promising trends identified in our previous study on a few samples of conizations. Further analysis using additional available polarimetric parameters is in progress.

9540-35, Session PWed

Anticancer photodynamic therapy based on the use of a microsystem

Elzbieta Jastrzebska, Natalia Bulka, Kamil Zukowski, Michal Chudy, Zbigniew Brzozka, Artur Dybko, Warsaw Univ of Technology (Poland)

In this paper, we present the evaluation of photodynamic therapy (PDT) procedures in a microsystem. Two cell lines were used in the experiments, i.e. human lung carcinoma - A549 and normal human fetal lung fibroblast MRC 5. Three kinds of cell cultures (mono-, coculture and mixed culture) were performed simultaneously. The microsystem designed allow for the cell introduction and their culture. The chip was made of transparent materials (glass and poly(dimethylsiloxane) - PDMS). The microsystem consists of four microstructures with a network of microchannels and eight pairs of culture microchambers. The arrangement of the microchannels' network on the plate creates a V-shaped structure with three pairs of microchambers without connecting microchannel and five pairs of microchambers connected with additional microchannels (a width of 100 µm, a length from 200 µm to 1000 µm). At the end of each microstructure, the common microchamber was placed. There is also a concentration gradient generator (CGG) in the microsystem, which enabled to obtain four different concentrations of a photosensitizer in a single step. The microchannels' geometry ensured proper flow of cells' suspension or substances and allowed for simultaneous introducing of two cell lines Balb/3T3 and MRC 5 into separate microchambers. A high power LED was used to test photodynamic therapy effectiveness in the microsystem. Viability of the cell was tested with the use of solutions of calceine AM and propidium iodide, which were pumped into the microsystem.

9540-36, Session PWed

Second harmonic generation (SHG) and two-photon fluorescence (TPF) contrast imaging in biomaterial analysis

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Collagen hydrogels are natural biomaterials that comprise 3D networks of high water content and have viscoelastic properties and biocompatibility similar to native tissues. Consequently, these materials play an important role in tissue engineering and regenerative medicine for quite some time. Second harmonic generation (SHG) and two-photon fluorescence (TPF) contrasts transpire as valuable label-free spectroscopic probes for analysis of these biomaterials and this presentation will report the structural, mechanical and physicochemical parameters leading to the observed optical SHG and TPF effects in synthesized 3D collagen hydrogels. We will present results regarding understanding the dependency of collagen fiber formation on ion types, new results regarding strengthening of these biomaterials with a non-toxic chemical cross-linker genipin and polarization selection of collagen fibers' orientations.

9540-1, Session 1

Optical blood coagulation sensing in patients *(Invited Paper)*

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Impaired blood coagulation or coagulopathy, results from several conditions associated with severe trauma, surgery or illness, and can cause life-threatening bleeding or thrombotic disorders. Deficient coagulation can lead to 'hypocoagulable' states resulting in uncontrolled bleeding, which may cause severe anemia, shock and multiple organ failure. In other cases, coagulation defects may manifest as 'hypercoagulable' states, causing increased clotting that can result in potentially fatal complications such as deep vein thrombosis and pulmonary embolism. During surgery, trauma care and chronic disease management, clinicians often encounter the challenging task of maintaining a precarious balance between bleeding and coagulation. In order to achieve optimal outcome, the early detection of coagulation defects and coagulation monitoring during therapy is therefore essential. Unfortunately, the diagnosis and treatment monitoring of impaired coagulation is currently problematic because of the long turn-around time of standard laboratory tests. In my talk I will discuss a novel coagulation sensing technology termed Optical Thromboelastography (OTEG) that directly addresses the unmet clinical need to access a patient's coagulation status within minutes at the point of care. The technique involves placing a drop of blood in a small cartridge. A laser source illuminates the blood sample and a CMOS camera images laser speckle patterns reflected from the sample over time. By analyzing laser speckle intensity fluctuations to measure blood viscoelastic properties during coagulation, we can recover information about multiple coagulation metrics related to clotting factors, platelet function and fibrinolysis that are critical to hemostasis. Our recent results of OTEG coagulation testing in 200 patients have shown close correspondence with standard methods. Thus, by enabling the comprehensive evaluation of blood coagulation status in a rapid manner, OTEG may advance clinical capability to identify patients at high risk for hemorrhage or thrombosis events, tailor and monitor treatment based on individual coagulation deficits and improve blood resource management.

9540-2, Session 1

Organic optoelectronic sensors for biomedical applications

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Organic semiconductors are important optoelectronic materials that are now of growing interest for sensing applications. They offer the potential for compact, light and flexible sensors that are simple to fabricate. Here we will present recent progress using organic light-emitting diodes and photodiodes for biophotonic applications with two examples. In the first example we will discuss a haemodynamic sensor using organic LEDs and photodiodes to measure changes in tissue oxygenation. The results of tissue oxygenation during a forearm ischemia experiment will be presented. In this experiment a blood pressure cuff was used to restrict blood flow, and the resulting changes in oxygenation of forearm muscles were measured. In the second example we have made a flexible organic optoelectronic muscle contraction sensor that can distinguish between isotonic and isometric types of muscle contraction. The sensor consists of a light source and four photodetectors made from semiconducting polymers arranged and assembled as a flexible bandage. We will also show the feasibility of this sensor for prosthetic actuation by actuating a robotic arm using the signal detected from a volunteer's real arm. These results provide another interesting direction for organic optoelectronics, and the possibility of measuring a range of important biomedical processes. These findings would be of significant interest across the biophotonics, clinical medicine and plastic electronics community.

9540-4, Session 1

Near infrared surface plasmon resonance imaging system

Aurore Olivéro, Alexandra Sereda, Lab. Charles Fabry (France) and HORIBA Scientific (France); Julien Moreau, Michael T. Canva, Lab. Charles Fabry (France)

Surface plasmon resonance imaging (SPRI) systems exhibits the intrinsic asset of transducing binding events in real time without requiring labelling of biomolecules, inversely to fluorescence detectors. Few publications are reporting SPR measurements in the Near Infrared (NIR), while we demonstrate that the capabilities of SPRI detection could be push forward at this wavelength range. The possibilities offered by NIR SPRI such as high sensitivity and large penetration depth would benefit from the study of membrane phenomena or the thorough characterization of the plasmonic behavior of emergent nanostructures. This work depicts the promises and the restraints of NIR SPRI sensing.

The setup relies on the conventional Kretschmann-Raether configuration using a prism covered by a thin layer of gold, whose 40 nm thickness is optimized for NIR. Reflectivity images in the TM polarization are taken with an InGaAs camera, sensitive between 750 nm and 1600 nm.

A figure of merit (FOM) is defined to evaluate the sensitivity, the accuracy and the quality of coupling of the instrument. We show that this FOM is improved by 50% above 1100 nm compared to 750 nm and is mainly limited by the angular divergence of the illuminating beam. However, the reduction of the angular divergence using a smaller fiber core diameter strongly increase speckle noise and degrade the signal-to-noise ratio as shown in this study.

9540-5, Session 1

Array sensor: plasmonic improved optical resonance methods and instrument for biomedical diagnostics

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The most widespread sensor schemes based on optical resonance of whispering gallery modes applied for monitoring biological agents are based on discrete microspheres, cylinders, disks, microtoroids, planar ring resonators on a silicon substrate, fiber ring resonators, photon crystals, and the mostly recent - plasmon - photon structures, sensing separate molecules and even viruses. Neural network technologies of experimental data processing allow on parameters of WGM optical resonance biological agents can be identified.

The next step to improve sensitivity of biomedical sensor is microcavity arrays. Developed array are designed as a series of columns with microspheres. These columns are separated from each other by polymer boxes or trapezes which are connected with each other at the ends. An array construction process consists of: choose of a material for structure; preparation of 3D model of needed construction; erection of developed construction on coverslip; drying to achieve a strong connection between coverslip and polymer construction; fixing microspheres inside channels mechanically or with adhesive by spin coating procedure. Arrays with different functionalized microsphere rows were also constructed.

Different classes of agents: Cefatoxim, Ethanol, Gentamicin, Glucose, Penicillin, Albumin, and Fibronectin in de-ionized water solution have been measured. Results for singe microsphere and array were compared using ethanol solutions and demonstrated difference in results between schemes less than 1%.

Micro cavity array, plasmon improvement and neuron network based data processing can improve a diagnostic tools for different biological molecules as well as in different experimental contexts: cellular and tissue imaging, blood components monitoring, drug monitoring and targeted delivery, remote control of cancer etc.

9540-6, Session 2

Non-mydriatic video ophthalmoscope to measure fast temporal changes of the human retina *(Invited Paper)*

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The analysis of fast temporal changes of the human retina is useful to get insight to normal physiological behavior and to detect pathological deviations. We developed a small, lightweight non-mydriatic video ophthalmoscope that allows taking video sequences of the human retina with at least 25 frames per second without dilating the pupil. The instrument is designed to image the region around the optic nerve head (ONH) with 20 x 15 degree? field of view. A LED (575 nm) is used for illumination and a CCD camera (640 x 480 pixels) for image acquisition. The entire instrument (LED and CCD camera) is powered via the USB interface of the acquisition computer or notebook. Acquired video sequences are registered offline using two-stage registration process. The large movements are firstly corrected via Fourier phase correlation approach. Then Lucas-Kanade blood vessel tracking is utilized for fine translation and rotation compensation, which allows sub-pixel resolution. The instrument is used to measure the time course of cardiac cycle induced changes in fundus reflection correlating with the changing blood volume in the ONH during systole and diastole. Another useful application is the measurement of eye movements during fixation tasks. Eye movements deltaX[t], deltaY[t] can be derived from image registration results with high temporal and spatial resolution: Due to the sub pixel resolution of the image registration eye movements can be assessed with a resolution of better than 3 arcmin.

9540-7, Session 2

Laser speckle contrast analysis for pulse waveform extraction

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The present paper shows a method for pulse waveform extraction using laser speckle contrast analysis. An experimental apparatus was constructed, using a green coherent light source and a digital video camera to record speckle patterns emitted from the radial artery. The speckle data were analysed by computing the speckle pattern contrast on a sequence of video frames. The speckle pulse wave signal was compared with a photoplethysmographic signal both in shape and dominant frequency. Thirty data-sets were acquired in 10 individuals. Subjects heart rate was identified with a root mean square error of 1.3 beats per minute. Signals similarity was evaluated using spectral coherence where a mean coherence of 0.63 was achieved. Speckle contrast analysis is a newly commercialized technique to monitor microvascular blood flow. These results demonstrate the ability of the same technique to extract pulse waveform information. The inclusion of this feature in the current speckle devices is only associated with a slightly change in the signal processing techniques and video acquisition parameters.

9540-8, Session 2

Laser speckle analysis synchronised with cardiac cycle

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Laser speckle imaging is combined with correlation analysis to investigate slow tissue dynamics. We developed an approach to detect blood flow and brain tissue dynamics during systolic phase of cardiac cycle, which allows to reliably monitor heart rate. By synchronising speckle analysis with the cardiac pulse we quantify correlation of speckle patterns from the same phases of the cardiac cycle. Sequential heartbeats are compared in terms of speckle realisation by averaging all phases of a cycle. Slow evolution of the heartbeats correlation in time reveals the dynamics of the tissue matrix on the time scale of at least one cardiac cycle separately from the blood flow.

As the first test application, cerebral haemodynamic response to whisker stimulation of spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto rats is compared. In both groups we observe a pronounced signal indicating tissue dynamics during vasodilation and vasoconstriction phases after functional activation. However, for SHR group tissue response is lower during both phases. The maximal level of blood flow characterised with speckle contrast is also lower for SHR group, which is in agreement with previous observations of hypertension-induced impairments.

9540-9, Session 2

Spatially offset Raman spectroscopy for photon migration investigations in long bone

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Raman Spectroscopy has become an important technique for assessing the composition of excised sections of bone, and is currently being developed as an in vivo tool for detecting bone disease using spatial offset Raman spectroscopy (SORS). The sampling volume of the Raman technique (and thus the amount of bone material that is interrogated by SORS) depends on the nature of the photon scattering in the probed tissue. Bone is a complex hierarchical material and to date little is known regarding its diffuse scattering properties which are important for the development and optimization of SORS as a diagnostic tool for characterizing bone disease in vivo. SORS measurements at 830 nm excitation wavelength are taken on stratified samples to determine the depth from which the Raman signal originates in bone tissue. Measurements are made using a 0.38 mm thin Teflon slice inserted in between layers of stacked 0.60 mm thin equine bone slices. The results show that larger SORS offsets were able to predominantly probe deeper layers within the sample. It could further be demonstrated that different Raman spectral signatures can be retrieved through 3.5 mm of overlying bone material with a 6 mm offset. Comparing the stack of bone slices with and without underlying bone tissue below the Teflon slice showed that thin sections of bone can lose appreciable numbers of photons through the unilluminated back surface. These findings have direct impact concerning potential diagnostic medical applications; for instance in the detection of bone tumor margins or areas of infected bone.

1. INTRODUCTION

Raman spectroscopy can provide chemically specific information and is therefore widely applied as an analytical tool in a number of areas. However, in its conventional form confocal Raman microscopy restricts the sample volume probed to depths of, typically, around 100-200 μm. The advent of spatially offset Raman spectroscopy (SORS) enables to breach these limits and has proven successful in numerous applications1. The SORS technique is based on a spatial separation of the point of sample illumination by the excitation laser beam and the point at which the Raman scattered photons are collected. A small spatial offset will favor the detection of photons emerging from near the surface while larger offsets increase the chances of detecting photons from deeper layers. Near infrared excitation wavelengths facilitate higher penetration depths into the probed sample, in particular human tissue, as this spectral region exhibits low light absorption for the vast majority of (biological) samples.

The ability of SORS to extract chemical information from deep inside the sample (up to several mm) has paved the way for numerous applications in non-destructive sub-surface analysis2. Especially in the biomedical field SORS is rapidly becoming a valuable tool for tissue analysis3. Application areas include the identification of breast calcifications4 and the detection of tumors5. Intensive research has also been performed on transcutaneous bone characterization to assess material composition for potential bone disease diagnosis6,7. Despite the success of SORS, a key issue remains. It is not entirely clear to what depth information is extracted for a certain spatial offset and how light scattering properties of tissues influence the SORS process at the NIR wavelengths. This paper aims to address this by carrying out investigations to gain information about photon migration inside bone.

2. MATERIALS AND METHODS

The Raman investigations were performed using a custom built Raman system (Cobalt Light Systems Ltd., Oxfordshire, UK) delivering 300 mW of 830 nm radiation to the sample surface. The spatial offset was achieved by means of annular laser illumination zones with selectable radius and a fixed signal collection zone in the center of the illumination ring1. The spatial offsets between excitation and collection areas ranged from 0 mm to 6 mm. The scattered Raman radiation from the collection zone was focused into a low-loss Optran WF fiber bundle (CeramOptec, East Longmeadow, MA) and transferred into a spectrograph (Raman Explorer, Headwall, MA) equipped with a CCD detector (Andor iDus 420 BR-DD; Andor, Belfast, Northern Ireland).

Tissue samples comprised a section cut from the diaphysis of a horse metacarpal, 4 cm in length. By means of a bandsaw 6 slices 0.60 mm thick were cut along the long axis of the bone section. The top section was not used in the measurements due to its small size and curvature. For the photon migration studies the bone slices were stacked together in the same order they originally had within the bone before cutting, forming a stack of 4 bone-bone interfaces between layers. For SORS measurements a slice of Teflon (polytetrafluoroethylene) with a thickness of 0.38 mm was inserted between individual bone layers within the stack, and below the stack. For each Teflon depth position three lateral positions were probed and at each spot 200 spectra with an integration time of 0.1 s were collected. To investigate a possible influence of an additional bone volume below the slices experiments were repeated with the remaining bone sections below the stack of bone slices. Raman intensity ratios were determined considering the most prominent bands of Teflon at 733 cm-1 as well as bone at 961 cm-1.

3. RESULTS AND DISCUSSION

Teflon was selected as it has a strong single band at 733 cm-1 which is in the vicinity but completely resolved from the strong bone phosphate signal at 961 cm-1. The material thickness of 0.38 mm was selected to achieve band intensity in the same order of magnitude as the bone phosphate band. Figure 1 displays Raman spectra for SORS offsets of 0 mm, 2 mm, 4 mm, and 6 mm when the Teflon layer is located below 3 mm of horse metacarpal diaphysis and with the remainder of the bone placed below. For clarity the broad background due to fluorescence interference was removed by subtracting a 5th order polynomial from each spectrum8. When the Teflon layer was measured through 3 mm of bone material the prominent Teflon band at 733 cm-1 was still observable in the spectra. Although the absolute intensities were slightly higher for small spatial offsets the Teflon to bone ratio increased from about 1.7 % for zero offset to 7.7 % for a SORS offset of 6 mm.

When investigating the Teflon below the 5 bone slices, without solid bone material underneath, the same trend can be observed albeit with the Teflon to bone ratios reduced by up to 50 %. This dramatic reduction is ascribed to the missing bone material underneath. Without a diffusely scattering medium underneath photons passing through the Teflon slice retain their propagation direction and therefore cannot contribute to the Raman signal intensity. In contrast, with the bone underneath the Teflon laser photons (as well as) which have already travelled through the thin Teflon sample have a certain probability to reverse their propagation direction by means of multiple diffuse scattering inside the underlying bone. This diffuse scattering can partly be regarded as a “photon mirror” redirecting at least some photons back towards the overlaying Teflon. Hence, these additional laser photons can undergo Raman scattering inside the Teflon hereby increasing the Raman signal intensity.

The Teflon to bone intensity ratios were calculated for all investigated SORS offsets and selected data are displayed in Figure 2 as a function of the overlaying bone thickness above the Teflon slice. The decrease in the intensity ratio is much more pronounced for small SORS offsets than for large offsets. The overall reduction going from 0.6 mm depth to 3 mm depth amounts to 94 % and 67 % for SORS offsets of 0 mm and 6 mm, respectively. This can be explained as smaller offsets will give a higher ratio for smaller depths as the Teflon volume and the probed volume have a maximum overlap in that case. Moving the Teflon deeper inside the bone will reduce the spatial overlap and hence the Teflon to bone ratio resulting in the observed dramatic decrease. In contrast, using a 6 mm spatial offset, i.e. probing greater depths, results in a small ratio when the Teflon sample is located at a small depth. Here, increasing the Teflon depth inside the bone will consequently increase the overlap with the sampled volume leading to larger values for the Teflon to bone ratio. However, in all cases this effect is accompanied by an overall signal decrease with increasing depth leading to the observed curve shapes.

From these data penetration depths for a given SORS offset were estimated assuming a Teflon-to-bone threshold ratio of 0.05 (dashed horizontal line in Figure 2). Even for zero spatial offset the depths of about 2 mm can be probed in the applied illumination and collection configuration. This is particularly advantageous when investigating biological samples as probing a larger volume helps to reduce inhomogeneities. Penetration depths of up to 3.5 mm can be realized using 6 mm spatial offset. Considering the bone slices only, i.e. without a diffusely scattering medium underneath penetration depths are generally lower. The reduction was ca. 10 % for small offsets and increased to 19 % for large SORS offsets due to the missing “bone mirror” effect. These findings have a direct impact for medical diagnostics using SORS, e.g. enabling the non-invasive detection of spectral changes caused by cancer or infection deep inside the bone.

9540-11, Session 3

Optical clearing of articular cartilage: a comparison of clearing agents *(Invited Paper)*

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Optical clearing technique was applied for the first time to the problem of OCT imaging of articular cartilage and subchondral bone. We show that optical clearing significantly enhances visualization of articular cartilage and cartilage-bone interface. The effect of different clearing agents was analyzed.

For the clearing, iohexol solution and propylene glycol (PG) were used as received. Clearing was performed in vitro at room temperature by immersion method.

Cylindrical osteochondral samples (d=4.8mm) were drilled from bovine lateral femur and stored in phosphate-buffered saline at -20°C until clearing.

Monitoring of clearing process was performed using high-speed spectral-domain OCT system providing axial resolution of 5.8?m at 930nm. Total duration of experiment was 90-100min to ensure saturation of clearing.

We have shown that iohexol solution and PG are capable to optically clear articular cartilage enabling reliable characterization of cartilage-bone interface with OCT. Being a low osmolarity agent, iohexol provides minimal changes to the thickness of cartilage sample. Clearing saturation time for the cartilage sample with the thickness of 0.9 mm measured with OCT is of 50 min. However, less than 15 min is enough to reliably detect the rear cartilage boundary. Alternatively, PG significantly (60%) reduces the cartilage thickness enabling better visualization of subchondral bone. It was observed that PG has higher clearing rate. The clearing saturation time is of 30 min, however less than 5 min is enough to detect cartilage-bone interface.

We conclude that iohexol solution is superior for OCT imaging of cartilage and cartilage-bone interface, while PG suits better for subhondral bone visualization.

9540-12, Session 3

Enhancement of upconversion deep-tissue imaging using optical clearing

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Intrinsic scattering and absorption of biological tissues pose a problem for diagnostic imaging. Spectral position of the so-called “diagnostic window” (650-1100 nm) is advantageous to offset these drawbacks. Contrast enhancement of tissue-embedded inhomogeneities (e.g. tumors) can be performed by administration of contrast agents such as fluorophores. However, utilizing the same spectral window for both fluorophore excitation and detection is not trivial.

In this paper, we use novel Tm-doped upconversion phosphors overcoming the mentioned challenges (excitation wavelength: 980 nm, detection wavelength: 800 nm) and glycerol as an optical clearing agent to enhance imaging from under 6-mm-thick porcine muscle tissue samples. We show that improvement of luminescent label visualization is caused by transforming of the diffuse label-emitted light into the direct component. This results in 5-fold increase in visibility (ratio of the sum and difference of the maximal and minimal intensity) of the label and 20-fold increase in maximal signal intensity thus making the combination of the phosphors and optical clearing promising for precise detection of tissue-embedded labelled inhomogeneities.

9540-14, Session 3

Polarization-sensitive autofluorescence spectroscopy of cutaneous tissues

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1. Introduction

Fluorescence polarization measurements could be applied to estimate various parameters of the fluorophore environment, and they have the potential role to discriminate between normal and malignant tissues for the needs of biomedical diagnostics. At polarized light excitation, the emission from a fluorophore in a non-scattering media becomes depolarized because of the random orientation of the fluorophore molecules and the angular displacement between the absorption and emission dipoles of the molecules. [1] The level of light depolarization in the tissues depends from the number of scattering steps of the excitation and fluorescence photons in their way to and from the given fluorophore in the sample investigated. In this way both – the excitation and the emission are depolarized in strongly scattering media, such as human skin. Nevertheless, the normal and diseased tissues could have different scattering coefficients, and respectively – different depolarization levels could be obtained. In malignant skin the extracellular matrix is changed and the scattering due to the structural proteins, such as elastin and collagen is different from normal to diseased tissue, and these changes give diagnostically important information about the tissue condition. [1, 2]

Many investigators work on polarization fluorescence applications for skin cancer detection. Several groups already reported newly developed imaging systems for polarization-sensitive fluorescence for tissue analysis with very promising results. [3-6]

Our group has significant experience on autofluorescence investigations of skin tumours and polarization technique could allow us to improve the diagnostic accuracy achieved up to now (93 %) for such severe pathologies, as malignant melanoma lesions. [7-9] We are interested to analyze the feasibility of fluorescence polarization technique for the clinical needs of primary diagnosis of skin neoplasia, without adding of contrast agents, due to the patient convenience.

2. Materials and methods

In our study we measured excitation – emission matrices (EEMs) for normal human skin in vivo, and ex vivo non-melanoma cutaneous tumours. The tumours are received after surgical excision during standard cancer treatment procedures. Ethical approval for our investigations of human skin was received from the Ethical Committee of University Hospital “Queen Jiovanna-ISUL” – Sofia, in the frames of research project DMU-03-46.2011.

We used spectrofluorimeter FluoroLog 3 (HORIBA Jobin Yvon, France) with fiber-optic module - F-3000 with fiber optic probe - 1950-1M that allows to measure the fluorescent properties of samples which cannot be put in a standard cuvette. Using this system could perform measurements of the excitation and fluorescence spectra, excitation-emission matrix and time-resolved regime of the fluorescence signal of biological tissues including in vivo. Three different situations were evaluated and corresponding excitation-emission matrices were developed – with parallel and perpendicular positions for linear polarizer (for the excitation) and analyzer (for the emission), and without polarization of excitation and fluorescence light detected from the forearm skin surface.

Autofluorescence spectroscopy measurements, using different excitation wavelengths for the needs of EEM development of fluorescence data, were carried out. Excitation applied was in 280-440 nm region. The fluorescence emission was measured between 300 nm and 650 nm for all three situations of polarizer positions in the spectrofluorimeter set-up. Analysis of the spectra on intensity and spectral shape changes for the three variants of polarization, as well as evaluation on the principal fluorescent maxima observed of the polarization P are carried out.

Ratio of the maxima at 450 and 400 nm, related to the emission of co-enzime NADH and collagen fluorescence, is also evaluated. We found that this ratio could be used as indicator of structural changes in the human

skin in vivo and could be useful indicator for the forthcoming needs of development of a polarization fluorescence spectroscopy modality for cancerous skin diagnosis.

3. Results and discussion

The fluorescence spectra obtained reveal differences in spectral intensity, related to general attenuation, due to filtering effects of used polarizer/analyzer couple. Significant spectral shape changes were also observed for the complex autofluorescence signal detected, which correlated with collagen and protein cross-links fluorescence, that could be addressed to the tissue extracellular matrix and general condition of the skin investigated.

The EEM matrices are developed for parallel and perpendicular positions of the polarizer/analyzer couple. When polarized excitation is applied the fluorescence signal is also polarized. [1, 2] The depolarization depends from the number of multiple scattering processes in the tissue during the light transport – of the excitation and of the induced fluorescence. In biological tissues, such as skin, where strong scattering has place the depolarization effects are very strongly revealed. However, non-scattered excitation and fluorescence part could be evaluated in a comparison of the fluorescence signals detected in parallel and perpendicular positions of the polarizer-analyzer couple. Decreased fluorescence intensity is related to the losses of non-depolarized fluorescence component coming from the fluorophore emitting in the given spectral range.

Fluorescence maxima observed in our measurements are addressed according our knowledge and the previous reports of different investigators, working in the field of autofluorescence detection of skin tissues. In general, the fluorescence intensities depend from the skin photo type of the person investigated. [10]

Significant differences in the autofluorescence signals obtained for the cross-polarized case were observed, as the maximum at 400 nm, corresponding to the collagen autofluorescence, depends strongly from the ages of the person investigated. The fluorescence signal at 400 nm, obtained from older persons after excitation at 280-320 nm region, is higher by intensity that for the younger ones. In the longer wavelength spectral region, for excitation range longer than 360 nm, when the co-enzimes, such as NADH and flavins are excited to fluoresce, no significant spectral shape changes for the parallel and perpendicular positions of polarizer/analyzer couple were observed. Only the fluorescence maxima related to structural proteins and their cross links reveal spectral shape differences in that comparison.

Some of the biologically important fluorophores are anisotropic by their nature, due to the specific structure of chirality. Typical example of scattering and fluorescence anisotropic molecule in the human skin is its major structural compound – collagen, due to its fiber structure. The structure of collagen fibers could be responsible for the higher degree of polarization effects observed for the fluorescence signal obtained from this fluorophore as well.

Other researchers reported that all compounds - collagen, elastin, co-enzymes NADH and flavins contribute to the polarized fluorescence spectra and the spectra received at a longer wavelength of 460 nm. The contribution of NADH dominates with excitation at 340 nm and different forms of flavins dominate with excitation at 460 nm. [11]

The slight spectral shift of the maxima between the parallel and cross-polarized fluorescence spectra was observed for thick tissue layers (≈ 2mm), or as in our case of in vivo study, that could be associated to the absorption properties of the tissue investigated. In these measurements, the most significant alterations observed between parallel and cross-polarized fluorescence were for the short wavelength spectral region of excitation lambda applied – 280-360 nm, where the structural proteins excitation spectra have place.

When the polarized fluorescence signals are compared, the polarization ratio P = (I? – I?)/(I? + I?) could be calculated for the parallel and cross-polarized signals detected from the different patients. The polarization is decreased with increase of the fluorescence wavelength for the patients below 40 years old and for the higher ages the polarization is increased with the wavelength of the fluorescence detected. These relations were observed for the different excitation wavelengths applied. Polarization is calculated for the principal fluorescence maxima observed, addressed as follow: tryptophan - 360 nm, collagen- 400 nm, NADH – 450 nm and collagen cross-links – 490 nm.

4. Conclusions

Skin cancer diagnostics could be developed based on the difference in metabolism and structure for normal and diseased tissues. The fluorescence anisotropy measurement of skin shows its high sensitivity to the structural and morphological changes, related to aging of the skin. The correlation between such small changes, related to the extracellular matrix decrease of structure and integrity could be more pronounced in the case of skin lesions, where such integrity and structural matrix are partially demolished due to the lesion growth. The polarization fluorescence measurements may provide a noninvasive method for cancer detection. In order to further demonstrate the feasibility of this method, studies need to be carried out to measure the fluorescence anisotropy of skin benign and malignant lesions. Our preliminary investigations on measurements of healthy human skin in vivo allow detecting the correlation between polarization vs. wavelength of the fluorescence detected and age of the skin samples investigated.

5. Acknowledgements

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9540-15, Session 3

Experimental analysis of bruises in human volunteers using radiometric depth profiling and diffuse reflectance spectroscopy

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Time evolution of traumatic bruises is governed by mass diffusion of extravasated hemoglobin and its biochemical decomposition into bilirubin as a result of the inflammatory response. We recently showed that extravasated hemoglobin dynamics (i.e., hemoglobin mass diffusion and biochemical decomposition) can be successfully monitored in vivo using pulsed photothermal radiometry (PPTR).

In our clinical study of incidental bruises we combine the above approach with diffuse reflectance spectroscopy (DRS). The technique provides information in a wide range of visible wavelengths (400-850 nm) and thus offers an additional insight into dynamics of the hemoglobin degradation products (biliverdin, bilirubin). A simple mathematical model of the bruise evolution processes and numerical simulations of reflection spectrum and laser energy deposition in bruised skin allows us to predict the corresponding PPTR signals and DRS spectra. We perform automated multi-dimensional parallel fitting of PPTR and DRS measurements with simulated data. This enables us to assess the key structural properties of the affected site as well as the parameters describing the temporal evolution of the bruise.

Our combining of two complementary techniques improves the robustness and accuracy of the inverse analysis and enables a comprehensive analysis of bruise evolution dynamics. Analysis of PPTR and DRS measurements at various times post injury provides an insight into temporal variations of bruise evolution parameters over the course of bruise resolution.

The obtained results advance our understanding of the bruise evolution process and present an important step toward development of an objective technique for age determination in forensic medicine.

9540-24, Session 3

Comparative studies of fluorescence spectroscopy and optical coherencetomography for nonmelanoma skin cancer diagnosis

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Skin cancers, both melanoma and non melanoma skin cancers (NMSC), such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are still the most common of all human cancers and a growing health problem in most countries. Although ionizing radiation based medical imaging continues to play an essential role in the diagnosis of cancer in several medical disciplines, in dermatology the discrimination of cancerous from healthy skin tissue is rather difficult with the conventional CT, PET or MRI techniques. The common routine diagnostic procedure for BCC and SCC is still the visual inspection, followed by skin biopsy and subsequent histopathology examination that takes days or weeks to produce a result. However, as early diagnosis of cancer is associated with improved prognosis, several non-ionizing radiation based, optical diagnostic, methods were developed to enable earlier detection of skin cancer, as laser induced fluorescence spectroscopy, diffuse reflectance spectroscopy, confocal microscopy, and optical coherence tomography. These techniques have the potential to provide diagnosis of malignant and precancerous skin tissue, reducing the need for biopsy and the potential risk of tumor growth and/or metastasis, in some cases, because of time-extended procedures for histopathology based diagnosis. Our previous investigations show that the laser induced fluorescence spectroscopy (LIF) is a useful tool to differentiate healthy from malignant (e.g. basal cell carcinoma - BCC, squamous cell carcinoma - SCC) skin tissue, eliminating the invasiveness of the biopsy and the cost and delay from histopathology procedures. Furthermore, our related research prospects are benchmarking fluorescence spectroscopy with other biophotonic techniques, e.g. optical coherence tomography (OCT).

OCT has been previously used in ophthalmology and cardiology among other disciplines. In dermatology, OCT has been used in various pre-clinical studies and, in the latest years, it implements in medical practice. The optical coherence tomography is based on interferometry, where low coherent infrared light (e.g. at ?=1300 nm) is projected into the tissue. The reflection of light is processed, and the sum of the different light refractions produces an image of cross-section images of tissue, analogue to ultrasound, but at a significantly higher resolution, in the range of <10?m. OCT is able to visualize the epidermis, the upper dermis of skin, and blood vessels. OCT images have been shown to be useful in non-invasive monitoring and classification of non melanoma skin tissues. The optical coherence tomography provides also an important tool for optical, noninvasive, delineation of the pathological area borders in both surgical interventions and photodynamic therapy of skin lesions, which also contributes to minimization of psychological impact of malformations / scarring that causes the diagnostic biopsy and surgical removal of skin lesions.

In this work, comparative studies of LIF and OCT results for non-melanoma skin cancer ex vivo diagnosis will be presented. The use of OCT in monitoring any change in the upper dermis and in deeper layers of skin tissue and the potential relation between the skin tissue optical properties in the relevant wavelengths for the LIF and OCT signals will also be discussed. Some representative results are shown in figures 1 and 2. The ex vivo samples, as that of figure 1a, were analyzed histopathologically, after LIF spectra acquisition, classified as normal, BCC or other skin lesion type.

Imaging with OCT could clearly differentiate the epidermis from the upper dermis, so that selected skin areas could be accurately measured and suspected skin areas are able to be revealed. LIF signals exhibited low intensity signals and a characteristic spectral shift in malignant skin tissues, while there were diagnostic difficulties in abnormalities below epidermis skin layer in wavelengths with low skin penetration (e.g. for 3rd harmonic Nd:YAG laser excitation at ?=355 nm). Specificity and sensitivity of LIF and OCT methods are compared to conventional histology and/or visual inspection analysis.

The object of this comparative study is to establish the possibilities of a relatively portable system that could combine a laser induced skin autofluorescence measurements device and an OCT to differentiate malignant from nonmalignant skin lesions. The optimization of the data analysis and the potential use of the biophotonic emerging imaging techniques for clinical applications will be discussed. For that, special care will be addressed on considering the safe levels of non-coherent and coherent (laser) radiation used for skin cancer diagnostic purposes, by consulting the relevant European and International guidelines on optical radiation exposure limits for both patients and personnel.

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9540-16, Session 4

Optical measurement of temperature in biological cells under infrared laser light exposure (?=800 nm) *(Invited Paper)*

David Moreau, Claire Lefort, Philippe Leveque, Rodney O’Connor, XLIM Institut de Recherche (France)

Biological studies and infrared laser light are increasingly combined with multiple applications such as the neural stimulation. In spite of the recent works proving the efficiency of the infrared laser light for neural stimulation, the nature of the interaction between the infrared laser light and the neurons is still unclear. However, two prominent hypotheses were proposed based on a photothermal or on an electrostatic mechanism. In the first case, infrared laser light would induce an increase in temperature that activates the temperature sensitive Transient Receptor Potential Vanilloid (TRPV) channels. The opening of these channels induces an influx of cations and depolarizes the neuron. The second hypothesis suggests that infrared pulses are absorbed by water, producing a rapid local increase in temperature. This rapid heating reversibly alters the electrical capacitance of the plasma membrane, that depolarizes the neuron. In both cases, the temperature modifications play a crucial role in the interaction between infrared laser light and neurons. Therefore, we present here an all-optical thermometer using the dye Rhodamine B, with a setup constructed around a wide field fluorescent microscope. The main particularity of this dye is that its fluorescence quantum yield depends on the temperature: when the temperature increases, the level of fluorescence decreases. First, the experimental setup is presented and the calibration procedure is detailed. Then, the use of RhB as a fluorescent dye to measure temperature is demonstrated in cultured human glioma cells in response to infrared laser light stimulation.

9540-17, Session 4

Laser guidance-based multiple beam irregularly-shaped-cell patterning system

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Laser guidance-based cell patterning uses optical force to confine a cell in the axis of a laser beam and propel it along the beam axis to the substrate. Based on a similar mechanism, laser tweezer microscopes have been developed to trap individual cells. Since laser tweezers use a highly focused laser beam, the working distance is very short (< 300 μm). In addition, the acting range of optical force of a strongly focused laser beam is much smaller than that of the weakly focused one that is used in laser guidance. The limited trapping range in laser tweezers does not allow the trapped cell to follow the laser beam during high-speed 3D navigation in a large volume of culture media with strong convectional flow.

Our laser cell-patterning system uses the principles of laser guidance generated by a weakly focused laser beam, so the working distance can be greater than 30 mm, allowing precise placement of individual cells at a specific location in a typical culture dish.

Here, we report the development of a multiple-beam guidance system. A spatial light modulator is used to generate multiple beams according to cell shape and size to achieve real-time laser patterning of large, irregular-shape cells. The developed system has been successfully used to pattern adult cardiomyocytes, which shows that the technique has the potential to advance cell biology with the ability to manipulate large irregular-shape cells.

9540-18, Session 4

Erythrocyte-derived optical nano-vesicles as theranostic agents

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We have engineered nano-vesicles, derived from erythrocytes, which can be doped with various near infrared (NIR) organic chromophores, including the FDA-approved indocyanine green (ICG). We refer to these vesicles as NIR erythrocyte-mimicking transducers (NETS) since in response to NIR photo-excitation they can generate heat or emit fluorescent light. Using biochemical methods based on reduction amination, we can functionalize the surface of NET with antibodies to target specific biomolecules. We will present results that demonstrate the effectiveness of NETs in photothermal destruction of human cells as well as the capability of NETs in targeted imaging of cancer cells that over-express the human epidermal growth factor receptor-2 (HER2).

9540-19, Session 4

Possibilities of holographic techniques in laser scanning microscopy

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Laser scanning microscopy obtains an image by point-by-point acquest, digitizing and inlaying a mosaic picture. Laser scanning confocal microscopy improves longitudinal resolution. Moreover some scanning techniques with processing by appropriate software allows to overcome diffraction limit in transverse direction. As for longitudinal size measurements different interferometric techniques ensure it on the level of a few nanometers, one of them is digital holographic interferometry. But now all of them have realized only for widefield microscopy, whereas scanning confocal regime give some grate opportunities in functional imaging of living cells. In order to enrich capabilities of laser scanning microscope by digital hologram recording we have included to microscope construction reference beam mirror that formes reference wave. Reference wave interfere with reflected from specimen object wave and resulting intensity of light is transformed to one pixel of the discrete hologram at each scanning step. In this way whole scan stack create digital hologram. We call this technique as holographic scanning microscopy (HSM). Features of object illumination and light propagation leads to different hologram structure expression as compared to wide-field holographic microscopy (WFHM). So HSM-hologram necessitate special reconstructing processing. Moreover integral phase incursion of wave front in HSM differs from WFHM because only light from objective focal volume gives significant contribution to detector lighting. This feature gives opportunity to obtain tomographic data by Z-scan series and to separate contribution of refractive index distribution and path length inside sample. Reconstruction algorithm should be specific again. Additional advantage is combining of confocal microscopy with phase microscopy.

9540-20, Session 4

Digital holography for recovering 3D shape of red blood cells

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Three-dimensional reconstruction and biovolume estimation of cells are important information in different fields, from bio-technologies to medicine for diagnostics purposes. Recently, a simple holographic approach based on Digital Holography (DH) and shape from silhouette (SFS) algorithm, has been demonstrated for accurate calculation of cells biovolume and displaying their 3D shapes. Such approach has been adopted in combination with holographic optical tweezers and successfully applied to cells with convex shape. Unfortunately, the method failed in case of specimen with concave surfaces. Here, we propose an effective approach to achieve correct 3D shape measurement that can be extended in case of cells having concave surfaces, thus overcoming the limit of the previous technique. We prove the new procedure for healthy red blood cells (RBCs) (i.e., discocytes) having a concave surface in their central region. The method can be also useful to classify, in terms of morphology, different kinds of RBCs. Moreover, for the first time RBCs are adopted as micro-biolenses, and their shape is recovered simply by analyzing their focalization spots. This novel idea could be of great help for diagnostic purposes.

9540-21, Session 4

Full 3D morphology of diatoms flowing in a microfluidic channel by digital holographic microscopy

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A key issue in optical metrology is the estimation of the three-dimensional shape of living cells. This estimation provides some important parameters, such as biovolume and biomass, which are useful in several research areas from taxonomy to the study of ecosystem health. The most common and well-established methods use a white light microscope to measure the linear dimensions of each cell in the ensemble. The length, width and height are used to calculate the biovolume and surface area of the cell by assimilating them into standardized geometric models. Despite these techniques being well known and used to classify biological specimens, they are invasive and contact-based which do not provide a precise measure. Recently, a new method for the measurement of a microscopic object’s volume by digital holography (DH) has been proposed. The method is all-optical and non-invasive, being based on the combination of optical tweezers (OT) and digital holographic set-ups. The key concept is the rotation of the sample under particular conditions and the simultaneous acquisition of holographic images from different angles, without touching or perturbing the sample. In this paper, we further improved the method which is tested to calculate the 3D shape and biovolume of several species of diatoms. The improvement consists in the rotation induced to the cell that flows in a microfluidic channel. More specifically, differently from the OT, here we are able to obtain a complete 360° rotation, thus obtaining a more accurate measure of the 3D shape.

9540-13, Session 5

Non-invasive diagnosis of cancerous tissues with circularly polarized light

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Polarization-based optical techniques have become increasingly popular in the field of biomedical diagnosis. We apply circularly polarized laser light which illuminates tissue samples of interest, and a standard optical polarimeter is used to observe the polarization state of the backscattered light. We use the Stokes vector depicted on a Poincaré sphere as a quantitative parameter to assess cancerous and non-cancerous tissue samples in vitro. The obtained results are discussed in the framework of a phe-nomenological model and the results of a polarization tracking Monte Carlo model, developed in house.

9540-22, Session 5

Wavefront shaping based on three-dimensional optoacoustic feedback *(Invited Paper)*

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Wavefront shaping techniques have recently evolved as a promising tool to control the light distribution in optically-scattering media. These techniques are based on spatially-modulating the phase of an incident light beam to create positive interference (focusing) at specific locations in the speckle pattern of the scattered wavefield. The optimum phase distribution (mask) of the spatial light modulator that allows focusing at the target location(s) is determined iteratively by monitoring the light intensity at such target. In this regard, optoacoustic (photoacoustic) imaging may provide the convenient advantage of providing simultaneous feedback information on light distribution in an entire region of interest, thus facilitate wavefront shaping. Herein, we showcase that volumetric optoacoustic images can effectively be used as a feedback mechanism in an iterative optimization algorithm allowing controlling the light distribution after propagation through a scattering sample. Experiments performed with absorbing microparticles distributed in a three-dimensional region showcase the feasibility of enhancing the light intensity at specific points. The advantages provided by optoacoustic imaging in terms of spatial and temporal resolution anticipate new capabilities of wavefront shaping techniques in biomedical optics.

9540-25, Session 5

Intraoperative model based identification of tissue properties using a multimodal and multiscale elastographic measurement approach

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Minimally invasive surgery has for many applications replaced the open surgery, since the amount of tissue, which has to be cut, is reduced, resulting in a quicker recovery of the patient connected with reduced post operational stress. Moreover, it offers some aesthetical advantages such as for facial operations. Besides all these advantages, minimal invasive surgery has restricted the working environment of the surgeon due to the loss of two mayor human senses, three dimensional vision and haptic feedback (important tools to guide the surgeon throughout the operation). Our research refers to the latter of the two, based on the application of elastography. The obtained elastographic data assists the surgeon in minimally invasive surgery by localising different types of tissue. In that manner small tumours and/or enlarged lymph nodes hidden underneath the peritoneum, which may not have been registered in the pre-operational data, can be found. Measurements are recorded on multiple scales of resolution (cell, tissue, organ) employing multiple elastographic techniques (e.g. AFM, 2D image correlation). Results are fed into a Finite Element (FE) model to generate an accurate description with regards to the elastic behaviour of an organ. Different scenarios with alternating position, shape and size of a tumour within the organ are simulated (databank generation). For real time classification and segmentation of tissue in the surgical environment, the highly complex FE model is either reduced (e.g. principle component analysis) and/or template matching is applied to the minimally invasive measured 2D displacement map, while maintaining the important data describing the geometry of the tumour.

9540-26, Session 5

Identification and collection of particles with optical fibers

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Optical fibers are inert, flexible, low-loss, low-cost waveguides, with small cross-section, which can guide high-power laser radiation. These features make them attractive in in-vivo life-sciences studies . The possibility of transporting light in a fiber into a living body already exploited in endoscopy and surgery, can be used for optically exciting samples and guiding back characteristic molecular signals, allowing for the study of cells through Raman scattering and fluorescence excitation. The introduction of larger capillary holes in the fiber cladding running parallel to the core considerably increases their functionality. The capillaries can be used for the removal of liquids or collection of species from the illuminated region for further analysis, for example cells that may be related to disease. The capillaries can also be used to deliver substances to the illuminated region, for example a drug or an optically activated chemical or medicine.

We have recently made initial studies with large hole fibers exploiting these possibilities. In these “proof of principle” experiments we used fluorescence for identification and microfluidic sample retrieval in a single fiber. We combined in a fiber low-loss light guidance and fluid flow. Our system excited, identified and collected particles considered of interest, mimicking cancer cells in a heterogeneous environment. The excitation light from a cw blue laser was guided through the fiber, and fluorescence from green or red tagged beads in a water solution was carried back through the fiber to a photo-multiplier. A pump was connected to the capillary. When the correct signature was seen, i.e. fluorescence at the right wavelength and intensity exceeding a trigger level that indicated acceptable proximity, a small volume of fluid (nl) was sucked into the fiber, catching the particle of interest together with a minimum extra liquid. This system was been able to select and sort red and green beads from a water solution with high specificity.

Present work is ongoing to replace the beads by GFP tagged living cells for identification and collection into fibers and applying the system in studies of photodynamic therapy in cancerous mice model.

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9541-1, Session 1

Optimal processing of Doppler signals in OCT

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Besides structural imaging, OCT can be used to estimate axial velocities of the sample resolved in depth by Doppler-processing. In Fourier domain OCT (FD-OCT), this is accomplished by measuring the phase difference (i.e. phase shift) between timely separated A-scans at the same depth. In most cases, these data are disturbed by noise caused by intrinsic noise of the OCT system, specified by the SNR, and decorrelation noise caused by the transversal movement of the optical beam relative to the sample. Since the first use of Doppler methods in OCT, many methods to reduce the phase shift noise by averaging have been presented. While all these methods use a fixed set of consecutive A-scans, the best method, exhibiting no bias and having the smallest standard deviation, was questionable. Recently, Doppler processing methods depending on the mentioned noise sources and delivering the most likely phase shift and thereby axial velocity became available. The relation of these methods to previously known methods like the Kasai estimator, maximum likelihood estimator (MLE) and joint spectral and time domain OCT (jSTdOCT) will be discussed. While the new methods seem to be optimal for fluids containing a large number of sub-resolution scattering particles, the question for the optimal method for fluids containing larger scattering particles, as for instance blood, seems to be still open.

9541-2, Session 1

Improved axial and transverse flow measurements in optical coherence tomography using resolution and dispersion diversity

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Blood flow measurements are of interest in a diverse range of clinical applications, including retinal blood-flow measurements in opthalmology, the assessment of stenoses in cardiology, the study of tumor angiogenesis and lymphangiogenesis for determining the viability of cancer treatments, in the study of cerebral pathophysiology, among many others. Swept-source optical coherence tomography (OCT) is able to provide high-resolution cross-sectional imaging and moderate penetration depths. Dynamic light scattering- (DLS-) OCT analyzes the time autocorrelation function of the complex OCT tomogram to determine the dynamics of the scatterers that produce the OCT signal. DLS-OCT requires the phase information of the tomogram to determine the diffusion, axial and the transverse components of the flow, which limits its implementation in fast swept-source OCT systems. In this work we present intensity-based dynamic light scattering OCT (iDLS-OCT), a new technique that allows the determination of the diffusion, axial and transverse components of motion based solely on the intensity of the OCT signal. This is accomplished by reconstructing a single tomogram with a diversity of axial resolutions and group velocity dispersions. This novel technique could open the way to new applications to DLS in OCT where the sample dynamics require faster acquisition times, a situation in which frequency-domain OCT excels, without being impacted by its phase instability.

9541-3, Session 1

Quantitative flow estimation in Doppler optical coherence tomography of the human retina

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In Doppler optical coherence tomography (OCT) the flow of moving particles is revealed from phase differences between repeated A-lines from the same location in the tissue. However, besides flow also noise contributes to these phase differences which is problematic for accurate flow quantification. Here, a maximum a posterior probability (MAP) estimator for the estimation of flow velocities in the human retina from in vivo Doppler OCT measurements is presented. The MAP estimator involves models from previous studies which describe the influence of shot noise, revisitation errors (RE) and flow to the Doppler OCT signal and combines them in order to isolate the contribution of the flow and to determine the flow velocities in retinal capillaries. The MAP estimator was compared to a direct calculation of the flow velocities from phase differences without taking noise influences into account. It was found that the MAP estimator significantly reduces bias which was otherwise caused by shot noise and RE in the direct calculation. The estimated velocities also present the whole range of velocities that are expected in retinal capillaries while the results of the direct calculation clearly exceed the maximum expected limit. These results lead us to the conclusion that the MAP estimator is more accurate than the direct calculation method and the considered noise sources cannot be neglected for flow quantifications in the human retina. This method is potentially interesting for studying retinal pathology such as age-related macular degeneration.

9541-4, Session 1

Quantitative blood flow imaging of functional hyperaemia in the mouse cortex using extended-focus optical coherence microscopy

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Optical microscopy, through its high temporal and spatial resolution, is of paramount importance to understand cerebrovascular function at the cellular level. Multi-photon Microscopy, the current gold-standard in optical neuroimaging, can perform quantitative measurements of blood flow velocity. Nevertheless, its small field of view and slow acquisition rate limit MPM as it fails to capture the heterogeneity of the hemodynamic responses throughout the vascular network. Optical Coherence Tomography is an alternative as it can perform three-dimensional imaging over a large area while maintaining a high-acquisition rate. Moreover, by measuring the Doppler shift experienced by light scattered by moving particles, classical Doppler Optical Coherence Tomography can compute the axial velocity component of these scatters. In the brain, as a large portion of the vascular runs horizontally along the cortical surface, this axial velocity projection can become significantly weak and thus difficult to detect. Several methods have been devised to recover the absolute velocity, either by measuring the “Doppler” angle, by integrating the axial projections over the lateral cross-section of the vessel or by measuring the broadening of the Doppler Spectrum. A generalisation of the latter

method was recently developed by our group, providing relations between the axial and lateral velocity components and the mean and variance of the Doppler Spectrum. We demonstrate here the advantage of total blood flow measurements performed with an extended-focus Optical Coherence Microscope to monitor stimulus-evoked changes in blood flow.

9541-5, Session 1

An approach to OCT-based microvascular imaging using reference-free processing of complex valued B-scans

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We propose an unconventional OCT-based approach to 3D microvasculature imaging. It is based on reference-free processing of individual complex-valued B-scans. This feature is essentially distinct from the earlier proposed correlation-mapping and speckle-variance methods in which two or several B-scans consequently obtained in the same plane are compared. In the proposed approach, the individual B-scans are formed by highly overlapped A-scans. In the lateral direction of such a B-scan, the amplitude and phase of speckles corresponding to vessel regions exhibit faster variability in comparison with the speckles formed by scatterers in the “solid” tissue. Therefore, the regions corresponding to vessels’ cross sections can be detected without comparison with other B-scans recorded in the same plane. The proposed method combines elements of several existing OCT-based angiographic techniques. In particular, utilization of the full complex-valued signal implies the use of both signal amplitude (like in speckle-variance methods and correlation mapping) and phase of the optical signal which is used in phase-resolved approaches. However, unlike the latter group, in the proposed method, we compare the complex-valued A-scans in an indirect manner via performing high-pass filtering of the spatial spectrum in the lateral direction of the recorded dense B-scans. The very principle of the proposed approach ensures such advantages as (i) enhanced robustness with respect to bulk tissue motion with velocities up to ~cm/s; (ii) resultant resolution of microvascular images equal to that of structural images and (iii) possibility of quantifying the vessels in terms of their decorrelation rates by varying the threshold of the high-pass filtering.

9541-6, Session 1

Flow rate estimation by optical coherence tomography using contrast dilution approach

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This paper describes experiments and methodology for flow rate estimation using optical coherence tomography and dilution method in single fiber setup. The single fiber is created from custom made glass capillary and polypropylene hollow fiber. As a data source, measurements on single fiber phantom with continuous flow of carrier medium and bolus of Intralipid solution as a contrast agent were used using Thorlabs OCT OCS1300SS. The measured data were processed by methods of image processing, in order to precisely align the individual images in the sequence and extract dilution curves from the area inside the fiber. An experiment proved that optical coherence tomography can be used for flow rate estimation by the dilution method with precision around 7 %.

9541-7, Session 2

Transmission matrix approach to light control in complex media (Topcon Invited) *(Invited Paper)*

Sylvain Gigan, Lab. Kastler Brossel (France)

No Abstract Available

9541-8, Session 2

Wavefront shaping for nonlinear microscopy: second-harmonic generation imaging enhancement through scattering media

Hilton Barbosa de Aguiar, Institut Fresnel (France); Sylvain Gigan, Lab. Kastler Brossel (France); Sophie Brasselet, Institut Fresnel (France)

Nonlinear microscopy (NLM) has emerged as a powerful technique for biological imaging in a label-free manner. Two-photon fluorescence imaging of neural activity, coherent Raman scattering imaging of lipid-rich assemblies, second-harmonic generation imaging of collagen structures and sensitive screening of pharmaceuticals, are just a few representative examples. Despite the tremendous impact of NLM, all of nonlinear imaging modalities can only image at shallow depths because deeper penetration imaging into biological specimens is generally hindered by scattering phenomena. Wavefront shaping (WS) is an emerging field with techniques that reestablishes a “focus” even after a strongly scattering medium. These refocusing capabilities are achieved by coherently controlling the spatial degrees of freedom with spatial light modulators. In this contribution, we show our first efforts in combining WS experiments with NLM. We image nonlinear sources of second-harmonic photons in order to evaluate which experimental parameters are most relevant for contrast enhancements. We show remarkably high nonlinear signal enhancements simply based on the control of spatial degrees of freedom.

9541-9, Session 2

Scanning-free and bend-insensitive imaging through a single fiber

Sylwia Kolenderska, Nicolaus Copernicus Univ. (Poland); Ori Katz, Institut Langevin (France) and Univ. Pierre et Marie Curie (France); Sylvain Gigan, Univ. Pierre et Marie Curie (France); Mathias Fink, Institut Langevin (France)

We present a simple 2D spectral encoding approach that is based on scattering from a random medium placed at the distal end of a single fiber. We show that even a simple diffuser can serve as a random 2D spectral dispersing element. As random scattering, even from a thin diffuser, is spectrally dependent, the light scattered from a diffuser is inherently encoded with different random spectral signatures for each spatial pixel position. This random, but fixed, encoding can be measured in a simple calibration procedure. Given the spatio-spectral encoding, the image of an object placed next to the distal end and illuminated by spatially incoherent broadband light can be reconstructed from the spectrum measured at the proximal end, using either a linear algebra based reconstruction, or a compressive sensing (CS) reconstruction algorithm. Apart from that, our method’s performance is insensitive to bending while using multimode fibers, because only one mode (speckle)

spectrum is measured which is an advantegous feature as far as the endoscopic applications are concerned.

9541-10, Session 2

Transillumination imaging through biological tissue by single-pixel detection

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Optical methods are growing into an essential tool in biomedical science. They are noninvasive, fast, cost-efficient and, unlike techniques based on ionizing radiation, they do not pose a health risk. In the realm of diagnosis, we have witnessed during the past few years how optical imaging has assisted clinicians in the detection and evaluation of suspicious lesions. However, the “elephant in the room” question that should be addressed is the short penetration depth of light into tissue, in contrast to ultrasound or X-ray technologies. Current knowledge is insufficient for early detection of small lesions located at a depth larger than 1-2 mm beneath the surface of tissue. In this work we present an optical scheme that achieves significantly deeper penetration depths inside scattering tissue. Concretely, our results demonstrate transillumination imaging of an object embedded within a 6-mm thick sample of chicken breast. Our technique is noninvasive, does not require coherent sources, raster scanning nor time-gated detection and works without increasing the cost and system complexity. The used optical system is based on a single-pixel detection scheme. This computational imaging modality allowed us to operate at illumination power levels that are three orders of magnitude lower than the tissue damage threshold.

9541-11, Session 2

High-speed single-shot multiwavelength lensless phase contrast microscopy

Dirk E. Boonzajer Flaes, Daniel W. E. Noom, Elias Labordus, Kjeld S. E. Eikema, Johannes F. de Boer, Stefan M. Witte, Vrije Univ. Amsterdam (Netherlands)

We demonstrate a highly compact lensless imaging system that is able to retrieve both amplitude and phase of a complex sample in a single shot, without requiring any a priori knowledge of the sample[1]. The numerically retrieved phase information provides real-time phase- and amplitude videos where the focal plane can be decided afterwards. We captured real-time images of moving beads in a flow cell at different focal distances and living, freely moving C. elegans with less than 2 micrometer resolution[2]. We will further present possible enhancements of the phase retrieval algorithm to compensate for a known diffuser in between the camera and the sample. This system provides a first step towards lensless imaging flow cytometry with quantitative phase contrast.

[1] Noom, Daniel W. E. and Eikema, Kjeld S. E. and Witte, Stefan, “Lensless phase contrast microscopy based on multiwavelength Fresnel diffraction”, Opt. Lett. 39, 2 (2014), pp. 193--196.

[2] Noom, Daniel W.E. and Boonzajer Flaes, Dirk E. and Labordus, Elias and Eikema, Kjeld S.E. and Witte, Stefan, “High-speed multi-wavelength Fresnel diffraction imaging”, Opt. Express 22, 25 (2014), pp. 30504—30511.

9541-12, Session 2

Speckle noise reduction by spatio-temporal optical coherence modulation

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In this work we show that the optical channel transmission capabilities detrimentally affected by random intensity fluctuations can be enhanced by diagonalization of the spectral coherence matrix G. To this end, we utilize the spatio-temporal coherence manipulation (STOC) technique, and modulate the coherent light temporarily such that its statistical properties can be controlled. As a result, the undesirable speckle noise can be diminished in a universal way, which does not depend on the structure of the particular medium generating random noise.

9541-13, Session 2

Enhanced adaptive focusing through ultra-thin scattering media

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Achieving control of light propagating in disordered media[1] has proved an emerging field with great impact in improving microscopy[2], biological and biomedical imaging[3]. Furthermore, wave-front shaping[4] is a powerful technique that allows to manipulate the optical paths through scattering media and makes possible the generation behind a scattering material of multiple light spots actively driven at user controlled positions[5]. These results are achieved by properly adjusting the wave-fronts in order to correct de-phasing produced due to the random propagation in the disordered medium with a Spatial Light Modulator (SLM)[6]. Vellekoop and colleagues[5] demonstrated that the minimum spot size achievable through adaptive focusing is defined by the speckle correlation function or in practice by the speckle grain[7,8]. When the scattering strength increases, the typical grain of the speckle pattern becomes smaller and the correlation function of the speckle pattern becomes sharper, this means that to achieve tight focusing one has to exploit optically thick samples[9,10]. In this work, we propose an experimental technique permitting to achieve sharp adaptive focusing through extremely low scattering samples. The core idea consists of selecting only the optical paths which underwent many scattering events filtering the ones which experienced little scattering. Once the filter is applied a speckle pattern with smaller size appears and a smaller focus can be achieved.

We describe the advantage yielded by implementing the standard wavefront shaping approach with a spatial filter which is able to select the transmitting modes for the best focus resolution. The spatial filters that we used are home-made beam stop filters (BeSt), we performed simple fabrication protocol which allowed us to produce filters with different diameter size. The setup we use consists in a SLM that modifies the wave-front of the coherent light (594nm) which impinges onto a scattering sample (S). During propagation through turbid material, the scattering decomposes the incident wave into multiple output components which generate a speckle pattern at the back of the sample. The speckle pattern is collected by an objective lens (OBJ) and is projected behind it. At the back of the rear face of the OBJ a lens L5 is placed, which reproduces the speckle pattern on a specific plane at its focal length. Exactly on this plane the BeSt filter is aligned in order to block the central components of the speckle pattern: those components are related to modes which underwent a few scattering events through the sample. Finally, lens L6 produces an image on the CCD camera plane which is at the conjugate plane of the image from L5. The different samples were illuminated with the same beam waist and the back surface of each sample has been aligned on the focal plane of the OBJ. We then define the Full Width at Half Maximum, FWHM (w) as the width of the intensity peak around the target spot. We compare the values w obtained with the standard focusing approach4 at different optical length L with the values obtained when the BeSt filter is used.

We initially study the focusing power, in terms of w, at different scattering regimes when the BeSt filter is not inserted in the setup transmittance. The transmittance of the sample is simply controlled by its thickness yielding L between one and ten transport mean free paths[8]. We show that turbid lenses reach the optimum efficiency when scattering becomes relevant (for L greater than 2 ?). The focus width, w, exhibits a fast reduction when transmittance decreases to 2L/? and stabilizes. We show, that for high range of transmittance (0.5?2 transport mean free paths) the focusing power is very weak due to a poor effective numerical aperture of the turbid lens. On the other hand, we demonstrated that an appropriate selection of the transmitting modes for focusing results to an enormous improvement of the focusing power for samples with optical length L

smaller than 2?.

Different results in term of FWHM are obtained for the foci achieved through the same set of samples when a BeSt filter is applied. We noticed that the minimum w is achieved with much less scattering and at higher transmittance we got much smaller foci. We therefore achieved an improvement in focusing resolution by using the spatial filter. We conclude that the BeSt filter significantly contributes to the focusing power when the optical length of the sample is short. In order to study and to characterize the efficiency of our approach we tested a sample at fixed transmittance and we collected the focus width, w, when the size of the BeSt filter varies. The best focusing quality was considered so far limited to scattering sample with multiple transport mean free paths[10,11]; On the contrary, our work demonstrates that either the minimum or near the minimum focusing power is attainable even when the scattering is not relevant.

9541-14, Session 3

Time-encoded Raman scattering (TICO-Raman) with Fourier domain mode locked (FDML) lasers *(Invited Paper)*

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We present a new concept for conducting stimulated Raman spectroscopy and microscopy by employing rapidly wavelength swept Fourier Domain Mode locked (FDML) lasers. FDML lasers are known for fastest imaging in swept-source optical coherence tomography. We employ this continuous and repetitive wavelength sweep to generate broadband, high resolution stimulated Raman spectra with a new, time-encoded (TICO) concept. This allows for encoding and detecting the stimulated Raman gain on the FDML laser intensity directly in time. Therefore we use actively modulated pump lasers, which are electronically synchronized to the FDML laser, in combination with a fast analog-to-digital converter (ADC) at 1.8 GSamples/s. We present hyperspectral Raman images with color-coded, molecular contrast.

9541-15, Session 3

Extended-focus optical coherence microscopy in the visible wavelength range for live cell imaging

Paul J. Marchand, Arno Bouwens, Séverine Coquoz, Miguel Sison, Jerome Extermann, Theo Lasser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Over the past decades, confocal microscopy has become the gold standard for 3D imaging of live cellular structures. Despite recent advances in light sheet microscopy, most volumetric high-resolution imaging schemes involve an inherently slow three-dimensional raster scan. Moreover, while fluorescent labeling offers molecular specificity, its influence on cell function remains often ambiguous mainly due to the potential interference with the cellular processes under study. Overall, due to this phototoxicity and the limited imaging speed, confocal microscopy is an ill-suited technique to image fast processes in live cellular structures. We present here an novel alternative to circumvent these caveats, based on optical coherence microscopy operating in the visible wavelength range. By combining a supercontinuum light source centered in the visible spectrum (?0 = 560nm, ?? = 180nm) with a high NA objective (effective NA = 0.86), the obtained axial and lateral resolution in water are 0.6 ?m and 0.4 ?m respectively. Decoupling the system into a Bessel illumination and a Gaussian detection mode offers the dual advantage of an extended depth of field and a dark field contrast enhancement. The extended focus maintains the sub micron resolution throughout the depth of the sample and thus allows fast imaging of three-dimensional volumes. Additionally, the dark-field detection suppresses the specular reflections originating from the coverslip, allowing to image sub cellular structures with a higher sensitivity. We demonstrate here three-dimensional imaging of live cells and fixed brain slices with a submicron resolution, revealing detailed subcellular structures.

9541-16, Session 3

Functional optical coherence imaging (FOCI) a novel, label-free approach for longitudinal, 3D visualization of autoimmune diabetes

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Longitudinal studies play a key role in clinical research and therapeutic evaluation. The application of high-resolution optical imaging to monitor animal models of disease has promoted valuable insight in disease mechanisms and treatments. However, many disorders, like type-1 diabetes, afflict tissues inaccessible to optical imaging in live subjects in vivo and in situ. Diabetes mellitus develops as a functional impairment in the insulin-production sometimes in association with insulin resistance. The progressive dysfunction of the ?-cell causes the disease development. Type I diabetes is a major health problem resulting from an autoimmune attack thereby destroying the insulin producing ?-cells. We present here a procedure for label-free, three-dimensional and quantitative OCM based detection of autoimmune inflammation and functional vascular imaging combined with the transplantation of pancreatic islets into the anterior chamber of the eye (ACE). We demonstrate that this method of functional optic coherence imaging (FOCI) allows for label-free tracking of the fate of individual islets of Langerhans during progressive autoimmune attack including the quantification of beta-cell volume and inflammation over multiple weeks in a live animal. We also show how OCM provides a powerful tool for visualizing three-dimensional vasculature structure and for assessment of microcirculary blood flow. Finally, applying this approach to a spontaneous mouse model for type 1 diabetes we find support for a strong correlation between the degree of insulitis and the density of the vascular network of the islet. The label-free nature of FOCI contributes an important dimension for transferring this imaging modality to human applications.

9541-17, Session 3

Performance of coherence-based imaging systems with rapidly tunable lens

Ireneusz Grulkowski, Krzysztof Szulzycki, Maciej Wojtkowski, Nicolaus Copernicus Univ. (Poland)

We develop high-resolution interferometric system with tunable lens. Different tunable lens technologies used in this study include: acousto-optic tunable lens and electrical focus tunable lens. Stroboscopic pulsed illumination is used for the first time to perform time-resolved optical

coherence tomography imaging with tunable focusing. The operation of ultrahigh-speed tunable acousto-optic lens is demonstrated theoretically and experimentally. Focal position tuning at different frequency ranges is experimentally shown in the coherence-based imaging system leading to OCT images with extended depth of focus. Imaging with active optical elements is helpful for improvement of photon collection efficiency, depth of focus and enhancement of the image quality.

9541-18, Session 3

Improved cancer diagnostics by different image processing techniques on OCT images

Rajesh V. Kanawade, Benjamin Lengenfelder, Tassiana Marini Menezes, Martin Hohmann, Stefan Kopfinger, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany); Tim Hohmann, Urszula Grabiec, Martin-Luther Univ. Halle-Wittenberg (Germany); Florian Klämpfl, Jean Gonzales Menezes, Maximilian Waldner, Michael Schmidt, Friedrich-Alexander-Univ. Erlangen-Nürnberg (Germany)

Optical-coherence tomography (OCT) is a promising non-invasive high-resolution imaging modality which can be used for cancer diagnosis and its therapeutic assessment. However, speckle noise makes detection of cancer boundaries and image segmentation problematic and unreliable. Therefore, to improve the image analysis for a precise cancer border detection, the performance of different image processing algorithms such as mean, median, hybrid median filter and rotational kernel transformation (RKT) for this task is investigated. Ex-vivo on OCT images acquired from an inflammation-driven mouse model of colon carcinogenesis. The preliminary results obtained from image processing confirm that the border between the healthy and the cancer lesions can be identified precisely. The obtained results are verified with histopathology. This research can improve cancer diagnosis and the detection of borders between healthy and cancerous tissue. Thus, it could also reduce the number of biopsies required during screening endoscopy by providing better guidance to the physician.

9541-19, Session 4

Scattered light microscopy (Topcon Invited) *(Invited Paper)*

Ivo M. Vellekoop, Univ. Twente (Netherlands)

Light scattering prevents microscopes to look inside tissue deeper than a few hundred micrometers. I will discuss alternative approaches that use scattered light, rather than ballistic light, for microscopic imaging; potentially increasing the penetration depth of microscopes by over an order of magnitude.

9541-20, Session 4

Effect on optical coherence tomography image quality of turbid tissue scattering using Gaussian or Bessel beams

Andrea Curatolo, Peter R. T. Munro, Parvathy Sreekumar, The Univ. of Western Australia (Australia); Christian C. Singe, The Univ. of Western Sydney (Australia); Brendan F. Kennedy, Dirk Lorenser, David D. Sampson, The Univ. of Western Australia (Australia)

Forward-directed light scattering from turbid tissue progressively degrades optical coherence tomography (OCT) image contrast and resolution with depth, in most cases long before the OCT signal reaches the system noise floor. Bessel beams have been proposed in OCT to image deeper into turbid tissue, by virtue of their extended depth-of-focus (DOF) and their ability to reconstruct after a small obstruction. Yet, to date, no comparative study has explored the effect of scattering anisotropy on OCT image quality, and the performance of Bessel, compared with Gaussian beams in these turbid tissue scenarios. We present such a theoretical and experimental analysis of the OCT point-spread function (PSF) and local contrast in structured scattering phantoms with thin scattering overlayers. We present both simulated and experimental results of imaging at high resolution through highly forward-scattering layers, using Gaussian or Bessel beams, with lateral resolution of 2.6 um. The scattering overlayers are 150 um thick and comprise mono-dispersions of spherical scatterers of varying diameter, with identical scattering coefficients of 3.7 mm-1. We demonstrate that the higher the scattering anisotropy of the mono-dispersions, the higher the on-axis background contribution of the forward scattered light and, therefore, the lower the image quality, regardless of the beam type. Furthermore, the Gaussian beam, in focus, suffers less reduction of local contrast, compared to the Bessel beam with equivalent input power, as the degrading background generated by either beam is very similar, whereas the Bessel beam peak power is sacrificed by redistribution along the optical axis.

9541-21, Session 4

OCT model of discrete random media links optical properties to micro-scale sample organization

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Optical Coherence Tomography (OCT) has the potential to provide for minimal invasive in-vivo optical biopsy by probing the changes in optical properties induced by the morphological changes in the tissue. Visualization of the lesion by OCT enables staging (invasiveness), and quantification of the optical properties by OCT can enable grading (aggressiveness) of the lesion. To achieve this goal a good physical model is required to link the OCT-derived optical properties to tissue properties, e.g. size and organization.

As a first step, we propose a model for the optical properties of a discrete random medium (DRM), consisting of randomly placed identical spherical particles. To take into account the organization of the scatterers, we introduce the pair correlation function and the structure-factor in the Percus-Yevick approximation. Where necessary, multiple scattering is accounted for using the Extended-Huygens-Fresnel formalism for the OCT-signal.

We have verified this model by comparing calculated optical properties with experimental OCT-data from a concentration series of silica spheres suspended in water. To this end the attenuation coefficient of calculation and experiment are compared. The results show that our model provides an adequate description of experimentally obtained values of the attenuation coefficients. Therefore, the results demonstrate how optical properties are sensitive to the micrometer-scale organization of the sample. This is an essential step towards a qualitative expression to link OCT-derived optical properties to clinically relevant morphological properties of tissue.

9541-22, Session 5

Multimodal mouse retinal imaging system with ocular aberration correction by wavefront sensorless adaptive optics

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Medical Ctr. (United States)

We present a multimodal mouse retinal imaging system combining Fourier domain optical coherence tomography (FD-OCT) with Scanning Laser Ophthalmoscopy (SLO). Combination of OCT with SLO allows for simultaneous detection of structural (back reflected light) and potentially functional (fluorescently emitted light) signals. Correction of ocular aberrations in the mouse eye was performed by a wavefront sensorless Adaptive Optics (WSAO) scheme utilizing the intensity of en face images acquired by FD-OCT as a correction metric. Our multimodal system has a simplified lens based optical design, including elimination of long focal length scanning optics and optical conjugation of vertical and horizontal scanners. This modification provides a large Field of View (low resolution) and small Field of View 'zoom' capability for high resolution aberration-corrected imaging. In the present system, a 0 Dpt mouse eye contact lens was used to better preserve the optical quality of the mouse cornea for long term imaging. To allow efficient use of the Deformable Mirror (MEMS based tip-tilt DM) stroke, defocus was controlled by the collimation of the sample arm entrance beam.

9541-23, Session 5

In vivo imaging of retinal and choroidal vasculature in the rodent eye using optical coherence tomography

Marco Augustin, Stanislava Fialová, Roberto Plasenzotti, Michael Pircher, Christoph K. Hitzenberger, Bernhard Baumann, Medizinische Univ. Wien (Austria)

Animal models play an important role in the fundamental research of ophthalmology. In vivo imaging technologies such as optical coherence tomography (OCT) and rodent models enable longitudinal studies of different ophthalmic pathologies and their underlying physiological changes.

In this work a custom-made high resolution polarization sensitive OCT system is used to image the posterior eye of small rodents. By using en-face projections at certain depths/levels in three-dimensional (3D) images, the ability to visualize different vascular structures in the retina as well as in the choroid is demonstrated. The levels are determined by a semi-automatic approach in which layers of the retina are segmented in a first step. In a second step the projection level can be adjusted by the operator to restrict the volume in which the en-face projection is carried out.

To demonstrate the functionality of this approach, rats of different strains (Sprague-Dawley, Brown Norway, Long-Evans) and a Black-6 mouse were imaged. The rodents were scanned with an angle of 11x11° and 21x21°. The signal-to-noise ratio was increased by averaging 5 repeated B-scans at each scanning position. Motion artefacts in the 3D images were compensated by post-processing.

The vascular maps based on the reflectivity information show that the resolution of the custom-made systems is high enough to resolve retinal capillaries in the outer plexiform layer. Further, larger vessels within the nerve fiber layer and the inner nuclear layer as well as in the choroid were visualized by the proposed approach.

9541-24, Session 5

Comparison of the polarization properties in the retinas of different rodents using high resolution polarization sensitive OCT

Stanislava Fialová, Marco Augustin, Roberto Plasenzotti, Sabine Rauscher, Marion Gröger, Michael Pircher, Christoph K. Hitzenberger, Bernhard Baumann, Medizinische Univ. Wien (Austria)

Animal models play an important role for understanding of pathophysiology of glaucoma and age-related macular degeneration. With these models, longitudinal studies can be performed and therefore there is need for non-invasive evaluation of disease progress. For that purpose histology can be substituted by optical coherence tomography (OCT) or even polarization sensitive OCT (PS-OCT). Since tissues with polarization properties are important in these diseases, PS-OCT could be valuable tool in preclinical research. In this work high resolution PS-OCT (HR-PS-OCT) system was used in-vivo for rodent retinal imaging. A superluminescent diode with a bandwidth of 100 nm was used as a light source that yielded an axial resolution of 5.1 ?m in air (3.8 ?m in tissue). A-scan rate was 83 kHz, a whole 3D dataset was acquired in a few seconds (1024x200x1536 pixels in 3.5 s) which reduced motion artifacts. Rats (Sprague-Dawley, Long-Evans and Brown Norway) as well as mice (Black-6) were imaged. High resolution reflectivity images showed all retinal layers in all animals. From acquired data also phase retardation and fast axis orientation images were calculated. On phase retardation images sclera was identified as birefringent and retinal pigment epithelium (RPE) and choroid as depolarizing tissues. Our results demonstrate suitability of the system for high speed/resolution imaging in follow up studies on rodents.

9541-25, Session 5

Real-time optical coherence tomography observation of retinal tissue damage during laser photocoagulation therapy on ex-vivo porcine samples

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Retinal laser photocoagulation represents an established treatment method for a variety of retinal diseases such as age-related macula degeneration. Its use, however, must be well controlled as local overexposure during photocoagulation can seriously harm or irreversibly destroy neural retinal. In retinal laser photocoagulation, assessment of the retinal lesions is carried out by visual inspection using indirect ophthalmoscopy or fluorescence imaging which is time-consuming and only offers limited interpretation of the laser damage. Optical coherence tomography (OCT) may be a promising modality to overcome those limitations.

Previous studies have carried out OCT imaging 1-2 hours after application of the laser energy. While this is adequate for imaging biological effects subsequent to retinal laser photocoagulation, immediate optical changes caused by laser application may occur on a much shorter timescale. Recent studies already showed promising performance of OCT measurements immediately after laser photocoagulation. Therefore, a real-time data recording might be beneficial extension in terms of the interpretation of immediate effects caused in the irradiated tissue.

In this manuscript, simultaneously acquired time-resolved point scans (M-Scans) of porcine eyes irradiated ex vivo with variable laser energies and recorded with a custom-made OCT system are presented. OCT M-Scans are segmented and parameters such as layer thickness and distortion are extracted and analyzed. Optical parameters extracted from time-lapse scans are evaluated and compared to a manual classification of the retinal lesions by ophthalmoscopic visibility. Statistical analysis of the extracted parameters is performed and parameters suitable for retinal laser photocoagulation interpretation are discussed.

9541-26, Session 6

Imaging the tympanic membrane oscillation ex vivo with Doppler optical coherence tomography during simulated Eustachian catarrh

Lars Kirsten, Technische Univ. Dresden (Germany); Anke Burkhardt, Jonas Golde, Julia Walther, Univ. Carl Gustav

Carus Dresden (Germany); Thomas Stoppe, Technische Univ. Dresden (Germany); Matthias Bornitz, Max Kemper, Thomas Zahnert, Univ. Carl Gustav Carus Dresden (Germany); Edmund Koch, Technische Univ. Dresden (Germany)

Recently, optical coherence tomography (OCT) was utilized in multiple studies for structural and functional imaging of the middle ear and the tympanic membrane. Since Doppler OCT allows both, the spatially resolved measurement of the tympanic membrane oscillation and high-resolution imaging, it is regarded as a promising tool for future in vivo applications. In this study, Doppler OCT is utilized for the visualization of the tympanic membrane oscillation in temporal bones with simulated Eustachian catarrh, which was realized by generating a depression in the tympanic cavity. The transfer function, meaning the oscillation amplitude normalized to the applied sound pressure, is measured frequency resolved in the range from 0.5 kHz to 6 kHz and with a lateral spatial resolution of 400 μm. Typical oscillation patterns could be observed in case of ambient pressure in the tympanic cavity. Under depression the characteristic oscillation patterns were observed with widely congruent appearance but at higher frequencies.

9541-27, Session 6

Real-time thermal therapy monitoring using dynamic OCT measurements

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In this work, we demonstrate the feasibility of using dynamic OCT measurements previously developed for angiographic applications for real-time thermal therapy monitoring in 2-D (and potentially 3-D), thereby enabling the non-invasive assessment of coagulation zone boundary at high spatial resolution. Conventional thermal therapy monitoring techniques based on temperature and impedance measurements provide only point sampling, while emerging techniques such as MR thermometry or photoacoustic thermography are limited by their spatial resolution. For epithelial applications such as laser therapy in the skin, the ability to directly visualize the thermal lesion boundary is critical to the accurate delivery of thermal energy to the target lesion. Here, we evaluate the use of three OCT angiographic reconstruction techniques that perform well in swept-source OCT systems: (1) speckle variance, (2) intensity-based Doppler variance, and (3) complex differential variance. We showed the ability to accurately delineate the coagulation zone boundary and therapy depth in ex-vivo porcine skin irradiated with a Thulium CW fiber laser and validated the results with both polarization-sensitive OCT and histological analysis. The ability to perform real-time thermal therapy monitoring non-invasively and at high resolution provides a powerful tool to guide treatment in a vast array of epithelial applications.

9541-28, Session 6

Investigating exogeneous modulators of mucociliary clearance using optical coherence tomography: effect of temperature and hyperoxia on cilia-driven flow velocity

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Premature infants are at a high risk for respiratory diseases due to an underdeveloped respiratory system that is very susceptible to infection and inflammation. One aspect of respiratory health is the state of the ciliated respiratory epithelium that lines the trachea and bronchi. The ciliated epithelium is responsible for trapping and removing pathogens and pollutants from the lungs and an impairment of ciliary functionality can lead to recurring respiratory infections and subsequent lung damage. Trachea in critically ill premature infants are exposed to a wide range of potential modulators of cilia-driven fluid flow, including pH, infection, and foreign bodies (e.g. endotracheal tubes). As the potential impact of exogenous modulators is incompletely understood, our research aims to close this critical knowledge gap. We are using optical coherence tomography (OCT) to visualize ciliary fluid flow in the tracheae of mice and quantify flow velocities through particle tracking velocimetry (PVT). To validate the method we statistically analyze the distribution of flow velocities and test the ability to detect changes in ciliary flow by varying temperature. Our measurements are performed ex vivo on tracheae of adolescent mice (PND21) under temperature controlled conditions. We found that changing the temperature causes a dramatic change in ciliary fluid flow, which has its maximum at 37 degrees Celsius. The impact of hyperoxia on ciliary functionality was investigated on mice that were exposed to hyperoxic conditions for 21 or 69 hours. We imaged flow in these tracheae and compared results with histology and found an exposure-dependent decrease of ciliary fluid flow with increasing exposure time to hyperoxia. We hope that elucidating more mechanisms of hyperoxia-derived alterations of the respiratory epithelium will contribute to an optimization of oxygen therapy.

9541-29, Session 6

Clinical validation of optical coherence tomography derived index of plaque attenuation

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Detection of coronary vulnerable plaques is important for early diagnosis and better therapy of ischemic heart disease. No single modality is able at present to detect vulnerable plaques with sufficient accuracy. We are developing optical coherence tomography (OCT) attenuation imaging as tissue characterization method. To investigate the ability of atherosclerotic tissue characterization in vivo, we are conducting the Optical Coherence Tomography Tissue Type (OC3T) study: a prospective single-center validation study of 62 patients, comparing OCT-derived optical attenuation to near-infrared reflection spectroscopy and intravascular ultrasound (NIRS/IVUS; Infraredx TVC).

We analyze the OCT data (C7XR, St Jude Medical) to create color coded maps of optical attenuation. To facilitate the comparison with NIRS/IVUS, we transform the pullback attenuation data in a longitudinal/en-face display by sampling a user specified depth window from the lumen border and match them longitudinally and circumferentially based on major side branches. To compare with LCBI (Lipid Core Burden Index) of the chemogram, we also compute a Index of Plaque Attenuation (IPA) which is the ratio of the high-attenuation pixels to the total number of pixels. In each pullback we selected a 4 mm plaque area in the chemogram with the highest LCBI and compared the IPA calculated for the same area from the OCT attenuation data.

LCBI and PAI, in general for all lipid plaques, have modest correlation (R=0.45). When categorizing lipid-rich plaques according to cap thickness based on OCT, there is no difference in LCBI between thin (<65 ?m) and thick cap fibroatheroma whereas IPA has significantly different value for both (p = 0.0005).

9541-30, Session 6

4D optical coherence tomography of aortic valve dynamics in a murine mouse model ex vivo

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The heart and its mechanical components, especially the heart valves and leaflets, are under enormous strain during lifetime. Like all highly stressed materials, also these biological components undergo fatigue and signs of wear, which impinge upon cardiac output and in the end on health and living comfort of affected patients. Thereby pathophysiological changes of the aortic valve leading to calcific aortic valve stenosis (AVS) as most frequent heart valve disease in humans are of particular interest. The knowledge about changes of the dynamic behavior during the course of this disease and the possibility of early stage diagnosis could lead to the development of new treatment strategies and drug based options of prevention or therapy.

ApoE-/- mice as established model of AVS versus wildtype mice were introduced in an ex vivo artificially stimulated heart model. 4D optical coherence tomography (OCT) in combination with high-speed video microscopy were applied to characterize dynamic behavior of the murine aortic valve and to characterize dynamic properties during artificial stimulation.

OCT and high-speed video microscopy with high spatial and temporal resolution represent promising tools for the investigation of dynamic behavior and their changes in calcific aortic stenosis disease models in mice.

9541-31, Session 6

Identification of parathyroid glands by using optical coherence tomography

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Background and Objective: The identification of parathyroid glands can be a major problem in parathyroid surgery. The purpose of this study was to evaluate the feasibility of optical coherence tomography (OCT) in distinguishing between parathyroid tissue, thyroid tissue, lymph nodes and adipose tissue. Apart from defining morphological criteria we investigated the backscattering intensity profiles of OCT images in order to determine whether significant differences between these tissue types exist.

Methods: Ex vivo and in vivo OCT images were generated from parathyroid glands, thyroid tissue, lymph nodes and fat in order to define significant morphological differences between the different tissue entities. All OCT images were separately evaluated by two blinded investigators and whenever posssible later compared to the corresponding histology. Sensitivity and specificity of OCT in distinguishing between the different tissues were determined. To assess the interobserver agreement, kappa coefficients were calculated from the ratings of each investigator for each OCT image seen. Furthermore, mean intensity profile was obtained from OCT. The profiles were analyzed employing Fisher's Linear Discriminant Analysis (LDA).

Results: A total of 320 OCT ex vivo images from 32 patients were compared with the corresponding histology. The sensitivity and specificity in distinguishing parathyroid tissue from the other entities was 84% (second investigator: 82%) and 94% (93%) respectively. Unweighted kappa was 0.97 (95% CI, 0.94 – 0.99) showing substantial agreement between both investigators. In vivo, 227 OCT images from 27 patients undergoing open or minimally invasive thyroid or parathyroid surgery were analyzed. Parathyroid glands were correctly identified in 69.2%, thyroid tissue in 74.5%, lymph nodes in 37.5% and adipose tissue in 69.2%. 43 OCT images (18.9%) could not be allocated to one of the tissue. Mean intensity profiles from 300 OCT images of 34 were analyzed. The overall rate of correct classifications was 96.15%.

Conclusion: Ex vivo, OCT is highly sensitive in distinguishing between parathyroid tissue, thyroid tissue, lymph nodes and adipose tissue. However, the excellent ex vivo results were not achieved in vivo, due to difficulties handling the sterile probe. Besides the individual assessment of OCT images by interpreting morphological criteria, backscattering intensity measurements can reliably distinguish between the diffrent types of tissue.

9541-32, Session 7

Non-confocal ophthalmic imaging of the photoreceptors, retinal vasculature and inner retina pathology (Topcon Invited) *(Invited Paper)*

Alfredo Dubra, Medical College of Wisconsin (United States)

No Abstract Available

9541-33, Session 7

High-speed, digitally refocused retinal imaging with line-field parallel swept source OCT

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MHz OCT allows mitigating undesired influence of motion artifacts during retinal assessment, but comes in state-of-the-art point scanning OCT at the price of increased system complexity. By changing the paradigm from scanning to parallel OCT for in vivo retinal imaging the three-dimensional (3D) acquisition time is reduced without a trade-off between speed, sensitivity and technological requirements. Furthermore, the intrinsic phase stability allows for applying digital refocusing methods increasing the in-focus imaging depth range. Line field parallel interferometric imaging (LPSI) is utilizing a commercially available swept source, a single-axis galvo-scanner and a line scan camera for recording 3D data with up to 1MHz A-scan rate. Besides line-focus illumination and parallel detection, we mitigate the necessity for high-speed sensor and laser technology by holographic full-range imaging, which allows for increasing the imaging speed by low sampling of the optical spectrum. High B-scan rates up to 1kHz further allow for implementation of lable-free optical angiography in 3D by calculating the inter B-scan speckle variance. We achieve a detection sensitivity of 93.5 (96.5) dB at an equivalent A-scan rate of 1 (0.6) MHz and present 3D in vivo retinal structural and functional imaging utilizing digital refocusing. Our results demonstrate for the first time competitive imaging sensitivity, resolution and speed with a parallel OCT modality. LPSI is in fact currently the fastest OCT device applied to retinal imaging and operating at a central wavelength window around 800 nm with a detection sensitivity of higher than 93.5 dB.

9541-34, Session 7

High definition in vivo retinal volumetric video rate OCT at 0.6 Giga-voxels per second

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We present retinal volumetric high speed OCT imaging at 20.8 volumes per second (V/s). The volumes consist of 255x255x450 voxels covering 20°x20° field of view. Imaging is performed with a swept-source OCT system with 1.6MHz A-scan rate, based on a 1060nm Fourier domain mode locked (FDML) laser. For fast axis scanning a 2x2691Hz resonant galvo in bidirectional scanning mode and for the slow axis a standard galvo scanner is used. The performance is analyzed with respect to various potential applications, like intraoperative OCT or OCT angiography.

9541-35, Session 7

Long-term follow up studies of retinal lesions in age-related macular degeneration by polarization sensitive OCT

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Polarization sensitive (PS) OCT is a functional extension of OCT that draws advantage from measuring the polarization state of light. Thereby, PS-OCT can generate tissue-specific contrast which can be used to segment retinal layers and lesions. In previous work, we have shown that the retinal pigment epithelium (RPE) depolarizes backscattered light. This effect can be used to segment the RPE and associated lesions. This feature makes PS-OCT very attractive for imaging eyes affected by age-related macular degeneration (AMD), since the RPE is among the earliest affected layers in this disease.

In this presentation, we report on the results of three long-term follow up studies using PS-OCT to analyze changes of RPE lesions in patients with AMD over time. The first study analyses the growth of GA in patients with dry AMD. 20 eyes of 13 patients were examined at 3 months intervals over at least one year. The second study analyses drusen volume progression to identify the pathway from drusen to the development of advanced AMD. 50 eyes of 30 patients diagnosed with early or intermediate AMD were examined every 3 months over three years or longer. The third study analyzed RPE alterations in fellow eyes of patients diagnosed with unilateral neovascular AMD. 31 eyes were imaged at baseline and over extended periods, with a mean follow-up time of 29 months.

9541-36, Session 7

3-beam Doppler optical coherence tomography for total retinal blood flow measurement

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We present a three beam Doppler optical coherence tomography (DOCT) technique suitable for 3-D velocity and mean flow measurements to evaluate the total retinal blood circulation from and to the optic nerve head (ONH).

The three beam DOCT consists of three independent channels. Superluminescent diodes with a central wavelength of 840 nm and a spectral bandwidth of 50 nm were used as source. The sources are coupled to collimators resting in a homemade mount to ensure a well-defined beam geometry, necessary for the full reconstruction of the three dimensional velocity vector. The reconstruction works without prior knowledge on the vessel geometry, which is normally required for DOCT systems with less than three beams. The beams share a common bulk optics Michelson interferometer, while the detection comprises three identical spectrometers.

In the sample arm a custom made facet prism telescope allows for variable beam separation, while the initial beam diameter is maintained (~ 0.5 mm at the collimator exit). A two axis gimbal less MEMS mirror is used for scanning to significantly reduce the effect of off-pivot scanning in comparison with a pair of galvo scanners.

To evaluate the total retinal flow a circular scan pattern around the ONH of a healthy human was applied. The total mean retinal blood flow, as well as the 3-D velocity profiles and vector orientations were obtained with the three beam DOCT. The measured total venous mean flow was 54.7 μl/min, while the arterial mean flow was 47.8 μl/min.

9541-37, Session 7

En face projection imaging of the human choroidal layers with tracking SLO: swept source OCT system and OCT angiography methods

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In this paper we test and compare the capability of OCT angiography techniques: phase variance, amplitude decorrelation and speckle variance to image the choroidal layers of the human eye. The imaging was performed with a combined swept source OCT (SSOCT) and tracking SLO (TSLO) system. The SSOCT setup uses a rapidly tunable light source (Axun Technologies) with 1040nm center wavelength and 100nm spectral width giving ~6?m axial imaging resolution in the ocular tissue. The sweep rate was 100,000 spectra/s. For the stabilization of spectral sweeps we have used a fiber Bragg grating method. For the correction of the transverse eye motion we utilized a tracking SLO system. The tracking is performed with cross-correlation methods applied to SLO images recorded with a custom-programmed field programmable gate array (FPGA) board. Imaging was performed in the eyes of healthy volunteers. Imaging of the choroidal layer in the human eye is feasible with OCT techniques and benefits from image stabilization provided by the TSLO system. However, optimization of imaging procedures are necessary to maximize the performance of the OCT angiography methods of choice. Also, image segmentation and surface fitting methods used in generation of en face projections play a critical role in visualization of the vasculature, especially of thin choriocapillaris bed.

9541-38, Session 7

1050nm handheld optical frequency domain imaging system for pediatric retinoblastoma patients: optical design and clinical study

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We show a novel optical coherence tomography (OCT) system with a handheld probe specifically developed and validated for complementary clinical imaging of retinoblastoma tumors in pediatric patients. Retinoblastoma is a malignant tumor of the retina, where treatment options aim at reduction of tumor (re)growth risks, and vision preservation. Optimal treatment strongly depends on skilled and detailed clinical assessment. Currently, the patients at risk undergo retinal imaging at monthly intervals under general anesthesia due to limitations of the existing real-time diagnostic tools. Three-dimensional mapping of tissue layer and microvasculature at improved axial and lateral resolution of interference-based OCT imaging answer improves sensitivity for detection of vital tumor tissue concurrent with local treatment. Our system uses 1050nm wavelength for deeper penetration into the choroid layers of retina. The prototype is designed and validated for children in supine position under general anesthesia, where ergonomically designed handheld probe is connected to fiber-based optical setup via umbilical cord. The system conforms to clinical safety requirements, including fully isolated low-voltage electric circuit. Focusing with mechanically tunable lens accommodates for the wide range of optical parameters of the eye that change rapidly in infancy. Imaging is performed at 101.6dB sensitivity with the 10-18μm lateral and sub-8 μm axial resolution varying with focusing.

We will present optical design, performance limitations, and results of the ongoing clinical study, including OCT diagnostic sensitivity, comparison with clinical imaging modalities. We will discuss images of early, active, and treated tumors, as well as follow-up on patients after local and systemic treatments.

9541-44, Session PTues

Detection system characterization and performance of spectral optical coherence tomography based on modulation transfer function

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The complete analysis of all signal roll-off components in spectral optical coherence tomography is investigated using the modulation transfer function (MTF). The analysis of these contributions in the frequency space translates into a simple multiplication of the respective Fourier transformed contribution and significantly simplifies the overall description of the spectral OCT detection system. Based on a proposed simple model we complemented the fundamental MTF description of a ?-spectrometer. It consists of three contributing MTFs, which have a basic influence on the registered signal: footprint, sampling and nonlinear phase averaging MTF. Using this model we addressed the roll-off of the OCT signal in depth caused by spectral sampling and nonlinear phase averaging. These contributions are mainly caused by a sparse and nonlinear sampling defined by the detector size and phase characteristic of detected signal. The interpolation method is commonly used to correct the nonlinearity of the spectrum in wavenumber based on the previously found dependence between wavelength and the pixel number. We compared the influence of linear and FFT interpolation on spectrometers roll-off and proved that it is possible to remove the influence of sampling and nonlinear phase averaging contribution to roll-off. Finally we verified our MTF simulations experimentally and we show that we can reach the efficiency of the k-spectrometer after application of proper spectrum interpolation. This is an important result of this SOCT detection system analysis.

9541-45, Session PTues

Iterative Otsu's method for OCT improved delineation in the aorta wall

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Degradation of human ascending thoracic aorta has been visualized with Optical Coherence Tomography (OCT). OCT images of the vessel wall exhibit structural degradation in the media layer of the artery, being this disorder the final trigger of the pathology. The degeneration in the vessel wall appears as low-reflectivity areas due to different optical properties of acidic polysaccharides and mucopolysaccharides in contrast with typical ordered structure of smooth muscle cell, elastin and collagen fibers. An OCT dimension indicator of wall degradation can be generated upon the spatial quantification of the extension of degraded areas in a similar way as conventional histopathology. The proposed OCT marker can offer in the future a real-time clinical perception of the vessel status to help cardiovascular surgeons in vessel repair interventions. However, the delineation of degraded areas on the B-scan image from OCT is sometimes difficult due to presence of speckle noise, variable SNR conditions on the measurement process, etc. Degraded areas can be delimited by basic thresholding techniques taking advantage of disorders evidences in B-scan images, but this delineation is not optimum in the aorta samples and requires complex additional processing stages. This work proposes an optimized delineation of degraded areas within the aorta wall, robust to noisy environments, based on the iterative application of Otsu's thresholding method. Results improve the delineation of wall anomalies compared with simple application of the algorithm. Achievements could be also transferred to other clinical scenarios: carotid arteries, aorto-iliac or ilio-femoral sections, intracranial, etc.

9541-46, Session PTues

Versatile long coherence length swept source for optical coherence tomography applications

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We present an inexpensive, stable and long coherence length swept source laser for optical coherence tomography (OCT). This laser consists of a polygonal spinning mirror and a multiple quantum well gain chip in a modified Littman-Metcalf cavity. The modification of the cavity consists of multiple illumination of the grating by changing the illumination angle onto the spinning mirror. By changing that, the reflected beam and incident beam onto the mirror are vertically displaced. The quadruple illumination of the grating results in a narrower intracavity filter bandwidth which results in better coherence length. The laser parameters of 24mm coherence length, 100nm bandwidth at 131nm center wavelength, an output power of 20mW, after the fiber, and a signal to noise ratio of 99dB make it ideally suited for imaging of biological samples. The imaging capability of the system was demonstrated by measuring the first layers of a sheep eye as deep as 6mm. The advantage of this design is that a novel intracavity filter is used to increase the coherence length and that almost any wavelength can be obtained simply by changing the gain chip.

9541-47, Session PTues

Frequency sweep jitter and wander of a Vernier-Tuned Distributed Bragg Reflector (VT-DBR) laser at 1550 nm in OCT applications

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The short-term jitter and longer-term wander of the frequency sweep profile of a Vernier-Tuned Distributed Bragg Reflector (VT-DBR) laser at 1550 nm used in OCT applications is characterized in this work. The VT-DBR has demonstrated success in source-swept OCT (SSOCT), performing both intensity and phase-sensitive OCT. The purpose of this paper is to investigate one of the unique aspects of the VT-DBR laser that makes it successful in OCT: the stability of the linear optical frequency sweep of the source. Jitter measurements of the optical frequency are recorded using a 3-cavity 100 GHz free spectral range solid etalon. A gas absorption reference cell is used for wander characterization. We report that the VT-DBR jitters by no more than 100 MHz in optical frequency while sweeping at an 8 kHz repetition rate. Longer-term wander provides insight into the accuracy of the VT-DBR self-calibration routine which produces an intrinsically linear optical frequency sweep. Over an 8-hour data collection period, the system maintains a linear sweep with an optical frequency step on the order of 105 MHz per 2.5 ns with +/- 0.02 MHz per 2.5 ns peak-to-peak deviation. We find that the absolute frequency drifts by 13.2 GHz (102 pm) over the 8-hour period. Results show that using calibration with a gas reference cell, picometer absolute wavelength accuracy over 1 sweep of the laser can be achieved. Stability and accuracy limits are thought to be due to electronic drive circuitry in the current design.

9541-48, Session PTues

Spectral domain optical coherence tomography for ex vivo brain tumor analysis

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Non-contact imaging methods to distinguish between healthy tissue and brain tumor tissue during surgery would be highly desirable but are not yet available. Optical Coherence Tomography (OCT) is a non-invasive imaging technology with a resolution around 1-15 ?m and a penetration depth of 1-2 mm that may satisfy the demands. To analyze its potential, we measured ex vivo human brain tumor tissue samples from 10 patients with a Spectral Domain OCT system (Thorlabs CALLISTO: center wavelength of 930 nm) and compared the results with standard histology. In detail, three different measurements were made for each sample. First the sample was measured directly after surgery, and then it was embedded in paraffin (also H&E staining) and examined for the second time. At last, the slices of each paraffin block cut by the pathology were measured. Each time a B-scan was created and for a better comparison with the histology a 3D image was generated, in order to get the corresponding en face images. In both, histopathological diagnosis and the analysis of the OCT images, different types of brain tumor showed difference in structure. This has been affirmed by two blinded investigators. Nevertheless the difference between two images of samples taken directly after surgery is less distinct. To enhance the contrast in the images further, we employ spectroscopic OCT and pattern recognition and compare the results to the histopathological standard.

9541-49, Session PTues

Optical wavefront aberrations in optical coherence tomography

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The transfer function for optical wavefront aberrations in single-mode fiber (SMF) based optical coherence tomography (OCT) is presented. Fresnel propagations and overlap integrals quantify the loss in measured OCT signal due to optical wavefront aberrations. The proposed transfer function models a Gaussian beam interacting with a scattering medium. The model predictions are validated with measurements on a scattering medium obtained with an adaptive optics optical coherence tomography setup. Knowledge of the transfer function can lead to better performance of adaptive optics through more efficient aberration correction algorithms and/or the development of accurate metrics for OCT.

9541-50, Session PTues

An algorithm for simulating image formation in optical coherence tomography for cylinder scattering

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An algorithm for the simulation of Fourier domain optical coherence tomography (OCT) images of a cylinder based on an analytical solution of Maxwell's equations is presented. The characteristics of the simulated OCT signal are discussed and the whispering gallery modes as well as the geometrical optics signals from the cylinder are identified. An OCT scanner with an incident Gaussian beam is implemented to simulate two-dimensional B-scans.

The basic phenomena of single cylinder scattering are reexamined with the OCT algorithm. The OCT signal is compared to the backscattered intensity of the cylinder. The Debye series expansion is employed to explain the resulting signals. An OCT scanning process with a focussed beam is simulated.

The simulated images show the front and back side of the dielectric homogenous cylinder and the additional signals arise from the periodic whispering gallery modes and the geometrical contributions (multiple reflection along the optical axis or complex star-like pathways). Various refractive indices can be chosen for the cylinder and the surrounding medium. The refractive index of the scatterer can be complex and various cylinder setups can be investigated. The algorithm can be extended to include a detector with an aperture to simulate images that can be compared to a laboratory experiment. In the future, it will be possible to investigate more complex geometries when a solution for the scattered electromagnetic fields is found.

9541-51, Session PTues

Optimal integration time in OCT imaging

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When measuring static objects with 3D OCT, two opposing trends occur: If the integration time is too short, the measurement is noisy resulting in granulated textures on measured objects. If the integration time is too long, drifts e.g. due to thermal effects or unstable laser sources lead to blurred images. The Allan variance is a scheme to find the optimal integration time in terms of reducing noise without picking up signal drift. A long-term measurement with short integration time of a reference target under realistic conditions is needed to obtain the database for the analysis. Longer integration times are simulated by taking the average of subsequent samples. The Allan variance being the mean of the squared differences between two consecutive measurements is calculated for different integration times. The optimal integration time is achieved for minimal Allan variance. The method is discussed with simulated data and applied in the practical example of the detection of water inclusions in calcite with a 3D OCT device. With the minimal integration time of 20 microseconds, the water inclusions appear with a stained surface. With the integration time increased towards the optimal time of 500 microseconds, the surfaces of the water inclusions get smoother and easier to discriminate from the background.

9541-52, Session PTues

Motion artefacts simulation in the imaging of the ocular media

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Eye motion is an essential function of our vision system. Even when fixating on a static object the eye undergoes "miniature" motion to prevent the image from fading away. This causes significant distortions for an ophthalmic investigation with high resolution imaging modalities, such as retinal or corneal optical coherence tomography.

Motion can be separated into two components: intrinsic and extrinsic. Intrinsic eye motion within the eye sockets has been extensively studied by researchers in application to neurophysiology. The extrinsic component (head and body motion, pulsation and respiration) is much less investigated but significantly contributes to the observed motion. It is difficult to describe theoretically the effect of motion on an imaging modality since it depends on the specific scanning geometry and frequency, number of repetitions and averaging approach.

Based on the available literature and experimental measurements we have developed a theoretical spectral model for stochastic simulation of three dimensional eye motion. This model is implemented in an intuitive software package which allows virtual scans of the defined eye structures to be obtained with a ray tracing algorithm. Simulation combines timing

information related to the user-defined scanning strategy and technical specifications of the imaging hardware with simulated motion in time. Imaging data is simulated further analysed and compared with the expected results to evaluate performance.

The framework allows the testing of different scanning geometries, sequences and averaging approaches by performing simulations of the scanning performance for different eye shapes and motion realisations. In this way the user can design the optimal scanning strategy and hardware requirements for the specific application.

9541-53, Session PTues

Investigation of changes in fractal dimension from layered retinal structures of healthy and diabetic eyes with optical coherence tomography

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OCT is usually employed for the measurement of retinal thickness characterizing the structural changes of tissue. However, fractal dimension (FD) could also character the structural changes of tissue. Therefore, fractal dimension changes may provide further information regarding cellular layers and early damage in ocular diseases. We investigated the possibility of OCT in detecting changes in fractal dimension from layered retinal structures. OCT images were obtained from diabetic patients without retinopathy (DM, n = 38 eyes) or mild diabetic retinopathy (MDR, n = 43 eyes) and healthy subjects (Controls, n = 74 eyes). Fractal dimension was calculated using the differentiate box counting methodology. We evaluated the usefulness of quantifying fractal dimension of layered structures in the detection of retinal damage. Generalized estimating equations considering within-subject inter-eye relations were used to test for differences between the groups. A modified p value of <0.001 was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed to describe the ability of fractal dimension to discriminate between the eyes of DM, MDR and healthy eyes. Significant decreases of fractal dimension were observed in all layers in the MDR eyes compared with controls except in the INL. The highest AUROC values estimated for fractal dimension were observed for the OPL and OS when comparing MDR eyes with controls. Our results suggest that fractal dimension of intraretinal layers could provide useful information to differentiate pathological from healthy eyes. Further research is warranted to determine how this approach may be used to improve diagnosis of early retinal neurodegeneration.

9541-54, Session PTues

Combining optical coherence tomography with single fiber reflectance spectroscopy to determine the scattering anisotropy of turbid media

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Both Optical Coherence Tomography (OCT) and Single Fiber Reflectance Spectroscopy (SFR) can be used to determine various optical properties. The scattering anisotropy is a property neither single technique can reliably determine, but combining OCT and SFR in a single measurement could make this possible. The scattering anisotropy is intimately associated with small scale tissue changes and therefore may change during e.g. tumor development. Therefore quantification of the scattering anisotropy with OCT and SFR may allow for improved minimally-invasive, in vivo discrimination between healthy and diseased tissue. We aim to provide a proof-of-principle by determining the scattering anisotropy of different dilutions of Intralipid-20%.

The scattering coefficient (μs) can be determined using OCT and the reduced scattering coefficient using SFR ($\mu s'$). Consequently, from SFR and OCT measurements at the same wavelengths, the – wavelength dependent – scattering anisotropy (g) can be calculated using $\mu s' = \mu s * (1-g)$. The scattering coefficient can be extracted from an OCT measurement by fitting the signal as a function of depth to a widely used model for the signal as a function of the attenuation coefficient (Faber et al., Optics Express 12(19), 2004). With SFR the reduced scattering coefficient can be calculated from at least two measurements of the same sample using fibers with different diameters (Kanick et al., Optics Letters, 36(15), 2011). We performed OCT and SFR measurements at 600 and 848 nm for different dilutions of Intralipid-20%, to determine the scattering anisotropy for these solutions and provide a proof-of-principle for scattering anisotropy determination with OCT and SFR.

9541-55, Session PTues

Speckle reduction process based on digital filtering and wavelet compounding in optical coherence tomography for dermatology

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Optical Coherence Tomography (OCT) has shown a great potential as a complementary imaging tool in the diagnosis of skin diseases. Speckle noise is the most prominent artifact present in the OCT images and could limit the interpretation and detection capabilities. In this work we propose a new speckle reduction process and compare it with various denoising filters with high edge-preserving potential, using several sets of dermatological OCT B-scans. For its assessment we used a custom-designed spectral domain OCT and two different data set groups. The first group consisted in 11 datasets of a single B-scan captured N times (with N<30), the second were two 3D volumes of 512 B-scans. As quality metrics we used signal to noise (SNR), contrast to noise (CNR) and equivalent number of looks (ENL) ratios. Our results show that a process based on a combination of a 2D enhanced sigma denoising filter and a wavelet compounding B-scans filter achieves the best results in terms of the enhancement quality metrics. In the first group of individual B-scans we achieved improvements in SNR, CNR and ENL of 13.9dB, 1.6 and 174.4 respectively; for the 3D volume datasets the enhancements were of 16.8dB, 3.21 and 1288. Our results suggest that the proposed enhancement process may significantly reduce speckle, increasing SNR, CNR and ENL and reducing the number of extra acquisitions of the same frame.

9541-56, Session PTues

Azimuthally-invariant Muller-matrix mapping of optically anisotropic networks of biological crystals

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Biological tissues and liguids represent structurally inhomogeneous optically anisotropic media with absorption. To describe the interaction of polarized light with such sophisticated systems, more general approximation based on Mueller-matrix formalism is required. Nowadays many practical techniques based on measuring and analyzing the Mueller-matrices of the samples under investigation are being used in biological

and medical researches. During recent 10-15 years a separate direction – laser polarimetry – has been formed in matrix optics [ ]. On its basis the interconnections between the set of statistical moments of the 1st-4th order, correlation, fractal parameters were determined, which characterize the distributions of Mueller matrix elements and the parameters of linear birefringence of fibrillar protein networks of human biological tissues. The diagnostics of pathological changes of skin derma, epithelial and connective tissues of the women's reproductive organs, etc. was realized on this basis.

This research work is focused on determining the potentialities of diagnostics of pathological changes in mammary gland basing on polarization analysis of Mueller – matrix images of polycrystalline networks of blood plasma albumins and globulins.

1. The model of blood plasma layer considering the mechanisms of optically anisotropic absorption – linear and circular dichroism of protein networks was suggested.

2. Mueller-matrix rotation invariants characterizing polarization manifestations of biological network optical anisotropy are determined.

3. The interconnections between the statistical, correlation and fractal parameters characterizing the Mueller-matrix images of blood plasma layer and the peculiarities of the mechanisms of optically anisotropic absorption biological crystals network were found.

9541-57, Session PTues

Autofluorescence polarimetry of blood plasma in differentiating of liver pathology

Yuriy A. Ushenko, Yuriy Fedkovych Chernivtsi National Univ. (Ukraine)

Biological tissues represent structurally inhomogeneous optically anisotropic media with absorption. To describe the interaction of polarized light with such sophisticated systems, more general approximation based on Mueller-matrix formalism are required. Nowadays many practical techniques based on measuring and analyzing the Mueller-matrices of the samples under investigation are being used in biological and medical researches. At the same time there practically no data concerning polarization manifestations of fluorescence effects in biological tissues in modern literature. Therefore, the task of complex uniting the diagnostic potentialities of the techniques of laser polarimetry and laser fluorescence proves to be topical.

In this research the model of complex optical anisotropy, possessed by protein networks of the tissues of liver is suggested, and on this ground the method of Mueller-matrix mapping of laser polarization fluorescence of blood plasma layers is developed.

1. The model of laser polarization fluorescence of biological tissues and liquids considering the mechanisms of optically anisotropic absorption – linear and circular dichroism optically-anisotropic networks was suggested.

2. Mueller-matrix rotation invariants characterizing polarization manifestations of laser fluorescence are determined.

3. The interconnections between the statistical, correlation and fractal parameters characterizing the Mueller-matrix images of laser polarization fluorescence and the peculiarities of the mechanisms of optically anisotropic absorption of blood plasma layers were found.

9541-58, Session PTues

Nonlinear amplification and detection for swept-source optical coherence tomography

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We propose that the broadband nonlinear optical process can be used to improve the axial resolution and the signal-to-noise ratio (SNR) of the swept-source optical coherence tomography (SS-OCT). A theoretical study of the system is presented. A comparison between the proposed system and SS-OCT is given in terms of axial resolution and SNR.

9541-59, Session PTues

Enhanced spectral and time domain OCT in dependence on transverse velocity and SNR

Julia Walther, Edmund Koch, Univ Carl Gustav Carus Dresden (Germany)

A variety of promising approaches for quantitative flow velocity measurement in OCT have been proposed in recent years. The question is: Which method gets the most precise flow velocity out of the interference signals detected. In this study, we describe first the link between joint spectral and time domain OCT (jSTdOCT) and phase-resolved Doppler OCT and introduce secondly a new model of enhjSTdOCT with dependency on the transverse velocity component and the signal-to-noise-ratio (SNR) of the OCT signal detected. By numerically and experimentally verified measurements, we present that enhjSTdOCT has the potential to significantly reduce the noise of the velocity measurement.

9541-39, Session 8

Heartbeat optical coherence tomography *(Invited Paper)*

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Intravascular optical coherence tomography (IV-OCT) has generated a wealth of data that has deepened our understanding of coronary artery disease and catheter-based interventions. However, IV-OCT images are affected by cardiac motion artifacts, undersampling and non-uniform rotational distortion (NURD). In this study, we demonstrate a new modality of IV-OCT, called Heartbeat OCT. Using a motorized catheter and a Fourier Domain Mode Locked laser, we achieved an imaging speed up to 5600 frames per second (fps) in vitro and 4000 fps in vivo, which is 25 times faster than commercial systems. We demonstrate the capability of Heartbeat OCT by in vitro and in vivo imaging experiments. Heartbeat OCT eliminated cardiac motion artifact by finishing data acquisition within one cardiac cycle. Using a motorized catheter also overcame the undersampling and NURD.

9541-40, Session 8

Single input state, single-mode fiber-based polarization sensitive OFDI by eigenpolarization referencing

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Fiber-based polarization sensitive (PS) OFDI is more challenging than free-space implementations. Using multiple input states, fiber-based systems provide sample birefringence information with the benefit of

a flexible sample arm but come at the cost of increased system and acquisition complexity, reduced acquisition speed and/or increased acquisition bandwidth. Here we show that with the calibration of a single polarization state, fiber-based configurations can approach the conceptual simplicity of traditional free-space configurations. Under reference to the eigenpolarizations of a wave plate, we control the polarization transformation of a single-mode fiber towards the sample, while solving for the fiber Jones matrix of the output fiber. The calibration can be obtained within a few seconds and with the sample in place. Our method is validated on several biological samples.

9541-41, Session 8

Fully automated 1.5 MHz FDML laser with more than 100mW output power at 1310 nm

Wolfgang Wieser, Thomas Klein, Wolfgang Draxinger, Optores GmbH (Germany) and Ludwig-Maximilians-Univ. München (Germany); Robert A. Huber, Univ. zu Lübeck (Germany)

While FDML lasers with MHz sweep speeds been presented four years ago, these devices required manual control for startup and operation. Here, we present a fully self-starting and continuously regulated FDML laser with a sweep rate of 1.5 MHz. The laser operates over a sweep range of 115 nm centered at 1315 nm, and provides very high average output power of more than 120 mW. We characterize the laser performance, roll-off, coherence length and investigate the wavelength and phase stability of the laser output under changing environmental conditions. The high output power allows optical coherence tomography imaging with an OCT sensitivity of 108 dB at 1.5 MHz.

9541-42, Session 8

Optical palpation: replicating the sense of touch at high resolution using OCT and a compliant stress sensor

Brendan F. Kennedy, Kelsey M. Kennedy, Shaghayegh Es'haghian, Lixin Chin, David D. Sampson, The Univ. of Western Australia (Australia)

Recently, we reported on a new tactile imaging technique for mapping stress at the surface of a tissue that replicates the sense of touch, or palpation, through optical coherence tomography (OCT) imaging of the deformation of a compliant stress sensor. In "optical palpation", a translucent, silicone layer is placed on the tissue surface and a compressive load is applied. Variations in the mechanical properties of the tissue below the layer result in a spatially varying layer thickness after compression. OCT is used to measure the initial and final thickness of the layer at each xy location and the corresponding stress is estimated using the stress-strain response of the layer as a reference. Optical palpation may be considered as a variant of optical coherence elastography (OCE). Unlike other OCE techniques, optical palpation is independent of the OCT signal acquired from the tissue, relying only on measurements of the layer thickness to form an image. As a result, optical palpation may be more reliable in tissue, as it is unaffected by issues such as attenuation of the optical beam. Here, we report the use of optical palpation in two ways. Firstly, we perform in vivo imaging of skin lesions, sensing the average mechanical properties at depths well below that imaged by OCT. We also show how measurement of the surface stress can be combined with compression OCE to provide quantitative maps of tissue elasticity in freshly excised malignant human breast tissue.

9541-43, Session 8

Rotational distortion correction in catheter-based optical coherence tomography using speckle decorrelation

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Optical coherence tomography (OCT) is being increasingly used in biomedical and clinical applications, in particular in gastrointestinal (GI) and intravascular imaging due to its capability for high-speed optical sectioning, high resolution and moderate penetration depths. Catheter probes are commonly used when imaging luminal organs, which provide rotational scanning with a motor that rotates the optical assembly at the tip of the probe. The flexibility required by the catheter impacts the fidelity of the torque transfer from the motor to the probe, and therefore the rotation measured by the motor encoder does not match 1:1 the physical angular position of the probe tip. This produces non-uniform rotation distortion (NURD) during image acquisition, which deforms the imaged structures due to the unknown true azimuthal position of the probe during each scan. NURD is not stable with time, which inhibits a calibration-based approach, and thus remains as an important image artifact in catheter-based OCT diagnostics. We present a new technique for the correction of NURD in any type of catheter, based on the statistical variations of speckle between adjacent A-lines using intensity-based dynamic light scattering, and demonstrate its suitability in a commercial GI OCT system deployed with a balloon catheter. We are able to determine quantitatively the rotational speed of the catheter during each frame independently, facilitating real-time correction. This technique provides high time resolution with respect to the determination of the rotational speed, which can correct for sudden spikes in NURD as well as for slow variations in rotational speed.

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9542-1, Session 1

Plasmonic photoionization for nanometric-scale radiation therapy *(Invited Paper)*

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Targeting individual cells within a heterogeneous tissue is a key challenge in cancer therapy, motivating numerous new approaches for cancer treatment that complement the shortcomings of conventional therapies. The small dimensions of isolated cell clusters require highly localized interactions that could be driven by focused laser beams; however, light-tissue interactions often involve macroscopic processes that may harm healthy nearby tissue. Here, we present a new technique for specific targeting of living cells using nanometric plasmonic photoionization. Using only a single, intense femtosecond laser pulse and specifically designed functional gold nanorods, we experimentally demonstrate rapid and effective cell death that is nonlinearly dependent on pulse duration and irradiance. The experimental results are supported by a detailed physical model for the pulse-particle-medium interactions. A good correlation is found between the calculated total energy of ionized electrons and the observed cell death rates, suggesting that photoionization plays the dominant role in this process.

9542-2, Session 1

Delivery of molecules in ex vivo corneal endothelium by femtosecond laser activated carbon nanoparticles

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Introduction

Human corneal endothelial cells (HCECs) form a monolayer, called endothelium, at the innermost face of the cornea and are the sole engine for corneal transparency. Nevertheless, they are a vulnerable population incapable of regeneration in humans and, in case of pathologies, the HCECs density can decrease resulting in permanent cornea opacity, called edema. Up to now, the only option for treating corneal edema is corneal grafting using a donor cornea. But a great global scarcity of corneal tissue is observed and needs alternative solutions. Several therapeutic molecules like proteins, peptides or DNA, have been identified to inhibit HCECs death and even promote proliferation of these cells [1]. The difficulty of gene and drug delivery is transport across cell membrane, normally impermeable to high weight molecules. Several methods were studied to deliver molecules into mammalian cells. Viral methods are the most used because of their high efficiency (until 100%) but represent high risks of immune and inflammatory responses, ontogenesis and mutagenesis. Chemical methods are non-viral methods which permit a safer molecules delivery without toxicity, but their efficiency is considerably lower (less than 10%) than viral methods. Finally, physical methods consist to generate nanoscale holes in cell membranes by physical stimuli in order to deliver genes and drugs directly into cytoplasm, we called this phenomena cell permeabilization. Membrane holes can be generated efficiently by several types of physical stimuli: electric pulses, ultrasounds, laser irradiation.

Femtosecond (fs) laser is well known and used in the field of ophthalmology, especially by the myopia surgery. Various single cells have been permeabilized with high efficiency and very low cell damage using a focused fs laser beam. However, this technique is only single-cell treatment and can't be applied on entire tissue like corneal endothelium. The use of micro/nanoparticles can multiply field of action of fs laser in order to perforate simultaneously a lot of cells. Three types of micro/nanoparticles have been used for cell permeabilization: polylactic spheres [2], gold nanoparticles [3] and carbon nanoparticles [4], each of these interacting differently with fs laser.

We decided to use carbon nanoparticles (CNPs) because of its low cost, ease of production and low incubation time. Irradiation of CNPs by fs laser prompts a photo-acoustic reaction able to disorganize ephemerally near cell membranes and hence to generate holes. Initially used for in vitro non-adherent cells, the aim of this work is to adapt this technique on ex vivo HCECs.

Materials and methods

Human corneas unsuitable for grafting for serological reasons and procured by Auvergne-Loire cornea bank (Saint-Etienne, France) were used after informed consent of the relatives, as authorized by French bioethics laws. All procedures conformed to the tenets of the Declaration of Helsinki for biomedical research involving human subjects.

Corneas were placed on homemade support with endothelial side upwards in order to flatten central corneal surface on 6 mm diameter. CNPs and dextran AlexaFluor 488, a fluorescent molecule of 4 kDa (ex:495nm/ex:519nm) were added at the corneal surface. Finally, half of the endothelium was irradiated with a 500 μm diameter Ti:Sa fs laser focalized spot as shown on fig. 1.

Different fluences from 5 mJ/cm? to 15 mJ/cm? were tested to assess their influence on delivery efficiency and toxicity. After laser irradiation, corneas were rinsed to remove CNPs and not delivered fluorescent dextran. Endothelial cells were then stained by Hoechst 33342 and propidium iodide to highlight nuclei of all cells and nuclei of dead cells respectively.

Three microscope images of irradiated surfaces and control surfaces were realized to assess delivery efficiency and toxicity. Total endothelial cell density (ECD) and dead ECD were determined in control and irradiated surfaces by image analysis (Image J). ECD of permeabilized cells containing fluorescent dextran (Dextran(+)), was realized manually. Efficiency, cell death, cell detachment and global toxicity were calculated by these equations:

Efficiency (%)=(Dextran(+)ECD*100)/(Total ECD)

Cell death (%)=([Dead ECD(irradiated)-Dead ECD (control)]*100)/(Total ECD (control))

Cell detachment (%)=([Total ECD(control)-Total ECD (irradiated)]*100)/(Total ECD (control))

Global toxicity (%)=Cell death+Cell detachment

Results

Microscope images of different fluences tested are shown in Fig. 2. At 5 mJ/cm?, no dextran(+) cell was observed showing that cells were not permeabilized at this fluence. The presence of dextran(+) cells were observed only from a fluence of 7.5 mJ/cm? and seemed to increase with the fluence.

Cell counts were realized with cornea presenting dextran(+) cells (7.5, 12.5 and 15 mJ/cm? fluences) and are summarized in Tab. 1.

Fluences Efficiency Cell death Cell detachment Global toxicity

7.5 mJ/cm? 1.29±0.3% 1.02±0.05% -5.74±6.77% -4.67±6.72%

12.5 mJ/cm? 5.97±2.86% -0.73±0.15% 14.96±7.07% 14.37±7.21%

15 mJ/cm? 10.57±4.72% -12.17±5.43% 24.4±4.57% 17.75±0.96%

Cell counts verified that delivery efficiency was dependent of the fluence used. With the maximal fluence of 15 mJ/cm?, we obtained of mean efficiency of 10,57%. Negative values of cell death mean that the dead ECD was higher for control surface than for irradiated surface. Negative values of cell detachment mean that the total ECD was higher for irradiated surface than control surface.

Global toxicity, determined by cell death and cell detachment, seemed also to increase with fluence. However, negative value observed at 7.5 mJ/cm? showed that the quantification method was not significant because irradiated surface was in better condition than control surface. This observation was probably due to a high ECD variability on a same cornea.

Discussion

We succeeded in ex vivo application of cell permeabilization by femtosecond laser activated carbon nanoparticles. This preliminary results show the potential of this method but also the need of optimizations to increase delivery efficiency and to better quantify toxicity.

Acknowledgments

We acknowledge Emmanuel baubeau, Julien Granier and Gregory Egaud from GIE Manutech-USD for their help to femtosecond laser settings.

9542-3, Session 1

Photodynamic effects of gold nanoparticles in a breast cancer cell line (MCF-7) in vitro

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Cancer refers to uncontrolled cell division of abnormal cells in organs. A substantial proportion of cancer could be prevented as they are lifestyle dependent and caused by both external and internal factors. Despites numerous efforts, the condition remains a dominant health-threatening issue worldwide. In United States, over 1.6 million new cases were expected to be diagnosed and would have resulted in 585,720 deaths in 2014. Breast cancer is one of the most frequently diagnosed cancer worldwide and is the leading cancer among South African women.

Among available treatments, Photodynamic therapy (PDT) is a targeted and light induced therapy that depends upon successful localization and specific activation of a chemotherapeutic agent to induce cell death. Nanotechnology in cancer therapy provides interesting possibilities in detecting and eradicating tumors with minimal damage to health tissues. This study aimed to synthesize and characterize a conjugate made of Zinc-Phthalocyanine (ZnPc) and gold nanoparticles (AuNPs), to identify subcellular localization as well as effects of the conjugate prior and post laser irradiation in a breast cancer cell line (MCF-7). This presentation will discuss the outcomes of the combined treatment on cell morphology, viability, proliferation and cytotoxicity. What is more, this work will also evaluate the induced cell death mechanisms and gene expression subsequent to this treatment.

9542-4, Session 1

Metal nanoparticles of different shapes influence on optical properties of multilayered biological tissues

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Diagnosis of pre-cancer states of biological tissues is still a challenge. Often the first changes occur in the deeper layers of tissue, which are hard to reach by optical range of wavelengths. The use of metal nanoparticles, which have plasmonic enhancement of special wavelengths, according to the geometry, material and media around nanoparticle, is strengthening its position in optical diagnosis. The experiments to reveal the influence of nanoparticle presence on optical properties of biological tissues, where second layer has a photosensitiser with or without nanoparticles of different sizes and shapes in different concentrations, were performed.

To measure changes in optical properties of epithelial tissue a set of two-layered phantoms was made. The first layer has Intralipid 2% and India Ink 0.0002% as absorbing media and was made of 4 thicknesses: 100:100:400 um mimics epithelium. The second layer, which models dermis and consists of Intalipid 3% as a scattering media and Gelatin 5% as absorbing media, has photosensitiser (Protoporphyrine IX) and metal nanoparticles of different diameters and concentrations. To excite fluorescence of PPIX 532 and 632.8 nm wavelength were used. The source and detector were placed on 6 distances from each other, from 200 um to 1300 um. The numerical aperture of fibres was 0.22, core diameter – 200 um. Spectra were collected by means of spectrometer .

The results are being discussed.

9542-5, Session 1

Review of dermatology use of 5-aminolevulinic acid photodynamic therapy in China from 1997 to 2013

Peiru Wang, Guolong Zhang, Shanghai Skin Disease Hospital (China); Xiuli Wang, Shanghai Skin Diseases and STD Hospital (China)

Abstract?The prodrug 5-aminolevulinic acid (ALA) and its ester derivatives have been used in photodynamic therapy (PDT) in dermatology worldwide. In China, ALA-PDT was first used to treat urethral condylomata acuminata and non-melanoma skin cancers in 1997. A powder formulation of ALA hydrochloride was approved by the Chinese Food and Drug Administration for the treatment of condylomata acuminata in 2007. Since then, ALA-PDT has been rapidly and sufficiently developed and numerous clinical studies as well as off-label use of ALA-PDT have been carried out in China. Large successful experience of treating condylomatas was accumulated compared with Western countries. Nowadays, ALA-PDT has been carried out in 600 domestic hospitals and completed a total of 200,000 cases of treatment, including warts, Bowen papulosis, actinic keratosis (AK), Bowen's disease, erythroplasia of Queyrat, BCC, squamous cell carcinoma (SCC), Paget's disease, acne, photoaging, atrophic lichen sclerosus, genital lichen planus, seborrheic keratosis, and fungal infectious granuloma. To reflect the progress of ALA-PDT in China, several major Chinese and English databases were searched and published data were reviewed in this article.

9542-6, Session 2

Optical properties of rabbit brain in the red and NIR: Changes observed under in-vivo, post-mortem, frozen and formalin-fixated conditions *(Invited Paper)*

Andreas Pitzschke, Blaise Lovisa, Olivier Seydoux, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Matthias Haenggi, Markus Oertel, Inselspital Bern (Switzerland); Matthieu Zellweger, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Yanik Tardy, Medos International Sàrl (Switzerland); Georges A. Wagnières, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Since the in-vivo determination of tissue optical properties is complicated, they are often determined ex-vivo and used for in vivo applications. However, the method of sacrifice, tissue preparation and storing techniques frequently alter them. Unfortunately, little is known about the changes of optical properties of post-mortem tissues. The objective of this study was to quantify the optical properties of brain tissues in-vivo and post-mortem for two methods of sacrifice (exanguination or injection of KCl), and assess changes thereto due to tissue handling post-mortem. The study was carried out on eight female New Zealand white rabbits. The fluence rate was measured in-vivo, just post-mortem, and after 6 weeks' storage of the head at -20 °C or in 10 % formaldehyde solution. It was measured with an interstitial isotropic detector placed in the brain when light at 635, 671 and 808 nm was delivered with a cylindrical optical fiber-based diffuser inserted into the brain. Only minimal changes in the

effective attenuation coefficient μeff were observed for the two methods of sacrifice. Under all tissue conditions, μeff decreased with increasing wavelengths. After long-term storage at -20 °C, μeff decreased on average by about 20 % at all wavelengths, whilst it increased by about 10 % at all wavelengths after storage in formaldehyde. We demonstrated that μeff is not very sensitive to the method of animal sacrifice, that tissue freezing significantly alters tissue optical properties, and that formalin fixation may affect the tissue's optical properties.

9542-7, Session 2

Optical spectroscopy for stereotactic biopsy of brain tumors

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A fiber-based mechano-optical device for stereotactic biopsies of brain tumors is developed. Two different fluorophores are employed to improve the safety and reliability of this procedure: The fluorescence of intravenously applied Indocyanine Green facilitates the recognition of blood vessels and thus helps minimize the risk of cerebral hemorrhages. 5-aminolevulinic-acid-induced protoporphyrin IX (PpIX) fluorescence is used to localize vital tumor tissue. Compact and efficient side-fire fibers with an outer diameter of 240 μm are manufactured for tissue characterization within the suction region of the biopsy needle. Moreover, the suitability of two different PpIX excitation wavelengths regarding practical aspects is investigated.

9542-8, Session 2

Spectral monitoring of stereotactic interstitial PDT of glioblastoma

Adrian Rühm, Herbert Stepp, Wolfgang Beyer, Maurice Hermwille, Julian Rudrof, Ronald Sroka, Friedrich-Wilhelm Kreth, Klinikum der Univ. München (Germany)

Objective:

The success of fluorescence guided resection (FGR) for the treatment of malignant glioma has also stimulated research efforts to utilize the underlying biophysical effects therapeutically by means of interstitial photodynamic therapy (iPDT). Being able to identify potential responders of such kind of treatment in advance would be highly desirable.

Material and Methods:

In the course of individual treatment attempts, in which iPDT based on 5 aminolevulinic acid (5-ALA) induced protoporphyrin IX (PpIX) has been applied to glioblastoma, light applicators have been stereotactically placed into the tumor region after careful dosimetry planning. The applicators could be used for light delivery to the tumor, but also for light collection and detection. The light propagating from each applicator to each other applicator could thus be recorded before and after the PDT treatment for monitoring purposes. Using a spectrometer for detection allowed to separately analyze a) the excitation light of 633 nm wavelength which is simply transmitted between two applicators and b) the PpIX fluorescence light of ~704 nm wavelength which predominantly arises from the vital tumor regions illuminated in each case. In order to identify potential predictors for treatment response, the obtained spectral data were processed in different ways and then statistically correlated with treatment outcome.

Results:

In all cases the PpIX fluorescence was bleached completely after the PDT treatment. The transmission of the treatment light usually decayed to some extent during PDT. The factor by which the transmission signal at 633 nm had decayed after the PDT treatment could be clearly identified as a potential predictor for treatment response. A sufficiently moderate decay correlates positively with the observation of a progression free survival time of at least 12 months. The transmission of the treatment light and the fluorescence intensity measured between each applicator pair did not show a monotonous decay with increasing applicator distance. The comparison of these data with model calculations points to local inhomogeneities of the optical properties within the treated tissue region. Based on simplifying assumptions, the mean value of the effective attenuation coefficient ?eff within the tumor region as well as local deviations of the absorption coefficient ?a from the corresponding mean value could be derived.

Conclusion:

Spectral data obtained during iPDT treatment attempts have provided novel additional information which might be suitable for predicting treatment response. Further analysis of the distance dependence of such data might allow to characterize the distribution of the treatment light within the inhomogeneous tumor volume in advance. This information could eventually be used to optimize the treatment planning and thus the treatment outcome for each individual patient.

9542-9, Session 2

Increasing the therapeutic index of PDT for glioma with hypothermia

Carl J. Fisher, Univ. of Toronto (Canada); Carolyn Niu, Princess Margaret Cancer Ctr. (Canada); Warren Foltz, Univ. Health Network (Canada); Yonghong Chen, Princess Margaret Cancer Ctr. (Canada); Lothar D. Lilge, Princess Margaret Cancer Ctr. (Canada) and Univ. of Toronto (Canada)

While PDT for glioma has been demonstrated successful in particular cases and small scale studies it is generally accepted that the photosensitizer selectivity towards highly proliferating cells can not be exploited for effective therapies due to this high intrinsic sensitivity of neurons and other normal brain cells. In order to improve the therapeutic index of ALA induced PPIX mediated PDT for glioma we investigated the use of mild hypothermia (32 C) as a co-therapy. In a murine brain tumour model (RG2) we monitored the PDT effects for edema, inflammation and necrosis using MRI T1, T2 and DCE imaging in a 7T magnet with high spatial resolution.

The T2 images showed that normal brain subjected to ALA induced PPIX mediated PDT under mild hypothermia had a reduced area of edema (reduced by ~50%) and faster recovery compared to normothermic animals.

In tumour bearing animals the same effect was seen for normal brain in proximity to the tumour.

These in vivo results confirm some of our prior work in vitro which demonstrated higher PPIX accumulation in highly proliferating cells and improved survival for normal brain tissue.

9542-10, Session 2

In vitro study for photodynamic therapy using Fotolon® in glioma treatment

Sara Mohamed, The German Univ. in Cairo (Egypt); Wolfgang Zimmermann, Ludwig-Maximilians-Univ. München (Germany); Dirk Hüttenberger, APOCARE Pharma GmbH (Germany); Rainer Wittig, Ulm Univ. (Germany); Mahmoud H. Abdelkader, The German Univ. in Cairo (Egypt); Herbert Stepp, Klinikum der Univ. München (Germany)

No Abstract Available

9542-11, Session 3

Thulium fiber laser damage to the ureter *(Invited Paper)*

Christopher R. Wilson, Luke A. Hardy, The Univ. of North Carolina at Charlotte (United States); Pierce B. Irby M.D., Carolinas Medical Ctr. (United States); Nathaniel M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: Our laboratory is studying the experimental Thulium fiber laser (TFL) as a potential alternative lithotripter to the clinical gold standard Holmium:YAG laser. Safety studies characterizing undesirable Holmium laser-induced damage to ureter tissue have recently been reported. The objective of this study is to provide similar safety data concerning use of TFL during lithotripsy by characterizing potential for TFL-induced damage to the porcine ureteral wall.

Methods: A TFL beam with pulse energy of 35 mJ, pulse duration of 500 ?s, and variable pulse rates of 150-500 Hz was delivered through a 100-?m-core, low-OH, silica optical fiber to the porcine ureter wall, in vitro. Ureter perforation times were measured and gross, histological, and optical coherence tomography images of the ablation zone were acquired.

Results: TFL operation at 150, 300, and 500 Hz produced mean ureter perforation times of 7.9, 3.8, and 1.8 s, respectively. Collateral damage zones averaged 514, 370, and 311 μm. Both perforation times and collateral damage decreased with increasing TFL pulse rate.

Conclusions: TFL operation using low pulse energy and high pulse rates produced mean perforation times exceeding 1 s at each setting, which is a greater safety margin than previously reported during Holmium laser ureter perforation studies.

9542-12, Session 3

Endovenous laser therapy for occlusion of incompetent saphenous veins using 1940nm

Ronald Sroka, Thomas Pongratz, Laser-Forschungslabor (Germany); Anna Esipova, Slobodan Dikic, Sahit Demhasaj, Florin Comsa, Evang. Diakoniewerk Schwäbisch Hall eV (Germany); Claus-Georg Schmedt, Ludwig-Maximilians-Univ. München (Germany)

Objectives: Several studies indicate that ELT using wavelengths of high water absorption showed advantages compared to conventional ELT. Thulium–Lasers emit nearby the local absorption maximum of water at 1940nm. In this clinical study the effectiveness, safety and the feasibility of 1940nm-ELT is proven.

Material and Methods: A single centric, prospective observational study was performed. 1940nm-laserenergy was applied using radial emitting fibres with continuous pullback (1mm/s). Treatment was performed under anesthesia (general, spinal, tumescent) thus simultaneous miniphlebectomy and ligation of perforators could be applied. Patient and technical details were systematically collected. Evaluation included: standardized questionnaire, clinical examination, color-duplex ultrasonography preoperatively, 3d, 4w, 6m postoperatively, statistic.

Results: The 1940nm-ELT study include 55 patients (female/men=34/21, mean age 55y, range 23-90y) treating n=72 vessels. The mean maximum diameter of great saphenous veins (GSV, n=59) was 7.5mm (range 3.7–11.3mm) and of small saphenous veins (SSV, n=13) was 5.3mm (3.0-10.0mm).

The mean applied longitudinal endovenous energy density (LEED) was 64.3J/cm (40.3-98.2J/cm) in GSVs and 51.0J/cm (37.6-72.7J/cm) in SSVs. Complete occlusion of the vein without sign of reflux was achieved in 100%. The mean length of non-occluded stump at the sapheno-femoral junction was 6.0mm (1.0-20.0mm). Postoperative reduction of the diameter of GSV was 1.6mm (21.3%) and 2.0mm (37.7%) in SSV. One (1.4%) endovenous heat induced thrombus (EHIT) was observed. Further adverse events were: paresthesia 10/72 (13.9%), ecchymosis 1/72 (1.4%), lymphocele 1/72 (1.4%), hyperpigmentation 1/72 (1.4%). The mean postoperative pain intensity was 1.3 and 1.8 single doses of analgesics were administered. Normal physical activity was reached after 3d (1-21d).

Conclusion: 1940nm-ELT using radial light application effectively eliminates the reflux in insufficient saphenous veins by a significant diameter reduction. The risk profile correlates with other endothermal treatment options. Low postoperative pain and analgesic requirements with rapid convalescence indicate a high level of patient comfort.

9542-13, Session 3

Impact of terahertz radiation on the epithelialization rate of scarified cornea

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Abstract: In this work the effect of terahertz radiation on healing of rabbit corneal epithelium was studied in the frequency range of 0.1-2.0 THz. Radiation power varied from 2.5 to 68.0 nW. Exposure duration was 5 minutes. The positive THz radiation influence on corneal epithelialization became obvious during the first hours after the corneal injury. It was shown that the total time for complete epithelialization did not change after irradiation and THz radiation had no analgesic effect.

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1. Introduction

Currently, studies of degenerative and dystrophic processes in the cornea associated with metabolic disorders, are of considerable interest for therapy and diagnostics of eye disorders. Recently, pharmaceutical drugs for neurite growth stimulation in the eye were developed [1]. A non-invasive technique based on the therapeutic effect of terahertz (THz) radiation on nerve cells represent a much more promising method. Our work was dedicated to this problem.

2. Object and methodology

The study was conducted on five eight-month old rabbits. Generated THz radiation had a bandwidth of 0.1 to 2.0 THz and pulse duration of 2.5 ps. THz radiation power was varied at different orders using filters in the range of 2.5 ÷ 68.0 nW. The irradiation time was 5 minutes. Experiments were performed at room temperature (? 25º C). Control of the temperature change for the sample was carried out using infrared temperature sensor.

The control (right) and irradiated (left) rabbit cornea were under investigation for 24 hours. The epithelialization of the corneal epithelium was controlled using an optical microscope at 1, 3, 6, 12 and 24 hours after deepithelialization of the damaged area.

3. Results

A positive effect of low-intensity terahertz radiation on the epithelialization of scarified cornea was observed in this study during the first hours, epithelialization was 10 % higher than for non-irradiated cornea. However when the power of terahertz radiation was increased to 68.0 nW, epithelialization process slows down. In addition, the total epithelialization time of the cornea is not affected by irradiation.

Terahertz radiation in 0.1 ÷ 2.0 THz range was well tolerated and safe for the rabbit eye. Terahertz radiation does not have analgesic effect, since the sensitivity of rabbit eyes was the same before and after irradiation.

Thus, experimental studies showed high tolerability, absence of toxic and allergic reactions or pathohistological changes in the eye tissues. Terahertz radiation does not affect normal eye physiology, stimulates reepithelialization of the scarified rabbit cornea at low-intensity doses.

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9542-14, Session 3

Healing process study in murine skin superficial wounds treated with the blue LED photocoagulator EMOLED

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A faster healing process was observed in superficial skin wounds after irradiation with the EMOLED photocoagulator. The instrument consists of a compact handheld photocoagulation device, useful for inducing coagulation in superficial abrasions. In this work we present the results of an in vivo study, in a murine model. Two superficial wounds were produced on the back of 12 mice: one area was left untreated, the other one was treated with EMOLED. Healthy skin was used as a control. The animals were sacrificed 3 hours, 12 hours, 1 day, 6 day after treatment. The treatment effects on back skin was monitored by visual observations, histopathological analysis, immuno-histochemical analysis, and non-linear microscopic imaging performed at each follow up time, finding no adverse reactions and no thermal damage in both treated areas and surrounding tissues. In addition, a faster healing process, a reduced inflammatory response, a higher collagen content, and a better-recovered skin morphology was evidenced in the treated tissue with respect to the untreated tissue. These morphological features were characterized by means of immuno-histochemical analysis, aimed at imaging fibroblasts and myofibroblasts, and by SHG microscopy, aimed at characterizing collagen organization, demonstrating a fully recovered aspect of dermis as well as a faster neocollagenesis in the treated regions. This study demonstrates that the selective photothermal effect we used for inducing immediate coagulation in superficial wounds is associated to a minimal inflammatory response, which provides reduced recovery times and improved healing process.

9542-15, Session 3

Comparison of four lasers (650, 808, 980, and 1075 nm) for noninvasive creation of deep subsurface thermal lesions in tissue

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Introduction: Lasers have been used in combination with applied cooling methods to preserve superficial skin layers (100's micrometers) during cosmetic surgery. Preservation of a thicker tissue surface layer (millimeters) may also allow development of noninvasive laser surgery beyond dermatology. Our laboratory is exploring noninvasive therapeutic laser applications in urology (including laser vasectomy and laser treatment of female stress urinary incontinence), which require surface tissue preservation on millimeter scale. In this preliminary study, four lasers spanning the optical window were compared for noninvasive creation of deep subsurface thermal lesions.

Methods: Laser energy from three diode lasers (647, 808, and 980 nm) and a Ytterbium fiber laser (1075 nm) was delivered through a custom built, side-firing, laser probe with integrated cooling. An alcohol-based solution at -5 C was circulated through a flow cell, cooling a sapphire window to -2 C , which in turn cooled tissue surface. The probe was placed in contact with porcine liver tissue, ex vivo, kept hydrated in saline and maintained at ~35 C. Incident laser power was 4.2 W, spot diameter was 5.3 mm (1/e2), and treatment time was 60 s.

Results: The 1075 nm wavelength, after further optimization, produced deepest subsurface thermal lesions while preserving surface layer of about 2 mm.

Conclusions: The optimal laser wavelength tested for creation of deep subsurface thermal lesions during contact cooling of tissues was 1075 nm. The Ytterbium fiber laser provides a compact, low maintenance, and high power alternative laser source to the Neodymium:YAG laser for noninvasive thermal therapy.

9542-16, Session 3

Anisotropy factor of biological tissue phantom and the effect to scattering coefficient in a strongly absorbing wavelength

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Anisotropy factor g is one of the most important parameter to determine the accurate scattering coefficient. However, there are a few reports about g for biological tissues, the wavelength dependence, and the absorption dependence. The purpose of this study was to obtain more highly accurate scattering coefficient for biological tissues. Biological tissue phantoms were prepared, the angular distributions of the scattered lights for a strongly absorbing and a weakly absorbing wavelengths were measured, and the g was determined. In the low Hb concentrations, the significant difference of g was not observed between the strongly absorbing wavelength and the weakly absorbing wavelength. In the high Hb concentrations, the g for the strongly absorbing wavelength was lower than that of weakly absorbing wavelength. Considering of the absorption dependency of g was thought to be important to determine accurate scattering coefficient.

9542-17, Session 4

Towards advanced OCT clinical applications *(Invited Paper)*

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Due to recent advances in OCT technology this technique is currently actively penetrating to other medical diagnostics areas beyond ophthalmology. However, OCT imaging of highly scattering media, such as skin or mucous tissues suffers from multiple scattering effects which may distort the tissue structure in an OCT image. These drawbacks play a critical role when a diagnostic OCT image is evaluated by a clinician

who is not familiar with physical principles of OCT imaging and has no wide experience in OCT diagnostics. Complications in subjective evaluation of OCT images are one of the primary factors preventing OCT from introduction into common clinical practice. In order to overcome this problem development of advanced approaches to OCT diagnostics is required. Such approaches may include development of novel OCT modalities revealing additional tissue features critical for accurate diagnosis, automated processing of diagnostic OCT images which helps a clinician to properly evaluate an OCT image, such as cross-polarization OCT (CP-OCT), or different techniques that allow to enhance an OCT image, such as optical clearing or contrasting which allow to increase OCT imaging depth or mark target areas in the image.

In this paper we present out recent advances in OCT diagnostics in gynecology (diagnostics of cervix and fallopian tubes), dermatology (diagnostics of psoriasis and atopic dermatitis and monitoring of treatment), urology (diagnostics of bladder), and otolaryngology (diagnostics of cheek inner surface) employing the abovementioned approaches which allowed to significantly increase diagnostic accuracy.

9542-18, Session 4

980 nm diode laser with automatic power control mode for dermatological applications

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Introduction

Diode lasers are widely used in medicine and particular in dermatology. Diode lasers are compact, easy to use, precise and minimally invasive instruments which provide hemostasis and antimicrobial effect. In dermatology diode lasers are used to remove of nosological neoplasms of human skin. It is known that near infrared diode laser radiation is not well absorbed by skin and its component, and can lead to a wide area of thermal damage of the surrounding tissue and may worsen healing of postoperative scars. As laser cutting of soft tissue and removal of neoplasms are based on thermal effect, which depends on laser power, speed of cutting, manipulation manner, type and consistency of the tissue, the ability to control temperature in the area of laser radiation action may provide additional safety of operation independently of the above factors.

This research considers the removal of nosological neoplasms of human skin by 980±10 nm diode laser radiation operating in continuous (CW) mode and automatic power control (APC) mode. APC mode allows to control and maintain constant temperature of the tip and size of coagulation zone independent from speed of cutting. It is expected that temperature control in the area of laser irradiation action will reduce collateral thermal damage of tissue and improve healing and reduce risk of postoperative scars formation in comparison with CW mode.

APC mode

In this study we used Alta-ST laser system (Dental Photonics, Inc., Walpole, MA) with a wavelength of 980±10 nm which was first developed for dental applications, but parameters of its radiation and the new APC operating mode can be used for dermatological applications and in particular for nosological neoplasms removal.

Diode laser light denatures tissue protein, which turns black and sticks to the fiber tip. The “dirty fiber tip” of the diode laser heats up to temperatures from several hundreds to more than a thousand degrees. These very high temperatures of the fiber tip carbonize and vaporize tissue, resulting in cutting and coagulation. However, the cutting effect is not constant, because the black deposit is continuously scraped away, leading to fluctuations in the cutting efficiency and the power. When using a computer-controlled diode laser as a power source its power is converted into thermo-optical power in the tissue. Real-time sensors continuously monitor the thermal power at the fiber tip. A regulating mechanism called Automatic Power Control (APC) continuously adjusts the optical power of the laser to ensure constant preset temperature at the tip, thus maintaining constant cutting conditions for soft tissue virtually independent of the speed of cutting. APC is a real-time power temperature sensing and adjustment mechanism, which allows the operator to maintain tip temperature independently of the speed of movement through the tissue, or the type and consistency of the tissue. This feature significantly reduces the variability of the depth of cut and collateral thermal tissue damage due to changes in the speed of movement observed with conventional thermal cutting systems. As an added safety measure, the Alta-ST laser system is also capable of sensing a stop in movement and shutting the power down to avoid overheating. This is extremely useful, since in most procedures the ability to accomplish smooth continuous movement of tip is often inhibited by anatomical and positional impediments.

Study design and results

For nosological neoplasms removal we used Alta-ST laser system (Dental Photonics, Inc., Walpole, MA) radiation with a wavelength of 980±10 nm operating in continuous (CW) mode with a power of 7-16 W and automatic power control (APC) mode with a power of 7-16 W and tip temperature of 700-1000°C. APC mode feature is cleared by FDA.

A total 81 patients, male and female, 15-85 years old, with 98 neoplasms such as nevus, papilloma, basal cell skin cancer, dermatofibroma, keratoacanthoma, capillary ectasia, keratoma, haemangioma, angioma, angiofibroma, and Bowen’s disease were involved in the experiment in vivo. Removal of 61 neoplasms was carried out in APC mode, removal of 37 neoplasms was carried out in CW mode. All neoplasms were photographed at the same conditions. It was demonstrated that using APC mode leads to formation laser wound with coagulation zone width more than 300 ?m in 50% of the cases, while using CW mode - in 75% of the cases. Long-term results will be obtained one month after treatment and scars will be investigated, measured and compared for CW and APC modes.

9542-19, Session 4

Analysis of radiation parameters to control the effects of Nd:YAG laser surgery on gastric malignancies

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Endoscopic laser surgery provides an advantageous alternative to Argon Plasma Coagulation, endoscopic tweezers or electro-ablation in gastroenterology that facilitates a selective ablation of stomach tumors with an additional hemostatic effect in the surrounding tissue. This coagulation effect can also be employed for the treatment of gastric ulcers. It is mandatory to control the laser parameters regardless of the desired effect, either cancerous tissue ablation or coagulation to prevent ulcerous bleeding, in order to avoid stomach wall perforation or an insufficient therapeutic outcome. Dosimetric models constitute an attractive tool to determine the proper light dose in order to offer a customized therapy planning that optimizes the treatment results. In this work, a model for Nd:YAG laser surgery is applied to predict both the coagulation zone in gastric ulcers and the removal in adenocarcinomas under different laser setups. Results show clear differences in the effective zone of the gastric malignancy affected by both coagulation and ablation. Therefore the current model could be employed in the clinical practice to plan the optimal laser beam parameters to treat a certain type of pathologic stomach tissue with variable morphology and without risk of perforation or undertreated parts.

9542-20, Session 4

Investigation of the potential of optical coherence tomography (OCT) as a non-invasive diagnostic tool in reproductive medicine

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Probe-based OCT up to a high-resolution level of nowadays-available OCT microscopic systems could open up new ways of in vivo imaging in the reproductive tract. Potential applications are OCT-guided testicular biopsy to improve sperm retrieval or microscopic evaluation of the oviduct by OCT-assisted fertiloscopy.

9542-21, Session 4

Investigation of the optical properties of normal fibroblasts and fibroblasts cultured with cancer cells in terahertz frequency range

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The optical properties of normal fibroblasts and fibroblasts cultured with cancer cells were studied in the frequency range of 0.1-1 THz. The possibility to distinguish healthy cells from corrupted ones using their optical parameters was shown.

9542-22, Session 4

Terahertz pulsed imaging study of dental caries

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Current diagnostic techniques in dentistry are not capable of monitoring dental caries in its early stages and it remains a challenging work in dental treatment. Terahertz Pulsed Imaging (TPI) has great potential for medical applications since is a nondestructive imaging method. It does not cause any ionization hazard on biological samples due to low energy of THz radiation. Even though it is strongly absorbed by water which exhibits very unique chemical and physical properties that contribute to strong interaction with THz radiation, teeth can be investigated in three dimensions. Recent investigations suggest that this method can used in the early identification of dental diseases and imperfections in the tooth structure without the hazards of using techniques which rely on x-rays. We constructed a reflection mode raster scan THz imaging system that enables us to investigate various teeth samples in three dimensions. The generated T-rays are focused on sample with a 30º degree to the normal and collected upon reflection from the tooth. Scanning the sample in 2D and acquiring the THz pulse at each pixel generated a 3D image of the tooth sample. The system could also be utilized to scan the teeth samples in x-y coordinates, for a fixed position in depth, allowing to image the surface of the teeth. After analyzing the measurements in both the spatial and frequency domains, the results suggest that the THz pulse is sensitive to variations in the structure of the samples that can be due to early formation of caries. By increasing the sampled data sets we hope to improve the preliminary analysis on monitoring the internal structure of various teeth samples.

9542-44, Session PD

Ultrafast laser ablation for targeted atherosclerotic plaque removal

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Coronary artery disease, the main cause of heart disease, develops as immune cells and lipids accumulate into plaques within the coronary arterial wall. As a plaque grows, the tissue layer (fibrous cap) separating it from the blood flow becomes thinner and increasingly susceptible to rupturing and causing a potentially lethal thrombosis. The stabilization and/or treatment of atherosclerotic plaque is required to prevent rupturing and remains an unsolved medical problem. Here we show for the first time targeted, subsurface ablation of atherosclerotic plaque using ultrafast laser pulses. Excised atherosclerotic mouse aortas were ablated with ultrafast near-infrared (NIR) laser pulses. The physical damage was characterized with histological sections of the ablated atherosclerotic arteries from six different mice. The ultrafast ablation system was integrated with optical coherence tomography (OCT) imaging for plaque-specific targeting and monitoring of the resulting ablation volume. We find that ultrafast ablation of plaque just below the surface is possible without causing damage to the fibrous cap, which indicates the potential use of ultrafast ablation for subsurface atherosclerotic plaque removal. We further demonstrate ex vivo subsurface ablation of a plaque volume through a catheter device with the high-energy ultrafast pulse delivered via hollow-core photonic crystal fiber.

9542-23, Session 5

Differentiation of tissue and kidney stones for laser lithotripsy using different spectroscopic approaches *(Invited Paper)*

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1. Introduction

The Holmium laser is the gold standard for ureteroscopic laser lithotripsy. However, there is a risk of damaging or perforating ureter or kidney tissue when the vision is poor and the treatment fiber is not placed correctly. An automatic stone/tissue differentiation would improve handling and safety of the procedure. First, in vitro studies were done with human renal calculi and animal tissue samples testing white light reflectance spectroscopy and fluorescence excitation for this purpose. Then, in clinical proof of concept studies those results were verified in vivo during endourological interventions.

2. Material, Patients / Methods

For in vitro measurements, 31 human ureter and renal calculi were stored at T=4°C in buffer solution. Porcine renal calices and ureters were used as tissue samples.

The illumination/detection geometry of a laser lithotripsy intervention was constructed in a simplified set-up: The in vitro samples were illuminated by a Xenon lamp via a liquid light guide (CombiLight PDD 5133, Wolf). Reflected / scattered light was directed to a grating spectrometer (AvaSpec-3648-USB2, Avantes) by placing an optical fiber onto the sample surface. With the same set-up, reflectance spectra of two porcine calices were taken. For measurements on ureter tissue, the fiber was inserted into the ureter, while it was illuminated from the outside.

Since illumination and detection geometries play an important role in reflectometry, an in vivo verification is necessary in the framework of a clinical proof of concept study. The clinical study was approved by the ethics committee of the University of Luebeck. Within this study, before the actual laser lithotripsy was performed, the treatment fiber was connected not to the laser, but to the spectrometer for some minutes. The surgeon placed the fiber in front of tissue/stone, the spectrum was recorded and then the fiber positioned on another place. In this way, a total of 49 stone and 72 ureter tissue spectra were recorded in 8 patients.

A simple fiber-based fluorescence measurement set-up was used to get signals from several human renal calculi, artificial stone and porcine renal calix and ureter. A measurement series on 42 human urinary calculi samples was performed using continuous wave excitation.

With a modified set-up, measurements on tissue and stone spots in

clinical laser lithotripsy procedures are possible. A proof of concept study was approved by the ethics committee of the University of Luebeck and fluorescence data taken with 8 patients.

3. Results

Since the signal amplitude is strongly dependent on a variety of conditions, including the fiber's position, it is necessary to find an intensity-independent differentiation criterion, such as the ratio of the reflected light at two different wavelengths. After reviewing all data, the ratio of the intensities was calculated at ?1=540 nm and ?2=475 nm. The reflectance spectra of tissue spots show a dip at ?1 = 540 nm due to hemoglobin absorption. This results in a ratio I(540nm)/I(475nm) that is smaller for tissue than for calculi. For the illumination configuration of the in vitro set-up (Combi Light PDD 5133, Wolf), the differentiation limit of stone and tissue was set to I(540nm)/I(475nm)=1.3.

The data of the first patient of the clinical study showed that the hemoglobin absorption is significantly lower in some tissue spots as in the in vitro case. The differentiation criterion had to be changed accordingly: To check if there are local absorption minima at ?=(542±8) nm and ? =(577±8) nm, the curves were differentiated with respect to the wavelength. If this was the case, the sample was classified as tissue. After definition of the new differentiation criterion, 64 stone and 43 tissue spectra were taken in 7 subjects (integration time t=10-800ms).

After smoothing and derivation of the raw data, 62 tissue (from 64) and 39 (of 42) stone points were correctly classified. This puts the probability of identification of calculi at 77%, and for tissue at 91% (significance level: 5%).

Contrary to tissue (porcine renal calix and ureter) and artificial stone, human urinary calculi show a strong fluorescence signal. In the clinical proof of concept study, first data were collected showing also a strong fluorescence signal on renal calculi.

4. Discussion

White light reflectance spectra obtained from in vitro samples show that differentiation of stone and tissue is possible due to the hemoglobin absorption dips in the tissue data. Here, taking the intensity ratio of the reflection signal at two appropriately chosen wavelengths is sufficient for differentiation. However, in in vivo situations, the hemoglobin absorption at some spots of the inhomogeneous rosy ureter is so low that calculating the signal ratio at ?1=540nm and ?2=475nm and comparing it to the differentiation limit results in a correct sample classification only for 75% of the tissue samples (calculi: 100%). If one classifies a sample as tissue if there are absorption minima at ?1=542nm and ?2=577nm and as stone elsewise, 77% of the calculi spots were correctly identified within the clinical study (significance level: 5%). This is sufficient, since in case of a false positive tissue classification the laser would not be fired and therefore, only the time needed for lithotripsy would be prolonged. A problem, however, is the probability for tissue recognition, which is 91% (significance level: 5%).

Contrary to tissue or artificial stone, human urinary calculi give a strong fluorescence signal. The results of the clinical proof of concept study show that also under in vivo conditions it is possible to discriminate stone and tissue by a simple fluorescence measurement.

5. Acknowledgements

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9542-24, Session 5

Fast and automatic depth control of iterative bone ablation based on optical coherence tomography data

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Laser surgery is an established clinical procedure in soft tissue ablation and ophthalmology. The presented experimental set-up for closed-loop control of laser bone ablation combines the working volumes of optical coherence tomography (OCT) and Er:YAG cutting laser. This addresses the need of a depth feedback system and enables safe ablation towards anatomical structures with high risk of damage. High level of automation in fast image data and tissue processing enables reproducible results and short time in the operating room. For registration of the two coordinate systems a cross-like incision is ablated with the Er:YAG laser and segmented with OCT in three distances. The resulting Er:YAG coordinate system is reconstructed. A parameter list defines multiple sets of laser parameters with discrete and specific ablation rates as ablation model. The control algorithm uses this model to plan corrective laser paths for each set of laser parameters and dynamically adapts distance of the laser focus. With this iterative control cycle consisting of image processing, path planning, ablation, and moistening of tissue the target geometry and desired depth are approximated until no further corrective laser paths can be set. The achieved depth stays within the tolerances of the parameter set with the smallest ablation rate. Specimen trials with fresh porcine bone are conducted to prove the functionality of the developed concept. A flat bottom surface and sharp edges of the outline without visual signs of thermal damage verify the feasibility of automated, OCT controlled laser bone ablation with minimal process time.

9542-25, Session 5

Investigations of the damage mechanisms during ultra short pulse laser ablation of dental tissue

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Several investigations of dental tissue ablation with ultrashort pulses usually suggest that these lasers enable precise and selective material removal and reduce the formation of micro cracks and thermal effects in contrast to ns-pulses. In this study, we present two damage mechanisms occurring during ultrashort pulse ablation of hard tissue using a 400 fs-laser at wavelength of 1040 nm and investigate their driving mechanisms.

First, it was found that nano cracks appear around the craters after single pulse ablation. These cracks are directed to the crater and cross the dentinal tubules. Transient investigation of the single pulse ablation process by pump-probe microscopy suggest that the driving mechanism could be a pressure wave that is released after stress confinement.

Second, squared holes were ablated using scan speeds between 0.5 mm/s and 2.0 m/s and fluences up to 4.5 J/cm?. It was found that deep cracks appear at the edges of the squared holes, if the pulse overlap is above 85%. In contrast, the fluence has only a minor impact on the crack formation. The crack propagation was investigated in the depth using x-ray micro tomography and optical coherence tomography. It was found that these cracks appear in the depth down to the dental pulp. These findings suggest that fast scanning of the laser beam is the key for damage free processing using ultrashort pulse lasers. Then, ablation rates of about 2,5 – 3,5 mm?/min/W can be achieved in dentine with pulse durations of 400 fs.

9542-26, Session 5

Impact of pulse duration on Ho:YAG Laser induced lithotripsy

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Objectives: Ho:YAG-laser lithotripsy is a multi-pulse treatment modality with stochastic effects on the fragmentation. In-vitro investigation on the single pulse and multi pulse applications were performed to identify

potential impacts of different Ho:YAG-laser pulse durations.

Material and Methods: A Ho:YAG laser system (Swiss LaserClast, EMS S.A., Nyon, Switzerland) with selectable long- or short pulse mode was tested with regard to fibre burn back, repulsion capacity and single pulse and multi pulse induced fragmentation (hand held, hands-free) capacity using artificial (BEGO) stones. The laser parameters were chosen in accordance to clinical application modes.

Results: Long pulse mode showed negligible fibre burn back compared to short pulse mode. The pendulum test showed that the deviation induced momentum of short pulses was by factor 1.5 to 2 higher compared to longer pulses at identical pulse energy. The crater volumes induced by single pulses fired in SP versus LP mode showed no difference. Differences in fragmentation rates between the two pulse duration regimes were detected with statistical significance for defined settings.

Conclusion: Fragmentation rates for long and short pulse durations at identical power settings remain at a comparable level. Dependent on the clinical situation the suitable pulse duration mode should be chosen like: longer pulse duration reduces stone repulsion thus convenient handling during clinical use without compromising fragmentation effectiveness could be obtained

9542-27, Session 6

IR and UV vortex-beams for ultraprecise plasma-mediated eye surgery *(Invited Paper)*

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

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

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

9542-28, Session 6

Ultrafast laser machining of porcine sclera

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The use of ultrafast lasers offers a possibility for a minimally invasive removal of soft ophthalmic tissue. The potential for using ultrashort pulses for modification of scleral tissue has been reported elsewhere and has resulted in the introduction of new, minimally invasive, procedures into clinical practice.

Our research is focussed on finding optimal parameters for picosecond laser machining of scleral tissue without introducing any unwanted damage to the tissue. Experiments were carried out on hydrated porcine sclera in vitro. Porcine sclera, which has similar collagen organization, histology and water content (~70%) to human tissue, was used.

In this research we present a 2D finite element blow-off model which employs a one-step heating process. It is assumed that the incident laser radiation that is not reflected is absorbed in the tissue according to the Beer-Lambert law and transformed into heat energy.

The experimental setup uses an industrial picosecond laser (TRUMPF TruMicro 5x50) with 5.9 ps pulses at 1030 nm, with pulse energies up to 125 ?J and a focused spot diameter of 35 ?m. The application of a scan head allows flexibility in designing various scanning patterns. Many scanning patterns including single line ablation and square and circular cavity removal were tested.

In this study we have shown that ultrashort picosecond pulses are capable of modification scleral tissue without introducing any unwanted damage which offers a possible route for minimally invasive sclerostomy.

9542-29, Session 6

Optical and acoustical feedback techniques for selective retina treatment (SRT): a clinical evaluation

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Selective retina therapy (SRT) is an ophthalmological laser technique which targets the retinal pigment epithelium (RPE) with repetitive microsecond laser pulses, while causing no thermal damage to the neural retina, the photoreceptors as well as the choroid. The RPE cells are damaged mechanically by the formation of microbubbles. Beneficial effects of SRT on Central Serous Retinopathy (CSR) and Diabetic Macula Edema (DME) have already been shown. The ophthalmic invisibility of selective RPE lesions and the pigmentation variation of RPE and choroid yield to a non-constant pulse energy threshold for the therapeutical effect of cell damage. Thus dosimetry-systems designed to detect signals correlated with RPE-cell damage are a mandatory elements in SRT devices. An actually existing dosimetry technique requires many pulses of constant pulse energy to detect signals induced by microbubbles. Approaches that can be used to develop automated treatment procedures where the pulse energy is increased stepwise from a sub threshold regime to a point to selective RPE damage are desired to reduce the necessary time and effort for a treatment. Hence feedback techniques capable to identify microbubble correlated characteristics form single pulses are required. A feedback technique based on the evaluation of backscattered light has already been proven to achieve this goal in animal experiments. Additionally an acoustic technique based on the evaluation of single opto-acoustic transients has been developed. We present the evaluation of clinical data of 10 patients in order to use the software algorithms to distinguish between undamaged regions and regions with selective cell damage.

9542-30, Session 6

Intraocular pressure from the laser induced cavitation bubbles dynamics

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The Intraocular pressure (IOP) is a mechanical property of the intraocular fluids in the eyeball. One of the main eye diseases that affect the IOP is the so called Glaucoma, it alters the intraocular aqueous humor balance, causing an elevation of the intraocular pressure, and therefore irreversible damage to the optic nerve terminal fibers. Currently, the IOP measurement is performed by using techniques based on the Imbert-Fick

principle (Chihara 2008); which states that the pressure of a liquid inside a thin-walled sphere is proportional to the force required to flatten part of its surface, divided by the area of flattening. This type of measurement requires physical contact to directly induce a mechanical deformation of the human eyeball.Several ophthalmologist reports point out the fact that patient movements, blood pressure changes, and the cornea physical characteristics which are distinct person to person, directly affect the IOP measure accuracy and precision.

The laser mediated cavitation process is generated in liquids starting from a laser-induced dielectric breakdown resulting in plasma formation. It generates two main phenomena: shock waves and cavitation bubbles. The cavitation bubble grows to a maximum size and then it collapses to generate another bubble (Evans and Camacho-López, 2010).The first collapse time depends on the liquid pressure. This work shows experimental results on the bubble collapse time and its relation with the actual liquid pressure. The results revealed the feasibility for pressure sensing in medical applications, specifically in the intraocular pressure measurement in the anterior eyeball chamber.

9542-31, Session 6

Pore size assessment during corneal endothelial cells permeabilization by femtosecond laser activated carbon nanoparticles

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Introduction

Human corneal endothelial cells (HCECs) form a monolayer, called endothelium, at the innermost face of the cornea and are the sole engine for corneal transparency. Nevertheless, they are a vulnerable population incapable of regeneration in humans and, in case of pathologies, the HCECs density can decrease resulting in permanent cornea opacity, called edema. Up to now, the only option for treating corneal edema is corneal grafting using a donor cornea. But a great global scarcity of corneal tissue is observed and needs alternative solutions. Several therapeutic molecules like proteins, peptides or DNA, have been identified to inhibit HCECs death and even promote proliferation of these cells [1]. The difficulty of gene and drug delivery is transport across cell membrane, normally impermeable to high weight molecules. Several methods were studied to deliver molecules into mammalian cells. Viral methods are the most used because of their high efficiency (until 100%) but represent high risks of immune and inflammatory responses, ontogenesis and mutagenesis. Chemical methods are non-viral methods which permit a safer molecules delivery without toxicity, but their efficiency is considerably lower (less than 10%) than viral methods. Finally, physical methods consist to generate nanoscale holes in cell membranes by physical stimuli in order to deliver genes and drugs directly into cytoplasm, we called this phenomena cell permeabilization. Membrane holes can be generated efficiently by several types of physical stimuli: electric pulses, ultrasounds, laser irradiation.

A new physical method published in Nature Nanotechnology in 2011 [2] consists in ephemerally permeabilizing cell membranes using a photo-acoustic reaction produced by carbon nanoparticles (CNPs) and femtosecond laser (FsL). Initially used for non-adherent cells, we have successfully adapted this technique on adherent HCEC monolayer in vitro and obtained maximal efficiency of 50% of cell permeabilization and less than 8% of cell toxicity. The pore size formed during permeabilization was never investigated and is yet strongly needed to determine adapted therapeutic molecules to this technique. Indeed, these molecules are of different size from less 1 nm to more than 50 nm. The delivery of different size molecules depends of pore size formed during cell permeabilization.

Therefore, the aim of this work is to assess pore size formed during cell permeabilization by femtosecond laser activated carbon nanoparticles.

Materials and methods

In vitro HCECs were cultured on well plate until obtain confluent cell monolayer. Prior to irradiation, 15 nm diameter CNPs were put in contact with HCECs to permit photo-acoustic reaction and hence membrane permeabilization. To investigated size pores formed at cell membrane, different fluorescent molecules size were added to CNPs and cells: calcein (1.2 nm diameter, Invitrogen), FITC-Dextran 4kDa (2.8 nm diameter, Sigma) and FITC-Dextran 70kDa (12 nm diameter, Sigma). These three marker molecules are fluorescent in the green (ex:495nm/em:519nm) that permit to detect them when they are delivered in cells. To induce membrane permeabilization, a femtosecond Ti:Sapphire laser system (Thales Bright, Neuilly-sur-Seine, France) at a wavelength of 800 nm, with a pulse duration of 150 fs and a frequency of 5 kHz, irradiated the entire cell surface. FsL beam was focalized by means of lens to obtain a spot diameter of 500 μm at cells level, controlled by an in situ system of beam observation (Fig. 1). In this way, with an output power of 0.8 W, the CNPs close to cells was irradiated at a fluence of 80 mJ/cm?.

After laser irradiation, cells were rinsed to remove CNPs and not delivered marker molecules and fresh culture medium was added. Controls were realized by incubation of cells with CNPs and marker molecules but without laser treatment. Cells were then removed from its supports by chemical action. To determine percentages of cells uptake with the different marker molecules, cells were analyzed by flow cytometry, a technique commonly used in biology and able to detect fluorescence into cells.

Results

We showed that calcein was delivered in 40% of HCECs, FITC-Dextran 4kDa in 25% of cells and finally, FITC-Dextran 70 kDa in 7% of cells. These results showed that pore size formed at cell membrane during laser irradiation was not homogeneously distributed. By investigating the size of marker molecules that can be delivered into HCECs, a large number of pores in the size ranging from 1.2 to 2.8 nm were observed. However, 12 nm and larger pores were more infrequent.

Discussion

For the first time, pore sizes formed at cell membrane by the technique of cell permeabilization by femtosecond laser activated carbon nanoparticles was investigated. The result indicated that the pore sizes are large enough for the efficient delivery of small therapeutics molecules on HCECs by this technique.

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9542-32, Session 6

Speckle-based monitored photocoagulation

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Photocoagulation is a laser treatment widely used for the therapy of several retinal diseases. Intra- and inter-individual variations of the ocular transmission, light scattering and the retinal absorption makes it impossible to achieve a uniform effective exposure and hence a uniform damage throughout therapy. A real-time monitoring of the induced damage is highly requested. Here, an approach to realize an optical feedback using dynamic speckle photography ex-vivo is presented.

A 532 nm continuous wave Nd:YAG laser is used for coagulation of enucleated porcine eyes. This process is monitored by dynamic speckle investigation. While coagulation, speckles are produce by a coherent object illumination using a 633 nm HeNe laser with a power of 300 ?W and analyzed by a CCD camera with a frame rate of 160 fps. In addition, a 75 ns/523 nm Q-switched Nd:YLF laser excites the temperature dependent ultrasonic waves for optoacoustic temperature measurement used for validation.

Radial temperature induced tissue motion in the micrometer regime have

been observed. This motion could be correlated to speckle movement and modulation using different speckle tracking algorithms. The speckle analysis showed the ability of this system to determine a threshold for evaluating RPE cell damage ex-vivo.

9542-33, Session 6

Anti-oxidant and anti-angiogenic potentials of laser-induced sublethal hyperthermia on retinal pigment epithelial cells

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Purpose: Aim of this study is to investigate the impact of laser-induced sublethal and lethal hyperthermia on retinal pigment epithelial (RPE) cells with a special focus on anti-oxidant and anti-angiogenic properties.

Materials and methods: Culture medium of confluent porcine RPE cell culture was heated with the radiation of a thulium laser (?=1940 nm) over 10 s with different power settings, such that the peak temperatures (Tmax) in the center of the culture dish at the cells reach from 40°C to 58 °C. At the indicated time points, cell viability, amount of intracellular heat shock protein (Hsp) 70 and vascular endothelial growth factor (VEGF) as well as extracellular VEGF were examined. Cellular antioxidant potential was investigated by measuring intracellular glutathione and hydrogen peroxide (H2O2)-induced VEGF secretion.

Results: Irradiation with Tmax≥50°C induced RPE cell death in the following 24 h. Intracellular Hsp70 after lethal irradiation was significantly higher (10-fold) than sublethal irradiations, which induced slight and time-dependent increase over 48 h (2 to 4-fold). VEGF concentration in the culture medium was slightly increased immediately following all irradiations, whereas the average secretion from each survived RPE cell was shown to be significantly higher after lethal irradiation. On the other hand, intracellular VEGF protein was significantly reduced after all irradiation over 48 h. The redox balance (reduced/oxidized) of glutathione was highest following the sublethal irradiation with Tmax=43°C, which inhibited H2O2-induced VEGF secretion.

Discussion: Sublethal hyperthermia on RPE cells might be effective to increase RPE cell functionality by enhancing anti-oxidant and anti-angiogenic potentials.

9542-34, Session PTues

Synchronous autofluorescence spectroscopy of gastrointestinal tumours – tool for endogenous fluorophores evaluation

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1. Introduction

Fluorescence spectroscopic investigations of gastrointestinal tissue samples of normal and cancerous tissue are performed in terms of identification of spectral characteristics in their emission spectra that can be used for differentiation between normal, dysplastic and malignant tissue. Fluorescence signal is obtained in two modalities – EEM – excitation –emission matrix and SFS – synchronous autofluorescence spectroscopy of the tissue. In the first case EEM is graphically represented with excitation wavelength on one axis, emission wavelength on the second, and fluorescence intensity forms the third axis. This method for three-dimensional fluorescence spectroscopy provides enough information about the fluorescence spectra of biological tissue samples for determining excitation wavelengths that gave emission fluorescence spectra containing the most diagnostic meaning for clinical diagnostic analysis.

Second approach for the fluorescence signal detection – synchronous autofluorescence spectroscopy appears to provide more information, in comparison with EEMs, based on its greater selectivity. Investigating fluorescence through the method of SFS is performed by maintaining constant wavelength interval between excitation wavelength and emission wavelength through the spectrum. This allows optimal excitation of the emission maxima, which result in narrower emission peaks. That is the main reason for the greater sensitivity of SFS in comparison with EEMs. [1] Narrower peaks in the obtained fluorescence spectra allows decrease the extent of spectral overlaps and this effect is useful in investigating multi-component samples which consist mixture of fluorescence compounds, like biological tissues. [2]

SFS high sensitivity, non-invasive character, relatively fast performing and lower cost are main factors for the rapid widespread exploitation of the method for qualitative and quantitative analysis of food and beverages. [3, 4] Its application in biomedical researches has emerged as a promising modality for investigation of blood and urine samples for cancer diagnostic purposes and for differentiation between normal and pathologically diseased cells and tissues. [5, 6] The diagnostic potential of SFS for identification and localization of dysplastic tissues has been investigated for breast, cervical and thyroid gland cancers. Conclusions of those investigations support the superiority of SFS in sensitivity and specificity for differentiating cancerous and healthy tissue on the basis of their fluorescence spectra. [7, 8]

One of the main drawbacks of autofluorescence diagnostic techniques is the lack of specificity, which arises from the similar metabolic alterations between cancerous and inflammation pathologies. [9] Implementation of SFS among fluorescence diagnostic techniques can lead to improvement in specificity of the fluorescence diagnostic techniques.

2. Methods and Materials

SFS was performed, along with EEM, over pairs of cancerous tissue and healthy tissue from the GIT from 9 different patients. The procedure of obtaining the investigated samples includes their excision during surgery for removal of GIT neoplasia lesions. After the surgical removal biological samples are transported in isothermal conditions and safe-keeping solution from the hospital to the spectral laboratory, where their fluorescence is investigated. All patients received and signed written informed consent and this research is approved by Ethics committee of University Hospital "Tsaritsa Yoanna", Sofia.

Spectrofluorimeter FluoroLog 3 (HORIBA Jobin Yvon, France) was used for the measurements. This system's light source is Xenon lamp with power 300 W, performance range of 200-650 nm and PMT detector with performance range of 220-800 nm for fluorescence detection. Since our samples vary in shape and dimensions, their fluorescence was investigated with additional module F - 3000 of Fluorolog 3, which allows investigation of samples outside of the sample chamber. Measurements of the fluorescence signals of the different tissues obtained in EEMs were performed with applied excitation in 280-440 nm spectral region and emission observed between 300 nm and 800 nm. SFS measurements were performed with excitation wavelength in the spectral range of 280-440 nm with increment of 10 nm and wavelength interval (offset) in the range of 10-200 nm with increment of 10 nm. After the performing of both spectroscopic measurements for healthy and cancerous tissue the samples were in formalin solution, for safe-keeping.

3. Results and Discussion

Main differences observed between the fluorescence spectra of healthy and cancerous tissue are in the intensity of the fluorescence originating from the amino acids –tyrosine and tryptophan, the enzymes and coenzymes NADH and FAD, and from the structural proteins elastin and collagen.

Those differences arise from the different metabolic rate and structural characteristic of cancerous cells to healthy cells. The higher metabolic rate of the cancerous cells results in intensive production of the amino acids tyrosine and tryptophan, hence we observe higher intensity of their fluorescence. Cancer cells undergo aerobic glycolysis which results in elevated NADH:NAD+ ratios, where NAD+ is the non-fluorescent oxidized form of NADH. This may be one of the reasons for the observed lower intensity of the fluorescence maxima of NADH in cancerous cells.

Abnormal oversized growths of cancerous cells results in lack of structural proteins in volume unite of the cancerous tissues. This reduction of the quantity of the structural proteins affects the fluorescence spectra of cancerous tissue by lowering the intensity of fluorescence maxima of structural proteins, in comparison with the same maxima in the spectra of healthy tissue.

In the SFS spectra of cancerous tissue, additional difference, in comparison with the SFS spectra of normal tissue is the spectral shift of the NADH and FAD maxima in the range of the FAD fluorescence, which we interpreted like increasing of FAD contained in the cancerous tissue or it's a result of the lower NADH fluorescence in combination with the superior sensitivity of the SFS to EEMs.

4. Conclusions

In SFS regime of detection we detect a signal only when the chosen wavelength interval matches the difference between the absorption and the emission maxima, which results in observing the fluorescence of a particular fluorophore in a multi-component tissues' sample. By choosing wavelength interval near the difference between excitation and emission maxima of the major fluorescence sources observed, we present SFS spectra of healthy and cancerous tissues with significant differences. Optimal SFS signals of normal and cancerous tissue are found for ??=60 nm, ??=90 nm and ??= 120 nm, respectively In terms of finding the wavelength ?? for performing SFS, which results in fluorescence spectra with diagnostically significant differences between fluorescence spectra of healthy and cancerous tissues, the presented ?? shows potential for further investigations of the potential implementation of SFS fluorescence technique in the family of optical diagnostic modalities.

5. Acknowledgements

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9542-35, Session PTues

Using the method of intracavity laser spectroscopy to study the optical characteristics of an optically thin layer of biological tissue with the small-scale inhomogeneities

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A mathematical model is constructed, which makes it possible to vary the characteristic sizes of roughness, the electrophysical parameters of the biological sample under investigation, and its geometrical characteristics and to establish the relations between these parameters and biological properties of the biological tissue being modeled, as well as to calculate theoretically the absorption spectra of optically thin biological samples placed into the cavity of an optical resonator

9542-36, Session PTues

Laser induced healing treatment for heart block removal

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Laser beam can be used to removing heart blockade or any sort of irregularities in blood circulation system without doing any sort of destruction in the tissue and body by using laparoscopic optical fiber for using laser healing treatment in the blockade area after diagnosing by using ecg or eeg or any other diagnostic process. Laser beam will used to melt the blockade by using quantized amount laser beam so that that can cause no burn and also by using liposuction mechanism we will remove as far as possible the fat there for proper circumcision of circulation system and hence regulate the healing system.The laser will be used in this process should be followed as ansi z 136 standard and also following the medical safety rules so that no beam and non beam hazard can happen .

9542-37, Session PTues

Assesment of the effective attenuation coefficient of scattering media illuminated by a led array: effect of the beam size

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The knowledge of the energy fluence rate distribution inside a biological tissue irradiated by a light source (LASER or LED) is fundamental to achieve optimal photodynamic treatment. In this paper, we present an analytical general model based on the two dimensional Fourier transform of the diffusion equation. This method can be applied to any fluence distribution (cylindrically symmetric or not) at the surface of the tissue. In this work, two particular beam shapes are studied : planar irradiation and flat Beam with finite radius. The total fluence rate along the axis of the beam was computed by adding the collimated and the diffuse components. The analytical solution is also used to study the effect of the radius of the beam on the attenuation along its axis. Measurements results were performed using a tank filled with a liquid-simulating medium (Milk) illuminated with a LED array (660 nm, 100 mm, 100 mm). Several circular diaphragms were used to obtain uniform circular beams. An optical fibre (with an isotropic tip) connected to a photodiode used to measure the fluence rate inside the medium. The experimental behaviour results are in agreement with theoretical predictions.

9542-38, Session PTues

Dynamics of accumulation of chlorin e6 photosensitizer in the mouse organs at lymphotropic administration

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A method to quantify the accumulation of photosensitizer (PS) Chlorin E6 ("Photoditazine") in various organs of the mouse, and an optimal algorithm for the analysis of the accumulation dynamics of the preparation in mice organs and tissues taking into account their optical properties were developed. A comparative analysis of the fluorescence intensities of PS accumulated in organs at two methods of administration was performed to improve the effectiveness of photodynamic diagnosis and therapy (PDD and PDT).

White laboratory mice were selected as biological models for the experiment. The PS was administered to the animals at two methods of administration: intravenous and lymphotropic.

Detection of the signal was carried out in real time in situ by spectrum analyzer «LESA-01-BIOSPEC» using fiber-optical probe. The experimental work was divided into two stages. In the first stage the features of absorption, scattering and autofluorescence of the studied organs were investigated and then the PS accumulation dynamics depending on time in the mice organs was studied. In the second stage the acute aseptic inflammation of the mouse testicle was provoked.

The features of diffuse reflectance and PS fluorescence in the organs at various time intervals and the influence of inflammation on the PS distribution and accumulation were examined. The algorithms for data processing and analysis necessary for calculation of the fluorescence index (the ratio of the fluorescence signal intensity to the intensity of back-scattered laser signal) were developed for each investigated mouse organ and for every time point. An optimal time of observation for the diagnosis of internal organs was also defined. Besides an optimal time for the PDT implementation basing on the dynamics of time of the PS accumulation and excretion for each target organ was determined.

The quantification of the PS accumulation in the parenchymatous organs of the abdominal cavity and in the pelvic organs, depending on the administration method and on the presence or absence of inflammation, was carried out at various time intervals. In particular it was shown that the lymphotropic injection is an optimal method of the preparation administration for prolonged PDT (the possibility of multiple PDT sessions after a single dose of the PS).

9542-39, Session PTues

Biological response and spectral characteristics of nerve cells in the frequency range 0.1 - 2 THz

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Research was devoted to the impact of broadband pulsed THz radiation 0.1 to 2 THz on the stimulation of neurite growth. Spectral characteristics of nerve cells were studied by terahertz pulse spectroscopy.

9542-40, Session PTues

Histological study of subcutaneous fat at NIR laser treatment of the rat skin in vivo

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The goal of this work is to quantify impact of in vivo photochemical treatment using indocyanine green (ICG) or encapsulated ICG and NIR (808 nm) laser irradiation through skin of rat with obesity by the follow up tissue sampling and histochemistry. After 1 hour elapsed since 1-min light exposure samples of rat skin with subcutaneous tissue of thickness of 1.5-2.5 mm were taken by surgery from rats in the limits of marked 4-zones of the skin site. For hematoxylin-eosin histological examination of excised tissue samples, fixation was carried out by 10%-formaldehyde solution. For ICG and encapsulated ICG subcutaneous injection and subsequent 1-min diode laser irradiation with a power density of 8 W/cm2, different necrotic sites and regions with lipolysis of subcutaneous fat were observed. The obtained data can be used for safe layer-by-layer laser treatment of obesity and cellulite.

9542-41, Session PTues

Electrical stimulation vs. pulsed and continuous-wave optical stimulation of the rat prostate cavernous nerves, in vivo

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Introduction: Identification and preservation of cavernous nerves (CNs) during prostate cancer surgery is critical for preserving post-operative sexual function. Due to their microscopic nature and close proximity to prostate gland, CNs risk injury during surgery. Electrical nerve stimulation (ENS) mapping has been tested as potential intraoperative tool for identification of CNs, but has proven unreliable. ENS is limited by need for electrode-tissue contact, poor spatial precision from electrical current spreading, and stimulation artifacts interfering with detection. Alternatively, optical nerve stimulation (ONS) has advantages of noncontact stimulation, improved spatial selectivity, and elimination of stimulation artifacts. This study directly compares ENS to pulsed/CW ONS to provide insight into ONS mechanism.

Methods: Eighty stimulations were performed in five rats, in vivo. ENS was studied using standard electrical stimulator and parameters (4 V, 5 ms, 10 Hz). Noncontact ONS was conducted using commercial (Capella) pulsed diode laser nerve stimulator (1873 nm, 5 ms, 10 Hz) or experimental continuous-wave, single-mode diode laser nerve stimulation system (1455 nm). Intracavernous pressure (ICP) response and nerve compound action potentials (nCAPs) were measured.

Results: All three stimulation modes (ENS, ONS-CW, ONS-P) produced comparable magnitudes for ICP responses. However, ENS consistently demonstrated more rapid ICP response times (1.5 s for ENS vs. 5 s for ONS) and well defined nCAPs compared to unmeasurable nCAPs for both ONS modes.

Conclusions: Further experiments measuring single action potentials during ENS and ONS are warranted to further understand differences in ENS and ONS mechanisms.

9542-42, Session PTues

Experimental investigation on light propagation through apple tissue structures

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The interaction of light with biological materials, such as fruits and vegetables, is a complex phenomenon involving both the absorption of this light and its diffusion. In a highly scattering material, the photons undergo multiple scattering and absorbing events. The absorption of photons is mainly due to chemical constituents. The optical properties of a material constitute a large family of physical parameters. The recording of the signal over the surface allows characterizing the fruit's ability to absorb or to scatter the exciting laser light. The absorption process is defined by means of the absorption coefficient and the scattering process by the reduced scattering coefficient ?'s. Measuring the optical properties of a fruit allows understanding the physical and chemical characteristics. In this paper, an optical bench based on the use of laser radiation including a CCD camera is developed to study the light diffusion inside apple tissue structures. The experimental results confronted with a diffusion model are then used to extract the optical properties of the flesh. To better understand the apple tissues structures, an investigation into the propagation of light through the apple sliced tissue was performed.

9542-43, Session PTues

Improved photoporation efficiency by fluorescence two-photon absorption

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Photoporation is an optical technique to create transient pores in cell to introduce exogenous substances. In this work we propose a new method for improving the photoporation efficiency using the two-photon absorption fluorescence technique to determine the location of the cell membrane.

9542-45, Session PTues

Miniature LED endoilluminators for vitreoretinal surgery

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200 000 retina and vitreous body surgeries are executed annually just in Germany [1]. For illuminating the region of interest, xenon or quicksilver light sources are usually employed in combination with disposable optical fibers. These fibers are inserted into the eye through small incisions to provide the surgeon an undisturbed view through the natural or artificial lens.

There are two variants of these optical fibers. The rigid handheld fiber is best suited for a bright and targeted illumination of a limited area (Fig. 1). This is in contrast to chandelier endo-illuminators who enlighten the whole intraocular space (Fig. 1). The necessary incisions are commonly executed at the pars plana, a region of the eye without retina or other functional tissue. Nevertheless these incisions should be as small as possible and as few as possible to reduce the infection risk and accelerate the healing process.

9542-46, Session PTues

Precision machining of pig intestine using ultrafast laser pulses

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Endoluminal surgery for the treatment of early stage colorectal cancer is typically based on electrocautery tools which imply restrictions on precision and the risk of harm through collateral thermal damage to the healthy tissue. As a potential alternative to mitigate these drawbacks we present laser machining of pig intestine by means of picosecond laser pulses. The high intensities of an ultrafast laser enable nonlinear absorption processes and a predominantly non-thermal ablation regime. Laser ablation results of square cavities with comparable thickness to early stage colorectal cancers are presented for a wavelength of 1030 nm and 515 nm using an industrial picosecond laser. The corresponding histology sections exhibit for both wavelengths only minimal collateral damage to the surrounding tissue. The depth of the ablation can be controlled precisely by means of the pulse energy. Additionally, by operating the laser at a wavelength of 515 nm, rather than its fundamental wavelength of 1030 nm, and by adjusting the overlap between successive laser pulses, deliberate heat transfer to the tissue and thermal damage can be achieved. This can be useful for haemostasis and laser coagulation. Overall, the application of ultrafast lasers to ablate pig intestine enables significantly improved precision and reduced thermal damage to the surrounding tissue compared to conventional techniques.

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Low level laser therapy improves necrosis area after transverse rectus abdominis myocutaneous flap surgery in rats

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Background and Objective: Breast cancer is the second common cause of cancer death among women especially in economically developing countries. Transverse rectus abdominis myocutaneous flap (TRAM) is one of the main options for breast reconstruction. However this muscle may present complications, mainly in smokers. In the literature there are studies applying Low Level Laser Therapy (LLLT) in order to increase the viability of flaps. The aim of this study was to analyze the LLLT effects on the TRAM's necrotic area post-surgery subjected to the deleterious effects of nicotine in rats. Materials and Methods: Twenty rats were divided into 4 groups: G1(sham): flap TRAM surgery; G2: surgery + nicotin; G3: surgery + LLLT; G4: surgery + nicotine + LLLT. Diode laser (?=660 nm), Power=15mW, Energy=0,30J;, Time=20s, spot area=0,025cm2 in contact mode was applied perpendicular to the flap at a single irradiation point onto vascular pedicle area. LLLT application started in the immediate postoperative period and 4 consecutive days (total: 5 applications). Results: G1 had a percentage of necrotic area of 42.2%; G2 - 47.6%; G3 - 22.9% and G4 of 35.4%. Conclusion: In this experimental model, nicotine caused increased area of necrosis when compared to the sham group, with statistically significant results. LLLT groups when compared to the sham group and the group treated with nicotine showed significant reduced necrosis area. Lasertherapy may aid a better recovery for breast cancer patients in post-radical mastectomy.

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Pulse mode irradiation at Radachlorin PDT shifted cell death to apoptosis in vitro

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Photodynamic therapy (PDT) is a clinically approved, minimally invasive treatment that can exhibit onsite cytotoxic activity toward tumor cells. One of the main factors limiting PDT supereffective antitumor activity is tissue hypoxia derived from photodynamic action. PDT with pulse mode irradiation at the same peak fluence rates as in continuous wave (CW) mode and with appropriate irradiation parameters could be more effective in the potency of 1O2 generation and the cytotoxic effect enhancement by tissue re-oxygenation. In this study, we demonstrated theoretically that the main parameter of pulse mode irradiation is the intermittency factor, which makes it possible to maintain the intended 3O2 concentration and to regulate the efficiency of 1O2 generation. We also showed experimentally that photodynamic treatment with pulse mode irradiation has congruent cytotoxicity to CW mode but induces preferable cell apoptosis. We assume that not only is cumulative 1O2 concentration is important in photodynamic cytotoxicity, but so is the temporal distribution of 1O2 generation, which determines the types of cell death. We expect our research to help to rebuild the irradiation protocols to improve the efficiency of photodynamic therapy.