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LALS

Laser Applications in Life Sciences

PLENARY 1:

Wider, Faster, Deeper: New Directions for Imaging at Depth

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The last decade has seen immense advances in photonics-based methods for imaging and manipulation. In this talk I will describe routes for obtaining wide field images that minimise photodamage and enable deeper penetration into tissue. The particular mode of imaging I will describe is light sheet microscopy using propagation invariant light fields, particularly Airy beams and Bessel beams. The second approach involves the use of temporal focusing for multiphoton imaging at depth. This latter approach allows us to retrieve images through scattering media in the absence of any form of aberration correction

Propagation invariant light fields, as the name suggests retain their transverse intensity profile upon propagation. Bessel light fields and Airy light fields are prime examples of such beams. In terms of imaging, single plane illumination (light sheet) microscopy (SPIM) offers a myriad of unique advantages. Orthogonal detection allows rapid imaging of large, three-dimensional, samples of living tissue. Illumination with a thin sheet of light ensures high contrast by minimizing the fluorescent background. Moreover, by restricting the sample exposure to a single plane, photo-bleaching and damage are minimized. I will discuss the use of propagation invariant light fields for the enhancement within this imaging modality [1-4]. We show that using the intriguing properties of the accelerating asymmetric Airy beam, a single-photon, single light sheet scan yields high contrast and resolution over an extended FOV.

More recent work has shown how using attenuation compensated fields can be used with both Airy, Bessel and potentially lattice light sheet modes [5]. This reveals a method for overcoming sample attenuation and scattering and increasing depth penetration in a facile manner.

The second approach will describe the use of temporal focusing in combination with single pixel detection for wide field multiphoton imaging at depth. This approach obviates the need for information or characterisation of the scattering medium.

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PLENARY 2:

Tissue optical clearing as a platform for *in vivo* optical imaging and treatment of hidden pathologies: from UV to terahertz

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Optical biomedical spectroscopic and imaging technologies from UV to terahertz suffer from a low penetration depth of a probing beam and blurring of images caused by light scattering and/or strong water absorption. One of the ways to overcome these problems is to use tissue optical clearing (TOC) method [1, 2]. In spite of recent great interest to *in vitro* TOC technologies and their unique achievements in neuronal connectivity in intact transparent small animals, it is not suitable for *in vivo* TOC caused by many reasons, including tissue fixation and long time of clearing (hours and days) [2-4]. *In vivo* TOC should be fast and reversible without any damage to tissue [1,2]. There are a few approaches which are capable to fulfill *in vivo* conditions, one is based on immersion optical clearing (IOC) with impregnation of a tissue with a biocompatible optical clearing agent (OCA), another uses a local mechanical compression, squeezing or stretching of a tissue – mechanical optical clearing (MOC), also optical adaptive systems and analytical methods with intensive computations can be applicable [1, 5-10].

The main goal of this lecture is to overview fundamentals and advances of tissue optics in context of TOC, and to demonstrate a wide range of biomedical applications of TOC beneficial due to increase of light beam probing depth and image contrast of human and animal tissues in *in vivo* conditions. A brief description of modified concept of 'tissue optical window' and IOC method will be done. Fundamentals and major mechanisms of IOC allowing one to enhance optical imaging facilities and laser treatment efficiency of living tissues and cells will be presented. Water transport in tissues and temporal tissue properties modification under OCA action, including reversible dehydration and shrinkage, balance of free and bound water will be analyzed. The enhancement of probing/treatment depth and image contrast for different human and animal tissues, including skin, eye sclera, muscle, cerebral membrane, cartilage, bone, blood vessels, and blood will be demonstrated using spectrophotometry, OCT, photoacoustic (PA) microscopy, *in vivo* PA flow cytometry (PAFC), upconversion nanoparticle luminescence, autofluorescence at multi-photon excitation, confocal, SHG and Raman microscopies, polarization and speckle imaging under action of glucose, glycerol, PEGs, iohexol (Omnipaque^{TM}), albumin, hemoglobin and other OCAs. Experimental data on diffusion and permeation coefficients of glucose, glycerol, PEG, iohexol (Omnipaque^{TM}) and other OCAs for normal and pathological tissues (cancerous and diabetic) will be presented. Perspectives of immersion optical clearing/contrasting technique aiming to enhance imaging of living tissues by using different imaging modalities working in the ultra-broad wavelength range from free electron beam excitation (Cherenkov light emission) and UV to terahertz waves will be discussed.

The technologies for effective OCA delivery due to hidden free diffusion, local heating, enforced tissue per meability (physical and chemical), OCA encapsulation, and blood and lymph vessel networking will be descr ibed. Impact of OCAs on tissue structure, free/bound water balance and blood microcirculation will be discu ssed. Combined TOC technologies by using combination of IOC, MOC, optical adaptive system and analytic al (computational) ones will be presented as well.

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Valery V. Tuchin is a Professor and holds the Chair/Institute of Optics and Biophotonics of Saratov State University (National Research University of Russia). He is also a Head of Laboratory on Laser Diagnostics of Technical and Living Systems of Institute of Precision Mechanics and Control of the RAS, a supervisor of Interdisciplinary Laboratory on Biophotonics of Tomsk State University (National Research University of Russia) and Laboratory of Femtomedicine at University of ITMO, Guest Professor of HUST (Wuhan) and Tianjin Universities of China, and Adjunct Professor of the Limerick University (Ireland) and National University of Ireland (Galway). His research interests include biophotonics, tissue optics, laser medicine, tissue optical clearing, and nanobiophotonics. He has published more than 700 papers (Web of Science), 27 monographs and textbooks, and 60 book chapters. Prof. Tuchin is also a holder of more than 50 patents.

He is a Fellow of SPIE and OSA, has been awarded Honored Science Worker of the Russia, Honored Professor of Saratov University, SPIE Educator Award, FiDiPro (Finland), Chime Bell Prize of Hubei Province (China), and Joseph W. Goodman Book Writing Award (OSA/SPIE). He has 20384 citations and h-index 66 (Google Scholar, August 6, 2018).

Track A: Fundamentals, hybridization and future approaches

KEYNOTE A1:

Laser assessment of red blood cells intrinsic properties and interactions

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Several laser and optical techniques were combined to perform a complex study of a number of parameters related to red blood cells (RBC) structure and dynamics by means of imaging and measurement. In particula r we used diffuse light scattering (DLS); laser diffractometry (LD); optical trapping and manipulation (OT) f or in vitro measurements with fresh samples of blood stabilized with EDTA drawn from either healthy dono rs or patients suffering from arterial hypertension, heart failure and/or diabetes mellitus. Video-capillaroscopy (VC), two-photon tomography (TPT) and fluorescence life-time imaging (FLIM) were used for in vivo mea surements and imaging of blood capillaries and cells therein. The parameters related to RBC aggregation in model solutions of certain blood plasma proteins known as aggregation agonists or inhibitors were also meas ured. In particular, we measured the forces of aggregation and disaggregation of individual RBCs with OT [1,2], as well as the aggregation index, characteristic half-time of aggregation and the critical shear stress wit h a whole blood aggregometer implementing the DLS technique. Using the conventional LD technique (ektac ytometry) we measured the average value of deformability of the RBCs in the sample and upgraded the tec hnique to enable measuring the parameters of deformability distribution, which is essential for clinical applic ation of the technique [3]. We performed in vivo imaging of the blood flows and individual blood cells in nailfold capillaries and the perivascular zone (PZ) around the capillaries with high resolution VC, TPT and FLIM techniques. Two-photon excited fluorescence of RBCs was shown to be applicable for their assessmen t both in vitro and in vivo. The corresponding fluorescence emission was ascribed to hemoglobin (Hb). Howe ver, as Hb is essentially non-fluorescent at single-photon excitation, the mechanism of two-photon excited flu orescence of RBC remains debatable. We show that a fluorescent photoproduct, characterized by an ultrafast decay of excitation, is formed after irradiation of Hb with femtosecond laser pulses with ca. 8 x10−5 quant um yield, and that it is also fluorescent at single-photon excitation. The kinetics of the Hb photoproduct for mation and its spectral properties were investigated. The obtained results clarify the processes responsible for RBC fluorescence observed in two-photon microscopy experiments.

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KEYNOTE A2:

Retinal prosthesis for restoration of sight – current and future technologies

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In outer retinal degeneration, such as Retinitis Pigmentosa or Age-related Macular Degeneration, the retinal photoreceptors degenerate while the inner retinal neurons are relatively preserved. Stimulation of these remaining neurons by various technologies was shown to elicit visual percepts and restore vision to some degree. One approach for such retinal stimulation is the use of wireless photovoltaic retinal prosthesis, in which camera captured images are projected onto the retina using pulsed near-IR light. Each pixel in the subretinal implant directly converts pulsed light into local electric current to stimulate the nearby inner retinal neurons. In the current talk I will present recent advances with retinal prosthesis while focusing on the photovoltaic approach. I will further show our advances in studying the complex brain responses to combined prosthetic and natural vision, which is an important issue in current retinal prosthesis design. I will finally show our novel approach toward restoration of sight with high acuity by a hybrid retinal prosthesis, composed of high-density electrode array integrated with glutamatergic neurons.

KEYNOTE A3:

Perspectives of application of circularly polarized light and optical angular momentum for diagnostic screening of biological tissues

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In turbid tissue-like scattering medium the conventional polarized light, scattered multiple number of times, is depolarized, and the depolarization rate depends strongly on the size and shape of scattering particles, as well as on the number of scattering events. In fact, the structure of light can be more complicated when the polarization of light across the laser beam can be radially or azimuthally polarized and carry orbital angular momentum. When these vector light beams, known as cylindrical vector beam (CVB) and Laguerre-Gaussian (LG) beams, propagates in turbid tissue-like scattering medium, either anisotropic or inhomogeneous, the spin or angular momentum are changed that leads to spin-orbit interaction. Such a spin-orbit interaction leads to the mutual influence of the polarization and the trajectory of the light propagation. We investigate the applicability of using CVB and LG beams for optical biopsy. In current presentation propagation of CVB and LG beams in anisotropic turbid tissue-like scattering media is considered in comparison to conventional Gaussian beams. We demonstrate that by applying CVB and LG beams the sensitivity of the conventional polarimetry-based approach is increased at least twice in comparison with the experiments utilizing 'simple' Gaussian polarized light. The results of the study suggest that there is a high potential in application of vector light beams in tissue diagnosis.

INVITED A1:

Liposomes and Exosome for Drug Targeting Applications: The current challenges and the need for improved imaging technologies

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In the last years a number of advanced nanotechnologies have been developed as carriers for drug delivery, providing possibilities to control drug release rates, as well as drug bio-distribution, while additionally preserving the stability of the drug. Between the various types of nanomedicines for drug delivery, our laboratory has expertise in the design and development of liposomal formulations.

Liposomes offer numerous advantages as drug carriers offering numerous therapeutic advantages, however, although many accomplishments have been achieved, several challenges are currently unresolved. One of the current challenges is the potential to target specific diseases and/or overcome biological barriers with ligand-targeted liposomes (a technology that has not been yet translated into pharmaceutical products).

The up-to-date accomplishments aa well as potential solutions to overcome current challenges (such as the use of topical administration routes, and the potential to boost liposome targeting potential by development of bio-inspired vesicles) will be discussed or proposed.

The current imaging methods usually applied in this research area and the need for improved imaging solutions will be discussed.

INVITED A2:

A short abstract of selected quantitative phase imaging techniques and their medical applications

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Quantitative phase imaging has become increasingly important in medical imaging. It enables the visualization of transparent thin biological tissue sections, which

are generally transparent and so do not show high amplitude contrast in a conventional optical microscope. It is therefore not easy to visualise such tissues unless a staining process is applied, which may harm or even destroy the natural morphological features and behavior of the tissue. Moreover, the phase data is quantitative, which enables objective evaluation of the sample supporting tissue discrimination. A large number of quantitative phase imaging techniques have been developed in the last couple of years. However, we will only focus on a small selection, which are characterized by robustness towards environmentally changing conditions, hence enabling the application outside the optical laboratory. The first two selected techniques are ptychography and the variable wavefront curvature approach, highlighting changes in the optical thickness and the scattering behavior.

Furthermore, shearing elastography is presented, which enables studying the elastic behavior of biological sample. Elastic parameters such as strain can be retrieved with high sensitivity, which supports the discrimination between different types of tissue.

In conclusion, quantitative phase imaging offers multimodal imaging capabilities since it is sensitive in changes of the refractive index and the topography. It can be combined with existing imaging techniques without too much effort since the necessary light sources and cameras are already available at low costs, such as in smart phone devices. The quantitative nature can be used in combination with a standardized probe preparation process (thickness and type of thin tissue slices) to enable automated tissue analysis and discrimination resulting in an increased detection accuracy.

INVITED A3:

Miniaturized photonic modules for biosensing and diagnostics

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There is a continuously increasing trend in finding miniature solutions that can be portable and cost effective. During the last few years we have been developing a number of technologies that help in building such miniature modules. Our research activities can divided into three interrelated activities to serve this purpose: (i) Liquid crystal active devices were developed and used in spectral and polarimetric imaging modules, extended depth of field camera and white light ellipsometric camera; (ii) Highly sensitive plasmonic biosensing substrates that can be read with miniature modules such as surface plasmon resonace (SPR) module of 5cm height, self-referenced nanograting structure, surface enhanced Raman spectroscopy (SERS) and fluorescence (SEF) substrates; and (iii) Fast parallel phase shift detection based methods for quantitative phase imaging, focus tracking, vibration measurement, ellipsometry and quantitative phase imaging. A quick review will be given with some demonstrated applications in biosensing and diagnostics.

INVITED A4:

SERS and TERS recognition of neuropathogenic proteins

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We present Surface-Enhanced and Tip-Enhanced Raman Spectroscopy detection and characterization of biomolecules, focusing on amyloid beta peptide oligomers, recently considered as precursors in the aberrant protein aggregation processes related to the Alzheimer's disease.

Plasmon-enhanced spectroscopies exploit the resonance effects between a laser excitation and the free conduction electrons in metal nanomaterials. As result of this interaction, a huge electromagnetic (EM) field in close proximity of the nanoparticles is produced, giving rise to an enhanced optical response from molecules adsorbed on their surface.

Surface-Enhanced Raman Spectroscopy (SERS) is an ultrasensitive analytical technique that couples the chemostructural information provided by the Raman scattering to a signal enhancement due to localized surface plasmon resonances in noble metal nanostructured substrates. SERS offers the possibility to rapidly investigate chemical composition and structure of analytes in traces down to the single molecule, inspiring several applications in the field of biomolecular detection and sensing [1-4]. The high chemical specificity and signal sensitivity of SERS can be also combined with the nanoscale spatial resolution of scanning probe microscopy (SPM) giving rise to Tip-Enhanced Raman Spectroscopy (TERS), in which the huge EM field localized on the apex of a sharp metallized tip is exploited for spectroscopically investigate the surface of samples with nanometrical resolution.

Here we present some strategies recently implemented in our laboratories for SERS and TERS detection of biomolecules and, in particular, of amyloid beta (Aβ) oligomers, which is considered at the basis of the aggregation processes that lead to the impairment of the cognitive functions in Alzheimer's disease (AD) [5]. This aberrant protein aggregation typically starts from 10 to 20 years before the AD symptoms become evident, so that the detection of Aβ oligomers at low concentration plays a key role for early diagnosis [6].

More in detail, we present a fast, low cost and industrially scalable method for the deposition of SERS-active silver nanowires on a hydrophobic polymeric substrate. The reduced size of the sensing area permits to work with very small aliquots of biomolecules, without affecting the sensing performances. Moreover, we show the potential of TERS in the characterization of single Aβ oligomers and fibrils, by distinguishing the toxic forms through a fine analysis of the exposed hydrophobic residues in the peptide chain.

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INVITED A5:

Hyperspectral imaging for functional characterization of skin and vascular system

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Spectral images of human skin and other tissues contain information about the spatial distribution and concentration of biological chromophores, such as hemoglobin (oxy-/deoxy), melanin, bilirubin, carotene and others. The combination of a hyperspectral camera with a broadband light illumination allows for multiparameter functional imaging of tissues. In this paper, we introduce a portable hand-held hyperspectral imaging setup for the functional diagnostics of skin and vascular system. The analysis of hyperspectral images aided by artificial neural networks (ANN) allows fast reconstruction of skin physiological parameters nearly in real-time. The developed device provides spatial distribution of blood volume fraction, oxygenation and melanin content within skin. The device was built on the basis of unique hyperspectral snapshot camera utilizing a micro Fabry-Perot filter providing real spectral response in each pixel (no interpolation is used in image formation).

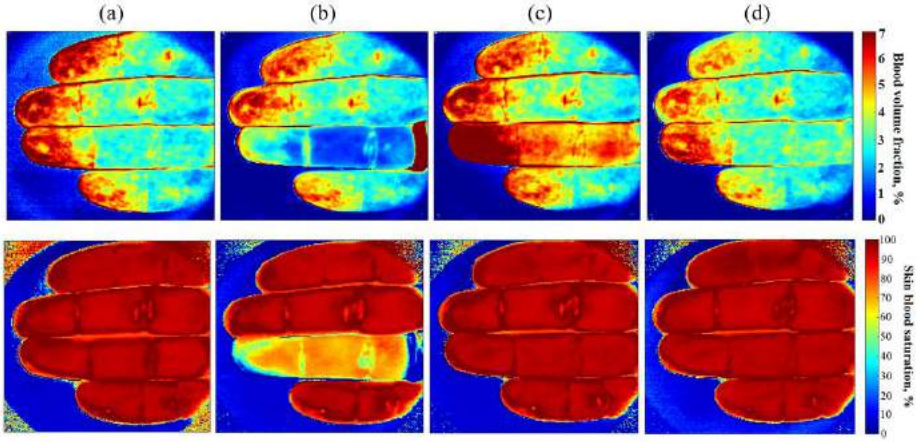

Fig.1 Obtained images of the blood volume fraction (upper row) and skin blood oxygenation (bottom row) before (a), during (b), 1 min after (c) and 3 min after (d) ring finger occlusion.

Specially developed ANN algorithm is used to perform the inverse problem solution for quantitative assessment of major parameters of skin based on the measured hyperspectral images. A set of diffuse reflectance spectra of human skin simulated by the Monte Carlo method developed in-house has been used extensively for the training of ANN. The volume fraction of blood, oxygen saturation, melanin content and thickness of the epidermal layer were used as variable parameters in the utilized seven-layer skin model. The total training set contained 45,198 spectra in the range of 510–900 nm simulated with a step of 5 nm. The developed imaging system has been successfully used to perform the occlusion tests with healthy volunteers (see Fig.1).

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INVITED A6:

Super-Resolution Light Microscopy of cellular Nanostructures

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Using laser based super-resolution microscopy (SRM) approaches [1], both optical and structural resolution have been enhanced far beyond the conventional resolution limit. At the present state of the art, they allow the imaging of specific molecules inside biological specimens down to an optical resolution in the few nm range and a structural resolution down to the 10 nm regime [2]. In this report, the focus will be on our experience obtained by Single Molecule Localization Microscopy (SMLM) [3]. The results comprise the disease related distribution of membrane bound receptors, transcription factors and intracellular microRNAs; of virus-membrane interactions; the monitoring of the repair of double strand DNA breaks induced by X-rays and heavy ion radiation in individual cells on the single molecule level [4]; the nuclear compaction status of individual disease related DNA sequences; the quantitative analysis of chromatin texture in normal and cancer cells, including first SMLM data on genome nanostructure of cells measured inside tissues; the SMLM imaging of chromatin nanostructure changes induced in cardiomyocyte cells under ischemia conditions [5]; or the cellular uptake of nanoparticles [6]. From the methodological side, perspectives for high throughput SRM as well as SRM at very large working distances (up to the cm range) shall be presented.

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INVITED A7:

A new achievement in multimodal microscopy imaging

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A multimodal microscopy system based on far field and near field was presented earlier [1,2]. The system integrates techniques having hundred nanometers resolution (confocal, two photon excitation, second harmonic imaging) and techniques with the nanometers resolution (stimulated emission depletion, apertureless scanning near field optical microscopy). The system has the main advantage that the same surface can be imaged using complementary techniques and in addition it is possible to make a correlation between the images at the nano scale and micro scale. Very few time ago we integrated in the multimodal system a new technique based on apertureless near field microscopy using a femtosecond Ti:sapphire laser that was used for the investigations on biological samples [3]. In our work we present the latest results that we had by using the new technique correlated with those obtained by using the old techniques.

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INVITED A8:

Laser-based spectroscopic methods as a versatile tools in investigating alive mammalian oocytes and embryos.

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To understand fundamental mechanisms underlying the process of mammalian preimplantation embryo development and to obtain important practical information for embryo evaluation and selection in assisted reproductive technologies (ART) new low-invasive and informative methods for study of embryo structure and biochemical processes are developed on base of modern laser-based spectroscopic methods. Among them Raman spectroscopy and 2-photon time-resolved spectroscopy can be employed to detect the biochemical changes which occur in the process of development or external treatments (medical, environmental, etc.).

One of important limiting factors for using laser methods in embryological studies and for medical applications is the adverse effect of laser beam on the investigated biological sample. The laser effect on biological system depends on laser wavelength and dose of laser radiation. Thus, one of the main goals of this work is to estimate possibility of non-invasive using laser spectral methods for embryology, and to analyze the optimal safe conditions and parameters of laser exposure in laser spectroscopy, applicable for studying live early embryos without affecting their development ability. The methods of analysis of non-invasively obtained information, correlation with developmental processes, criteria of estimation and further fate prognosis are discussed.

Raman spectra of mice living oocytes and embryos on different stages of development were measured with varied excitation wavelength (488, 532 and 785 nm), laser power in focal spot and total exposition. The developmental rate of embryos after the Raman measurements was characterized via estimation of the embryo ability to reach morphologically normal blastocyst stage and counting the cell number in the embryo. It has been shown that safe parameters of the laser irradiation can be selected, which allow acquisition of Raman spectra suitable for further analysis and the measurements don't affect significantly the early mouse embryo *in vitro* development.

Two-photon fluorescence lifetime imaging microscopy (2P-FLIM) was applied for functional mapping of the cellular redox state of the oocytes and embryos determined via ratios of oxidized and reduced forms of co-enzymes which takes part in the ATP production in the inner-mitochondrial membrane: nicotinamide adenine dinucleotide and flavin adenine dinucleotide. Fluorescence of these endogenous fluorophores was observed at excitation with Ti-Sapphire femtosecond laser (760 nm excitation, 140 fs, and 80 MHz repetition rate) and characterization of the oocyte and embryo state via analysis of distribution of fluorescence lifetimes and safe conditions of pulse laser application are discussed.

Principle Components Analysis (PCA) was applied to the obtained spectral information to compare embryos and oocytes of different stage of development and conditions. It has been shown that method on the base of PCA can be

developed and used for the prognosis of the embryo development.

Obtained results can be used for investigation of fundamental mechanisms of early mammalian development and further studies are aimed to suggest advanced method for estimation of state and embryo developmental ability in ART.

Acknowledgment

The work was supported by Ministry of Science and Technology, Taiwan, projects numbers 105-2923-B-320-001-MY3 and Russian Foundation for Basic Researches, grant 16-53-52046.

INVITED A9:

Biomedical applications at lab on Chip scale by advanced label-free coherent imaging

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Here we report on a smart solution to achieve biomedical diagnosis at lab-on-chip scale by digital holography in microfluidic environment. It will be show how different imaging methods and techniques that have been developed recently for assessing diagnosis by label-free modality. Example of applications will be demonstrated for detecting circulating cell by full phase-constrat tomography in the new paradigm of liquid biopsy. In particular, we performed the 3D imaging of human breast adenocarcinoma MCF-7 cells, opening the way for the full characterization of circulating tumor cells (CTCs). Furthermore, we will show that detection o several inherited anaemias is possible by the same method. Specifically Iron-deficiency Anaemia, Thalassemia, Hereditary Spherocytosis and Congenital Dyserythropoietic Anaemia,anamie. Perspectives about those novel approach will be also overviewed in order to give the framework of the challenging in the near future about for biomedical application at lab on chip scale.

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INVITED A10:

Light propagation in biological media: microscopic, mesoscopic and macroscopic views

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Light propagation in biological tissue is described in microscopic, mesoscopic and macroscopic scales. A newly developed numerical method, which solves accurately and very efficiently Maxwell's equations, is used to describe microscopically, for example, images obtained by optical coherence tomography and light focusing due to wave front shaping. Accelerated Monte Carlo simulations and analytical solutions of the radiative transport equation (RTE) are applied to investigate, for example, the accuracy of RTE compared to Maxwell theory, the anisotropic light propagation in complex media like a whole tooth with periodontium and the determination of mesoscopic optical properties like moments of the scattering function. Analytical solutions of RTE approximations, such as diffusion theory or P_3 approximation, are applied to determine, for example, the optical properties of layered media. A variety of experimental results are shown and compared to the theoretical solutions, for example, in the spatial frequency domain, in the spatial domain and in the time domain.

INVITED A11:

Histology of tissue with Mueller microscopy

Tatiana Novikova[1] *, Hee Ryung Lee[1], Pengcheng Li[1, 2], Enric Garcia-Caurel[1], Razvigor Ossikovski[1], Christian Lotz[3], Florian Kai Groeber-Becker[3], Sofia Dembski[3]

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Polarized light Mueller microscopy provides complete information on tissue optical properties (depolarization, retardance, dichroism) that can be used for the diagnostics during histological analysis. On the other hand, exploring polarimetric data as optical markers of the disease (e.g. inflammation, degeneration, cancer, etc) requires deep understanding of the process of interaction of polarized light with tissue. The combined approach, including measurements of unstained tissue cuts with Mueller polarimetric microscope in transmission configuration, decomposition of experimental Mueller matrices within the framework of differential Mueller matrix formalism and Monte Carlo simulations; was tested on the model system of human skin.

We confirmed experimentally and numerically the linear dependence of retardation / dichroism and parabolic dependence of depolarization on the thickness as it was predicted by theory. Our results prove that Mueller microscopy combined with phenomenological modeling may effectively separate the contribution of polarimetric properties of biological tissue and predict their evolution, thus, paving the way to digital histology of tissue.

INVITED A12:

Plasmonic Gap-Enhanced Raman Tags: Fabrication, Properties, and Applications

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Gold Au@RM@Au and composite Au@RM@Ag layered nanoparticles (also called gap-enhanced Raman tags, GERTs), in which Raman molecules (RMs) are embedded in a nanometer-sized gap between metal layers, have great potential in biomedical applications as highly efficient imaging SERS probes [1]. Compared to common SERS tags with outer RMs exciting by plasmonic near field, the embedded RMs of new probes are protected from environmental conditions and subjected to a strongly enhanced internal field in the gap. Another type of efficient SERS tags are the ti p functionalized Au(core)@RM@Ag(shell) nanorods (TFNRs) operating in off-resonance mode [2]. In this talk, we summarize our recent efforts in fabrication [3, 4], electromagnetic simulation [5], and bioimaging [1, 2] and analytic [6, 7] applications of GERTs and tip-finctionalized hybrid Au@RM@Ag SERS tags.

This research was supported by the Russian Scientific Foundation (project no. 18-14-00016) and by RFBR grants nos. 16-02-00054, 17-02-00075, and 18-52-7803.

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INVITED A13:

Lumpectomy margin assessment using hybrid optical and mass spectrometry technology

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Accurate assessment of tumour margins during breast-conserving surgery remains problematic, in that some residual tumour is often left behind and surgery must be repeated (15%-30% repeated surgery rate). A promising new method to definitively address this issue uses mass spectrometry (MS), a very sensitive and accurate technique at for distinguishing tumours. However, its very slow speed precludes its use in the operating room. If, however, MS could be directed to only examine suspicious cancer regions, the examination time could be cut down dramatically and made practical for the clinic. Here we use polarized light imaging (Mueller matrix "polarimetry") to get a rapid overview of the exposed breast tissue, identifying potential cancer regions that will benefit from targeted MS diagnosis. Polarimetry is indeed well suited for this guidance task -- it is fast, robust, able to image large tissue regions, etc - and yields useful information for directing MS. The unique combination of the polarimetry-targeted MS approach draws upon the strengths of the two technologies and should prove useful as a rapid, accurate and practical method to improve breast-conserving cancer surgery outcomes. Corresponding preclinical and initial clinical results will be presented and discussed.

INVITED A14:

High Dynamical Range Laser Imaging of Brain

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We are interested in the correlations between morphology of brain connections and functionality, which is one of the major issues in neuroscience in the comprehensions of many pathologies and mechanisms of behavior and computation. Elucidating the neural pathways that underlie brain function is also one of the greatest challenges in neuroscience.
Nowadays, there are several imaging techniques offering a complementary approach to visualize intact neural networks. Each of those offers a different strategy and furnish complementary information on the role of neural components.
We will describe different approaches enabling to move from single neuron details to whole brain imaging both on functional and morphological point of view.
Some examples of correlative microscopies, combining linear and non linear techniques will be described. Particular attention will be devoted to neural plasticity after damage as neurobiological application.

INVITED A15:

Optical coherence elastography as a tool to improve tumour margin assessment

Brendan Kennedy, UWA

Surgical excision of tumour is a critical factor in the management of breast cancer. The most common surgical procedure is breast-conserving surgery, where the surgeon's goal is to remove the tumour and a rim of healthy tissue surrounding the tumour. A major issue in breast-conserving surgery is the absence of a reliable tool to guide the surgeon in intraoperatively assessing the margin. Currently, in 20-30% of cases, additional surgery is required to remove residual tumour. It is clear that new tools are needed to address this issue. A number of optical techniques have been proposed, most notably optical coherence tomography (OCT). OCT readily delineates regions of adipose tissue, however, in many instances, provides low contrast, particularly in the important case between tumour and surrounding uninvolved stroma. To address this, we are developing optical coherence elastography (OCE), a technique that complements OCT by providing additional contrast based on the tissue's mechanical properties.

In this presentation, I will provide a perspective on what the requirements are for an ideal margin assessment tool and, also, will describe the technical and clinical advances we have made to meet these requirements. In particular, I will describe our latest clinical results describing the efficacy of OCE and the developments we have made towards a handheld OCE probe for use in the tumour cavity.

INVITED A16:

Functional optical imaging of biological tissue using laser speckle

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The blood vasculature delivers oxygen and nutrients to biological tissue. Abnormalities of the vasculature ma y induce a series of vascular diseases such as intracerebral haemorrhage, cerebral infarction,and varicose vei ns. It is important to monitor the morphology and blood flow of vascular network \for investigating the pat hogenesis and pathological changes in vascular disease, which is crucial for the improvement of human healt h. Laser speckle is a phenomenon that will be produced when a coherent light is illuminated to biolog ical tissues. Since the laser speckle is sensitive to the motion and\deformation of tissue, laser speckle related techniques are widely attempted to estimate the mechanicand rheological properties of biological tissue, suc h as blood flow velocity, viscosity, elasticity and so on. Moreover, the blood volume and blood oxygenation can also be obtained by using multiple wavelengths. By combined with fluorescent imaging, we can even a ccess the information of labeled molecule inside the biological tissue. Laser speckle contrast imaging (LSCI) is a full-field, non-contct and low cost optical imaging method for monitoring blood flow and vascula r morphology,which is attracting more and more applications in biomedical field. Here, the progress of meth odology of laser speckle techniques for functional imaging are discussed. Its pre-clinical applications on diag nosis and therapy of skin diseases, burn, and nerve block are also presented.

INVITED A17:

Noninvasive monitoring of nanoparticle clearance and aggregation in blood circulation by *in vivo* flow cytometry

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Nanoparticles have been widely used in biomedical research as drug carriers or imaging agents for live ani mals. Blood circulation is crucial for the delivery of nanoparticles, which enter the bloodstream through inje ction, inhalation, or dermal exposure1, 2. However, the clearance kinetics of nanoparticles in blood circulatio n has been poorly studied, mainly because of the limitations of conventional detection methods, such as insu fficient blood sample volumes3, 4 or low spatial-temporal resolution5, 6. In this study, a novel detection me thod based on in vivo flow cytometry7-10 (IVFC) is applied to monitor the clearance kinetics of nanoparticl es in the bloodstream in real time. Our results show that the rich information provided by IVFC can be em ployed to monitor nanoparticle concentrations and enumerate nanoparticle aggregates in blood circulation noni nvasively and continuously. Our work shows that IVFC could be a powerful tool for pharmacokinetic studie s of nanoparticles and other drug carriers in vivo.

ORAL A1:

The role of the meningeal lymphatic system in the brain drainage: *in vivo* visualization

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Here we studied the role of cerebral lymphatic system in the brain clearing using intraparenchymal injection of Evans Blue and gold nanorods assessed by fluorescence microscopy and optical coherent tomography for *in vivo* measurements in mice.

Our data clearly show that the meningeal lymphatic network plays an important role in the brain cleaning via the meningeal lymphatic vessels. The meningeal lymphatics drains the cerebral fluids (interstitial and cerebrospinal) from the brain into the deep cervical node, which is the first anatomical “station” for the brain fluids outflow.

These results shed light on the anatomy of lymphatic pathways of brain drainage and clearing and give a novel knowledge about physiological relationship between the meningeal and peripheral lymphatics.

The research was supported by grant of Russian Science Foundation № 18-75-10033.

ORAL A2:

Laser techniques for studying the adenylyl cyclase activity in regulation of human erythrocytes deformability

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INTRODUCTION: Deformability is an essential intrinsic property of erythrocytes (red blood cells, RBC) which allows them to reversibly change shape and linear sizes and realize the gas transport function. RBC deformability is determined by cells internal viscosity and cytoskeleton structure. Understanding molecular mechanisms of the RBC deformability regulation according to the metabolic demands of the organism is an important task required for an effective control of the blood microrheology.AIM: To study the effect of activation of RBC membrane protein adenylyl cyclase on RBC deformability. This process may play a key role in the receptor-mediated stimulation of signaling pathways leading to the conformational changes of RBC cytoskeleton proteins.

METHODS: We implemented laser ektacytometer RheoScan AnD-300 to assess changes of deformability of RBC extracted from heparinized blood at presence of forskolin – the direct stimulator of adenylyl cyclase.

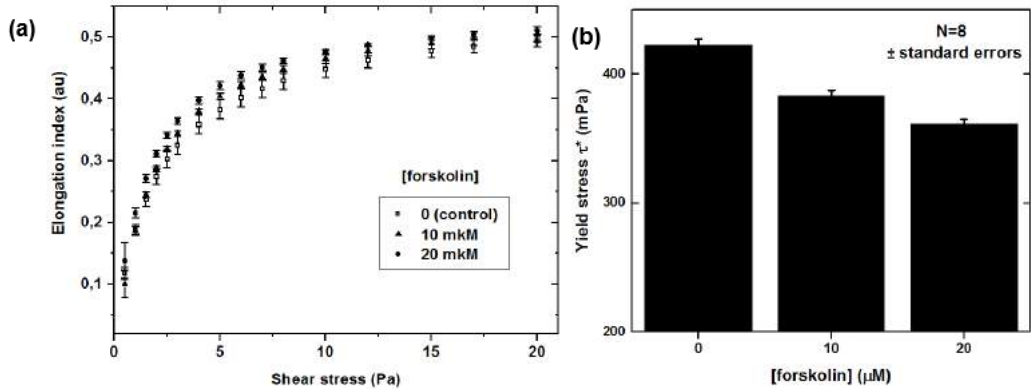

Fig. 1. RBC deformability curves and yield stress τ* at presence of forskolin.

RESULTS: We observed the dose-dependent rise of RBC elongation index curve (Fig.1(a)) at presence of forskolin in comparison with control RBC. Parametrization of these curves in semi-log scale revealed the decrease of yield stress τ*(the minimal stress required to initiate cells elongation) from 422.8 ± 4.2 mPa in control down to 361.3 ± 7.5 mPa at 20 μM forskolin (fig. 1(b)).

CONCLUSIONS: Results clearly indicate that adenylyl cyclase activation leads to the increase of RBC deformability. Decrease of yield stress supports the concept that the second messenger cAMP accumulated when the adenylyl cyclase is activated triggers the protein kinase A cascade resulting in the conformational changes of RBC cytoskeleton proteins.

ACKNOWLEDGEMENT: The study was funded by RFBR grant № 18-32-00756.

ORAL A3:

Dynamic Optical Coherence Elastography of Soft Tissues

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Abstract. Optical coherence elastography (OCE) is relatively new emerging method allowing to assess biomechanical properties of tissues in situ and in vivo in 3D. In this talk I will focus on overview of techniques and methods of *dynamic* OCE. For example, low-amplitude elastic deformations in mice and rabbit ocular tissues and mice hearts (both ex vivo and in vivo) were measured by the OCE system consisting of a phase-sensitive optical coherence tomography (OCT) combined with focused ultrasound (lens excitation) or air-puff (cornea and heart muscle excitation) systems used to produce a transient force on the tissue surface. The amplitude, temporal profile, and the speed of the deformations were used to reconstruct tissue biomechanical properties using novel analytical models. The results of these studies demonstrate that the OCE system can be used for noninvasive analysis and quantification of tissue biomechanical properties in 2D and 3D in normal and pathological tissues and as a function of tissue aging or therapy (e.g. CLX procedures). At the end, I'll introduce our recent advances in ultra-high speed imaging and assessment of the elastic waves using several configurations such as MHz laser swept source and optimizing scanning/imaging methods (such as line-field low-coherence holography).

ORAL A4:

Volumetric (3D) Ultrasound Medical Imaging Supported by Laser Micromachining

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LEEOAT Company
Hariena 11, Netanya

Volumetric (3D) ultrasound imaging is of high interest for modern medicine, enabling early cancer detection, medical guiding, surgery staging and other medical noninvasive procedures. Based on our innovative laser micromachining in ferroelectric relaxors, we developed high frequency ultrasonic transducers for diagnostic and therapeutic volumetric medical imaging. The transducers consist of orthogonal phase arrays, to allow horizontal and vertical 3D rastering of the focused ultrasound beam. Our prototype revealed real-time imaging of mile-stone targets in medical phantoms.

ORAL A5:

Remote Monitoring of Blood Pulsation with Infra-Red Illumination Compared to Electrocardiography

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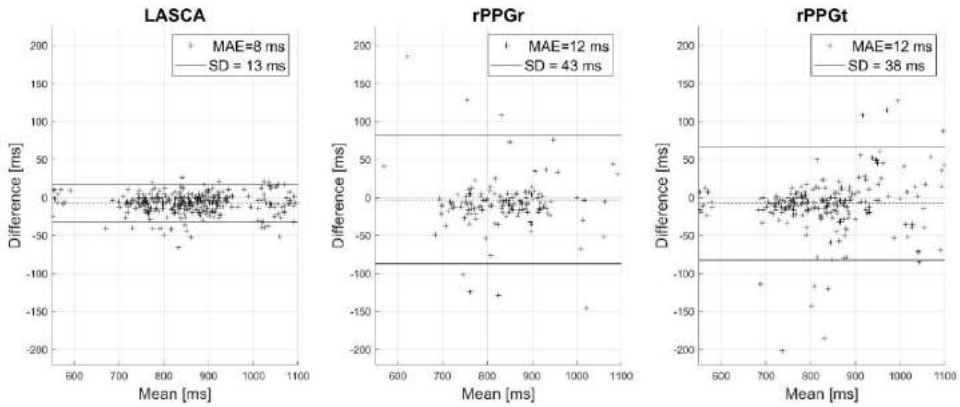
Remote sensing of blood pulsation enables the ability to measure human cardiac activity in a continuous way with minimum disturbance to the patient. In recent years remote PPG techniques (rPPG) gained interest as asolution to monitor blood pulsation, and to extract information about patient health, mood, and more [1][2][3]. Dynamic speckle is a physical mechanism in which motions of scattering particles in a medium can be evaluated by analyzing the time dependency of the laser speckle pattern [4]. Dynamic speckle can be realized by directly measuring the time evolution of the speckle pattern [5] [6] and also by measuring the time evolution of statistical properties such as contrast [7] [5] .Despite the fact that the time evolution of the speckle contrast had been studied extensively in the context of blood flow imaging [8] [9] [10], it was not yet employed to extract specific vital signs. In this work we evaluate the feasibility of using laser speckle contrast analysis (LASCA) to measure the inter-beat intervals (IBI) which may be used to calculate heart rate and the heart rate variability. We compare the results obtained from the speckle contrast analysis to intensity-based measurements in transmission (rPPGt) and in reflection (rPPGr) with an ECG as the reference device and conclude that LASCA provides excellent accuracy and that it is less sensitive to movements of the subject than intensity-based methods.



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ORAL A6:

Interaction-Free Ghost-Imaging of Structured Objects

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Correlated light (either classical or quantum) can be employed in various ways to improve resolution and measurement sensitivity. In an "interaction-free" measurement, a single photon can be used to reveal the presence of an object placed within one arm of an interferometer without being absorbed by it. This method has previously been applied to imaging. With a technique known as "ghost imaging", entangled photon pairs are used for detecting an opaque object with significantly improved signal-to-noise ratio while preventing over-illumination. Here, we integrate these two methods to obtain a new imaging technique which we term "interaction-free ghost-imaging" that possesses the benefits of both techniques. While maintaining the image quality of conventional ghost-imaging, this new technique is also sensitive to phase and polarization changes in the photons introduced by a structured object. Furthermore, thanks to the "interaction-free" nature of this new technique, it is possible to reduce the number of photons required to produce a clear image of the object (which could be otherwise damaged by the photons) making this technique superior for probing light-sensitive materials and eventually biological tissues. If time allows, I will discuss some follow-up works involving partial measurements and remote erasure/completion of images.

ORAL A7:

Real-time nuclear diagnosis using high-resolution coherence phase-interference microscopy

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The working-out and implementation of new optical technologies for bioimaging into clinical practice increases the effectiveness of diagnosis. We used the phase imaging of interphase chromatin as an original biosensor and detector of early changes in cell nuclei. Our quantitative phase diagnostic system is based on a Linnik interference microscope with high-numerical-aperture objectives.

CD4+ and CD8+lymphocytes of the peripheral blood were under study taken from 30 healthy volunteers and 57 multiple sclerosis patients in exacerbation stage. Exacerbation was determined basing on clinical picture and MRI. The patients underwent pulse-therapy with methylprednisolon in total dose 5,000 mg. The lymphocyte nuclei was assessed in the real time using the quantitative phase-interference microscopy “MIM” (“Amfora-laboratories”, Moscow, Russia): height accuracy 0,1 nm, coordinate accuracy 13 nm, image area 256x256 pixels, optical magnification 1000. The complex algorithm included the definition of optic and geometrical characteristics of living cells, statistical analysis of data and creation of medical documents.

It was established that the morphodensitometric indicators objectively reflect structural reorganization of the spatial organization of interphase chromatin. The following parameters were evaluated: relative intensity (for the phase image density), the relative distance between the centers of the segments, the number of segments in the core area and the perimeter of the kernel in pixels. We have used a few different segmentation algorithms: clustering, areas, levels and watershed. It is shown that as a reliable criteria for the nuclei during the exacerbation of the disease can be considered a relative increase in intensity, increasing the number of segments in the core and perimeter change image nuclei in pixels.

Acquirement of important quantitative information concerning cellular condition using technically available and not very expensive modalities of interference microscopy presents new opportunities for practical application of live functioning cell nuclei as prospective biosensors for diagnostic purposes.

ORAL A8:

In vivo real time assessment of water content in the human dermis using diffuse reflectance spectroscopy

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Edema is a swelling effect caused by accumulation of fluid in tissues under the skin. It may manifest itself due to pregnancy and medical conditions such as heart failure. The common method for edema characterizing is known as pitting, which is a procedure when pressure is applied to the swelling part of patient's body and restoration of the "pit" is observed. This method provides general picture and is not capable of quantitative characterization of the process. It is evident that swelling may be described by the water content in the tissue and it can be done using spectroscopic technique. In our recent work [1], we have suggested a method for characterizing edema severity using optical microscopy, which was based on the assessment of the viable epidermis size. However, this method is complicated when there is a need to produce a serial medical device. The most reasonable way for non-invasive measurement of water content in tissues is reflectance spectrometry. Currently, some commercial devices based on the method of diffuse reflectance spectroscopy were proposed to characterise human skin chromophore contents [2].

We assembled a setup for diffusive reflectance spectroscopy measurements, prepared different phantoms of human skin, including liquid and soft matter phantoms with varying water and chromophores contents, and carried out measurements of diffusive reflectance spectra for them. As expected, reflectance spectra demonstrated correlation with chromophores contents. Since the reflectance spectra is mainly determined by two processes, namely scattering and absorption, it was necessary to develop the method for characterization of skin absorption using data of reflectance spectroscopy. The process of light propagation in the human skin was simulated using Monte-Carlo method on the cluster of GPU nodes [3] with the modified version of GPU-MCML program [4]. The results of simulations was utilized to develop statistical methods of diffusive reflectance spectra processing which allowed to estimate contribution of absorbance into observed spectra. These methods were applied for processing of data obtained using skin phantoms. After that, a series of experiments on humans were performed, which allowed to estimate reliability of the proposed method for clinical applications.

The research was carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University. The work was supported by the Russian Science Foundation (grant №18-15-00422).

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ORAL A9:

Fiber photonics for broad spectra biomed applications

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Fiber photonics development for laser medicine was started around 50 years ago – from flexible cables and waveguides used to deliver laser beam for medical operations, while much longer history is related to imaging fiber bundles used for endoscopic diagnostics and minimal invasive operations *in-vivo*.

Present review will highlight the latest results in advanced fiber optic solutions for a broad spectral range of 0,4-16µm: multi-spectral tissue diagnostics to detect malignant tissue *ex-vivo* and *in-vivo*, minimal invasive laser angioplasty, inter-corporal InfraRed-imaging of tissue during RFA procedures in heart and the other ways to use fiber optics in intraoperative therapy control. Thus fiber optics enables to fuse diagnostics and therapy procedures in so called "theranostic" applications.

Spectral fiber sensing for label free analysis of tissue composition helps to differentiate malignant and normal tissue to secure minimal invasive, but complete tumor removal or treatment. All key methods of Raman, fluorescence, diffuse reflection & MIR-absorption spectroscopy will be compared when used for the same spot of tissue - to select the most specific, sensitive and accurate method or to combine them for the synergy enhanced effect. Examples of multi-spectral tissue diagnostics will be presented for several organs together with the preclinical trials of the 1st tumor sensor prototypes.

The 1st results on Mid IR-fiber endoscopy imaging will be presented for our Polycrystalline fiber cables produced for advanced system of Securus Medical/ Boston Scientific – used for thermography control of RadioFrequency Ablation (RFA) for pulmonary vein isolation (PVI) - the common treatment used against atrial fibrillation (AF).

The other achievements will be described multi-fiber catheters we produce for EXIMO Medical – the innovative company from Israel providing a hybrid laser and blade technology for a variety of interventional vascular procedures enhancing their performance and safety for different lesions.

Unique advantage of PIR-fiber transmission in Mid IR-range 3-16µm enables also to run non-contact temperature control for various laser-tissue operations: ablation, coagulation and welding of vessels, - providing non-contact control of tissue temperature at the spot of laser beam on tissue. This feature helps to design “smart” laser systems for minimal invasive operations.

ORAL A10:

Three-level approximated helical phase with a carrier wave for beam delivery for optical trapping applications

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Abstract: A spiral phase is approximated using three phase levels and is propagated in free space. The amplitude and phase patterns generated by the beam are investigated for optical trapping applications. Carrier waves are engineered using diffractive optical elements for delivering the spiral phase to unconventional regions in space.

Summary

Optical trapping has shown great potential to isolate and study biological cells [1]. The conventional optical traps which use a Gaussian intensity profile may create absorptive heating on certain samples due to the presence of higher intensity at the optical axis. Vortex beams on the other hand exhibit a useful intensity profile with an intensity value of zero at the optical axis [2]. The former uses gradient force to trap the particle while the later uses orbital angular momentum. The generation of a tightly focused Gaussian intensity profile is relatively simpler as it requires only a microscopic objective lens with a larger NA. On the other hand, the generation of vortex beam can be achieved only using complicated phase elements such as spiral phase plates [3] which are difficult to manufacture [3], or forked gratings [4] which produce higher diffraction orders [4].

In this study, we investigate the possibility of designing a spiral phase plate with only three phase levels. The spiral phase generated with only three phase levels is propagated in free space whereas its the intensity and phase profiles are studied. The Poynting vector fields are calculated for the three-level spiral phase and have been found to possess orbital angular momentum suitable for optical trapping applications. Three carrier waves are engineered using diffractive optical elements for delivering the helical phase to a point in space, to a pre-defined longitudinal interval in space and to a pre-defined curved line in space. The phase masks consist of two elements namely the three-level spiral phase and the diffractive optical function for beam delivery which are combined using a modulo-2π phase addition method.

The experiment was carried out using a He-Ne laser spatially filtered and collimated which is incident on a spatial light modulator (SLM). In the SLM, the phase masks are displayed, and the resulting intensity patterns are recorded by an image sensor. The theoretical analysis of the three-level spiral phase plate and the experimental results for the three different beam delivery modules will be presented.

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ORAL A11:

Wideband optoacoustic detectors for multi-scale characterization of the vasculature

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***Abstract*— The paper reviews our recent experience in multi-scale biomedical optoacoustic imaging using wideband ultrasonic detectors based on polyvinylidene fluoride films. The experimental setups for optoacoustic microscopy and tomography are presented, numerical algorithms for versatile characterization of tissue vasculature are discussed.**

Keywords— Photoacoustic imaging; angiography.

Optoacoustic (OA) imaging is based on remote detection of ultrasonic signals generated in biological tissue as a result of the absorption of pulsed laser radiation by optical inhomogeneities. The major biomedical application of OA methods is angiography with VIS-NIR range of optical wavelengths [1]. Shorter optical wavelength range (<630 nm) allows for high-contrast angiography of smaller and superficial blood vessels (<150 μm in diameter at <1 mm depths), while optical transparency wavelength range (630-1300 nm) is important for OA angiography of deeper and larger blood vessels (>150 μm in diameter located at > 1 mm depths).

Limited bandwidth of conventional piezoelectric detectors does not allow for effective detection of broadband OA pulses. We develop custom-made wideband (0.1-50 MHz) ultrasonic detectors based on 30 μm polyvinylidene difluoride (PVDF) films. Elasticity of such PVDF films allows fabrication of spherically-focused detectors (with up to 0.6 numerical aperture) for OA microscopy [2]. Chemical resistance of such PVDF films allows photolithographic etching of 64-element linear arrays for OA tomography [3].

Along with the development of ultrasonic detector with the optimal frequency and geometry, practical realization of an efficient OA imaging system requires algorithms for acoustic and optical reconstruction. To perform acoustic reconstruction from raw OA B-scan acquired by linear detector array we use synthetic aperture focusing technique (SAFT) algorithm developed by the group of Prof. Frenz [4]. Virtual-detector concept [5] allows to use the same SAFT algorithm [4] in OA microscopy to improve the spatial resolution above and below the detector focal plane. To compensate frequency-dependent ultrasonic attenuation, we use Tikhonov deconvolution filtration [6]. For optical fluence compensation, we estimate effective in-depth fluence profiles by Monte Carlo simulation of light transport accounting for complex geometry of laser illumination and acoustic detection [7]. Fluence compensation allows for significant increase of OA signal level corresponding to deeper blood vessels, however due to the limited dynamic range of optoacoustic detector the same procedure inherently increases the noise amplitudes. The unwanted background noise in angiography images caused by melanin and myoglobin can, however, be effectively suppressed by vessel-specific filters, such as Frangi's multiscale vessel enhancement technique [8].

To characterize the oxygen saturation within the tissue vasculature (Fig. 1), we develop indirect approach based on estimations of effective optical attenuation $\mu_{eff}(\lambda)$ in given blood-containing vessel based on temporal characteristics of OA signal from this vessel. Our preliminary experiments demonstrate that wideband (0.1-50 MHz) PVDF ultrasonic detectors can be used for calibration-free quantitative optoacoustic measurements of effective optical attenuation in whole blood in the range of 532-1064 nm.

The study is supported by Russian Science Foundation, project 18-45-06006.

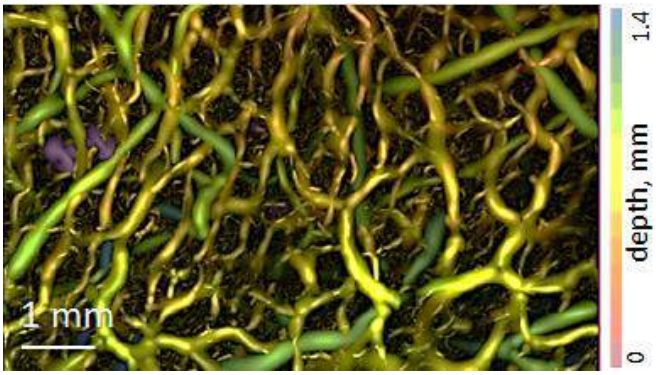

Fig. 1. Optoacoustic angiography of human palm at 532 nm wavelength.

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ORAL A12:

The theory behind the full scattering profile

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Optical methods for extracting properties of tissues are commonly used. These methods are non-invasive, cause no harm to the patient and are characterized by high speed. The human tissue is a turbid media hence it poses a challenge for the different optical methods. In addition the analysis of the emitted light requires calibration for achieving accuracy information. Most of the methods analyze the reflected light based on their phase and amplitude or the transmitted light.

We suggest a new nanophotonic method for extracting optical properties of cylindrical tissues based on their full scattering profile (FSP), which mean the angular distribution of the reemitted light. The propagation path of each photon was calculated from the scattering constant and the scattering was measured experimentally. The FSP of cylindrical tissues is relevant for biomedical measurement of fingers, earlobes or pinched tissues. We found the iso-pathlength (IPL) point, a point on the surface of the cylinder medium where the light intensity remains constant and does not depend on the reduced scattering coefficient of the medium, but rather depends on the spatial structure and the cylindrical geometry. However, a similar behavior was also previously reported in reflection from a semi-infinite medium. Moreover, we presented a linear dependency between the radius of the tissue and the point's location. This point can be used as a self-calibration point and thus improve the accuracy of optical tissue measurements. This natural phenomenon has not been investigated before. We show this phenomenon theoretically, based on the diffusion theory, which is supported by our simulation results using Monte Carlo simulation, as well as experimental results with phantoms and real fingers.

ORAL A13:

Development of histomorphometry using multiple sequential polychrome-labeling and confocal laser scanning microscopy for quantitatively estimation of new bone formation rate in repaired defects

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Physiological remodeling of bone is a continuous process by mineralization and forms regular deposition pattern microstructurally, which can be traced using multiple sequential polychromelabeling (MSPL). However, the pattern of newly regrown bone in pathological conditions, such as osteoporosis, osteonecrosis, fracture repair, and Paget disease of bone, form irregular microstructure. A quantitatively objective assessment of bone formation and microstructure in those conditions would not only assist with a better understanding of bone pathogenesis but also provide a measurement of the new bone growth rate essential for estimation of ossification particularly in clinical repairs. The ex vitro new bone formation and its microstructure in defect repair model were evaluated by in vivo MSPL using confocal laser scanning microscopy (CLSM), which is equipped with acousto-optic tunable filter (AOTF) of argon and He-Ne laser to generate images using the multi-tracking scan (MTS) mode with transmitted light mode (Zeiss LSM 510 META system) with excitation wavelengths of 488 and 453 nm, and emission at 520 and 615 nm for calcein green and xylenol orange, respectively. The method generated clearer and more reliable images of thick bone sections than conventional fluorescence microscopy (CFM), showed fine details of the bone microstructural features, including the mineralization fronts, quiescent versus active osteons, and Volkmann's channel. Histomorphometrical analysis using CLSM combined with differential interference contrast (DIC) microscopy revealed that the functionality of bone remodeling was reflected by histomorphological changes. New bone formation and its microstructure can be evaluated more adequately using a combination of CLSM and DIC microscopies. Furthermore, histomorphometrical evaluations using the ratio of labeled areas of new bone formation by CLSM measure growth rate of new bone in repairs, which provides a powerful tool to evaluate the effectiveness of bone repairs using any tissue engineering materials.

ORAL A14:

Quantitative detection and staging of cancer tissues using label-free Mueller matrix microscope

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Polarization imaging is a promising technique for probing the microstructures, especially the anisotropic fibrous components of tissues. Among the available polarimetric techniques, Mueller matrix polarimetry has many distinctive advantages, such as providing label-free and comprehensive descriptions on the properties of tissues. Mueller matrix polarimetry can help to improve the image contrast of the superficial layers of tissues by eliminating multiply scattered photons from the deep layers. The previous literature shows that more than 85% of cancers originate from the superficial epithelium, which means that Mueller matrix polarimetry has great potential in screening and identifying cancer at an early stage. Recently, we have developed a Mueller matrix microscope by adding the polarization state generator and analyzer to a commercial transmission-light microscope, and applied it to differentiate cancerous tissues with fibrosis. Here we apply the label-free Mueller matrix microscope for quantitative detection and staging of different cancer tissues including human breast ductal carcinoma, liver cancer, and colon cancer at different stages. The Mueller matrix polar decomposition (MMPD) and Mueller matrix transformation (MMT) parameters of the abnormal tissues in different regions at in situ and invasive stages are calculated and analyzed. For more quantitative comparisons, several image texture feature parameters derived from the gray level co-occurrence matrix (GLCM) are also calculated to characterize the difference in the polarimetric images. The experimental and simulation results indicate that the Mueller matrix microscope and the polarimetric parameters can facilitate the quantitative detection of cancer tissues with fibrosis at different stages.

ORAL A15:

Automated Analysis of Sperm for *In Vitro* Fertilization

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Abstract: Selecting sperm cells possessing normal morphology and motility is crucial for intracytoplasmic sperm injection (ICSI). Sperm quality directly affects the probability of obtaining healthy pregnancy. We recently presented a novel platform for real-time quantitative analysis and selection of individual sperm cells without staining. Towards this end, we developed an integrated approach, combining interferometric phase microscopy (IPM), for stain-free sperm imaging and real-time automated classification [1], with a disposable microfluidic device, for sperm selection and enrichment [2] (Fig. 1(a)). Over 1,400 human sperm cells from 8 donors were imaged using IPM, and an algorithm was designed to digitally isolate sperm cell heads from the quantitative phase maps while taking into consideration both the cell 3D morphology and contents, as well as acquire features describing sperm head morphology (Fig. 1(b)). A subset of these features was used to train a support vector machine (SVM) classifier to automatically classify sperm of good and bad morphology. The SVM achieves an area under the receiver operating characteristic curve of 88.59% and an area under the precision-recall curve of 88.67%, as well as precisions of 90% or higher. The microfluidic device was then manufactured and tested. Upon testing the device, we obtained successful selection of sperm cells with a selectivity of 89.5 ± 3.5%, with no negative-decision sperm cells being inadvertently selected. Based on these results, we believe that the presented integrated approach has the potential to change the way sperm cells are selected for ICSI and other assisted reproduction procedures, making the selection process more objective, quantitative and automatic, and thereby increasing success rates in obtaining healthy pregnancies.

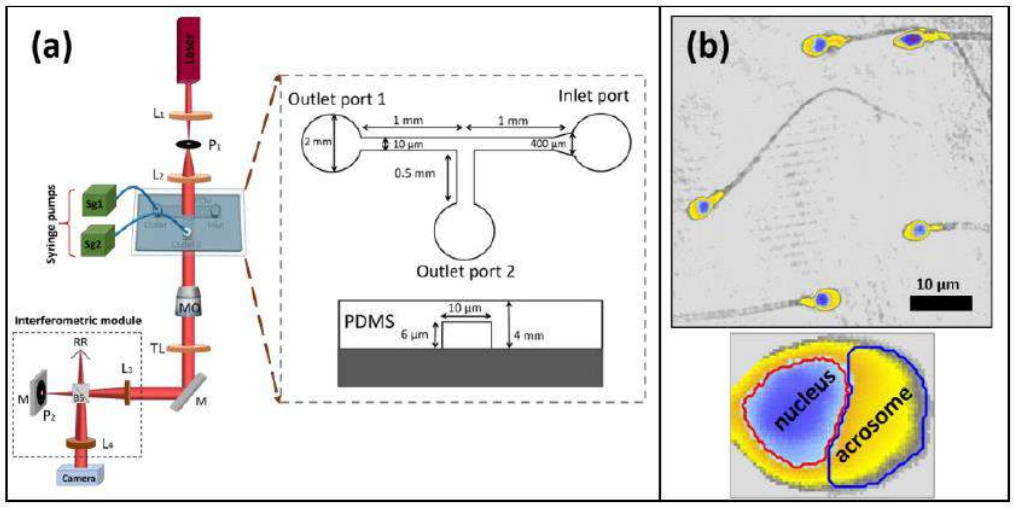

Fig. 1. (a) Schematic of the experimental setup containing a disposable microfluidic device integrated with the IPM setup. The inset shows the top-view and cross-sectional view of the microfluidic device. L1-L4, achromatic lenses. MO, microscope objective. TL, tube lens. Sg1, Sg2, syringe pumps. P1, P2, pinholes. M, mirror. RR, retro-reflector mirror. BS, beam splitter. Figure is modified from Ref. [2]. (b) IPM images of sperm cells obtained without staining, and detection of the sperm organelles. Figure is modified from Ref. [1].

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ORAL A16:

Singlet oxygen phosphorescence *in vivo*

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Background and Objective

The visualization of singlet oxygen interaction with the target tissue *in vivo* is aspired since long. The distribution of the photosensitizer in tissue can be determined via fluorescence, but the localization is not necessarily related to the photodynamic effect. Only direct time-resolved supervision of the singlet oxygen phosphorescence may report this.

In this work we report successful *in vivo* measurements through the skin of mice after systemic drug application. Using special high transmission optics centered around 1200, 1270 and 1340 nm, we can clearly distinguish singlet oxygen luminescence from other signals. Our results give new insight into the influence of the local oxygen concentration during PDT on the treatment efficiency.

Materials and Methods

N-(2-hydroxypropyl) methacrylamide copolymers conjugated with pyro-pheophorbide-a were used for measurements. This drug exhibits highly selective accumulation in tumor.

Central part of the work is the technical development of a setup, ready for *in vivo* singlet oxygen phosphorescence detection. It consists of a laboratory version of the TCMPC1270 (SHB Analytics – a Humboldt spin off). It is operated with a high aperture multifurcated quartz fiber (Ceram Optec) for excitation and detection, together with six pulsed laser diodes at 660 nm (Thorlabs). All fiber cores are combined in a sealed single tip that can be placed directly at the skin of the mouse. This way the efficient light collection of a high aperture optics is combined with the flexibility of a fiber setup, as required for *in vivo* application.

Results and Discussion

We measured the singlet oxygen phosphorescence 24h to 96h after injection into the tail vein with so far unprecedented SNR. The phosphorescence kinetics in tumors clearly differ from those at other places. Also, in tumors we can reliably identifiy singlet oxygen signals of two different origins, of which one indicates acute anoxia right from the start. This explains, why the ratio of singlet oxygen phosphorescence amplitudes in tumor (A) vs. normal tissue (B) is below one, while the ratio of the fluorescence intensities at the same spots is 8 ± 2. Obviously, local oxygen depletion has major impact on photosensitizer activity *in vivo*, which underlines the importance of direct singlet oxygen supervision.

Figure: Corrected singlet oxygen phosphorescence kinetics and raw data (insert) of the measurements at the tumor. The dashed curves represent the singlet oxygen phosphorescence corrected for the short time artifact comprising a fast and a slow signal component therein. (submitted to EJPB)

Conclusions

Direct supervision of singlet oxygen phosphorescence in tumors located directly below the skin is principally possible now. First measurements reveal pronounced local oxygen depletion in the tumor making direct singlet oxygen supervision a must-have during PDT.

ORAL A17:

Fluorescence time-resolved macro-imaging

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Abstract.

While laser scanning fluorescence lifetime imaging (FLIM) is a powerful approach for cell biology, its small field of view (typically less than 1 mm) makes it impractical for imaging of large biological samples that is often required for biomedical applications. Here we present a system that allows to perform FLIM on macroscopic samples as large as 18mm with a lateral resolution of 15μm. The performance of the system is verified with FLIM of endogenous metabolic cofactor reduced nicotinamide adenine dinucleotide (phosphate), NAD(P)H, and genetically encoded fluorescent protein mKate2 in a mouse tumor *in vivo*. We also use the system to make FLIM-FRET measurements on tumor xenografts and observe the activity of caspase-3 with genetically encoded sensor TR23K based on the red fluorescent protein TagRFP and chromoprotein KFP linked by 23 amino acid residues containing a specific caspase cleavage motif. We believe that this macro-FLIM system with a large field of view and cellular resolution opens the opportunity to explore biological processes *in vivo* in the whole tumor in animal and has a prospect for clinical use for intraoperative characterization of tumor or exploration centimeter sized samples.