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# LALS

Laser Applications in Life Sciences

# Track B: Sensing

**KEYNOTE B1:**

# Parametric approaches to optical coherence tomography and their applications

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Optical coherence tomography (OCT) has proven to be a successful label-free imaging technique; however, achieving resolution and contrast sufficient to visualise individual cells and their constituents remains challenging. Achieving such visualisation over the centimetre-scale fields of view key to many applications presents additional data capture and processing challenges, but accepting lower resolution has the disadvantage of reducing the contrast from the intrinsically scattering structures that OCT must rely on for imaging without labelling. In part, this challenge and trade-off have been the motivation for examining options for other sources of contrast in OCT; additionally, such sources convey extra information not available from morphological imaging. Many sources of contrast have been examined, including detection of the vascular and lymphatic systems via motion contrast, as well as the assessment of associated flow parameters. As well, imaging tissue micro-scale mechanical properties has been shown to be important in understanding function and revealing pathology. We have been exploring methods and applications of applying OCT to elastography, so-called optical coherence elastography. Polarization is another interesting source of contrast – probing the polarization state of the OCT signal reveals tissue birefringence and orientation of sub-resolution-scale fibrous structures, or the disruption of such structures caused by disease. Such systems have only recently matured to the extent necessary to reveal interesting phenomena in applications, such as imaging airway smooth muscle. We have been interested in the development of these methods (OCT parametric imaging of vessels, attenuation, birefringence, and stiffness), in the development of imaging systems using them, and in their applications in biology and medicine. Our main themes of application include intraoperative surgical guidance for breast cancer removal, airway physiology and asthma, wound healing, and glaucoma surgery.

**KEYNOTE B2:**

# High Throughput, Wide-Field Multiphoton Microscopy For Deep Structural Imaging of Neuronal Synapse Remodeling

**Peter T. C. So**
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Neuron structural remodeling is closely related to mammalian memory plasticity. Many pioneering studies in this field were enabled by point scanning multiphoton microscopy. In vivo, high resolution 3D imaging of the whole dendritic tree requires about 30 minutes. Significant remodeling on the synaptic level has been observed within a day but we hypothesize that there are important faster dynamics to be explored. To access events on the time scale of minutes to hours, we are developing high throughput wide-field multiphoton microscopy based on temporal focusing. While this method has been developed for over a decade, it has been limited by having modest penetration in tissues due to scattering of excitation and emission photons. We will explore several approaches to overcome this limitation including three-photon excitation and coupling structured illumination with computation image recovery.

**KEYNOTE B3:**

# Multi-channel optical coherence tomography

**Christoph K. Hitzenberger**

Conventional OCT measures backscattered light in a single sample arm channel. The simultaneous illumination by two or more sample beams and/or the simultaneous detection of light backscattered at different angles provides additional information and can be used to generate improved contrast and quantitative measurements. Various multi-channel OCT schemes will be presented and demonstrated for Doppler- and angle-resolved imaging in the human retina and in brain tissue.

**INVITED B1:**

# 3D imaging of thick specimens using Gradient Light Interference Microscopy (GLIM)

**Gabriel Popescu**

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Light scattering limits the quality of optical imaging of unlabeled specimens: too little scattering and the sample is transparent, exhibiting low contrast and too much scattering washes the structure information altogether. As a result, current instruments, target specifically either the thin (low-scattering) specimens or the optically thick (multiply scattering) samples. We developed gradient light interference microcopy (GLIM) to extract 3D information from both thin and thick unlabeled specimens. GLIM exploits the principle of low-coherence interferometry to extract phase information, which in turn yields strong, intrinsic contrast of transparent samples, such as single cells. Because it combines multiple intensity images that correspond to controlled phase shifts between two interfering waves, GLIM is capable of suppressing the incoherent background due to multiple scattering. Thus, GLIM yields real-time tomography of optically thick samples via full field imaging. These results indicate that GLIM can become a valuable label-free analysis tool for in-vitro fertilization, where contrast agents and fluorophores may impact the viability of the embryo. We demonstrate the use of GLIM to image various samples, including standard micron size beads, single cells, cell populations, and thick bovine embryos. Recently, we have developed a reflection-based GLIM setup *(epi-GLIM)*, which allows us to image topography of opaque samples, specimens on opaque substrates, as well as bulk tissues, such as surfaces of organisms *in-vivo.* GLIM/epi-GLIM operates as an add-on to a conventional microscope and overlays seamlessly with the existing channels (e.g., epi-fluorescence).

**Bio**

Gabriel Popescu is a Professor in Electrical and Computer Engineering, University of Illinois at Urbana-Champaign. He received his Ph.D. in Optics in 2002 from the School of Optics/ CREOL (now the College of Optics and Photonics), University of Central Florida. He continued his training with Michael Feld at M.I.T., working as a postdoctoral associate. He joined Illinois in August 2007 where he directs the Quantitative Light Imaging Laboratory (QLI Lab) at the Beckman Institute for Advanced Science and Technology. Dr.

Popescu served as Associate Editor of Optics Express and Biomedical Optics Express, Editorial Board Member for Journal of Biomedical Optics and Scientific Reports. He authored a book, edited another book, authored 150 journal publications, 200 conference presentations, 29 patents (12 disclosures under review), gave 190 lecture/plenary/invited talks. He founded Phi Optics, Inc., a start-up company that commercializes quantitative phase imaging technology. He is OSA and SPIE Fellow.

**INVITED B2:**

# Increasing Resolution in 3D Live Cell Microscopy

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Resolution in microscopy has been described by Ernst Abbe more than 100 years ago, giving values around 200 nm in lateral and 400 nm in axial direction, if high aperture objective lenses are used. Only recently smaller values were achieved by STED microscopy, methods based on single molecule detection or Structured Illumination Microscopy (SIM). However, some of these methods suffer from high light exposure in live cell imaging and are restricted to thin sample layers due to light scattering and low working distances of high aperture lenses.

The present paper shows how starting from conventional 3D microscopy over light sheet fluorescence microscopy (LSFM)[1], confocal microscopy, axial tomography[2] and SIM[3] down to Total Internal Reflection (TIR)[4] and Förster Resonance Energy Transfer (FRET) microscopy[5] lateral and axial resolution are enhanced step by step upon application of non-phototoxic light doses. Thus, larger cell or tissue samples as well as single living cells and cellular substructures (e.g. cell nuclei) can be imaged adequately. Possibilities are outlined how some of these methods may be combined for 3D super-resolution imaging.

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**INVITED B3:**

# Label-free differentiation of floating cells by polarized photon scattering

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It has been known that changes in the state of polarization (SOP) during polarized photon scattering encode rich information on the optical properties and microstructural features of the scattering samples. By adding a polarization state generator (PSG) and a polarization state analyzer (PSA) to an optical instrumentation based on non-polarized light detection, the instrument can be upgraded to a polarized version with minimum alterations on its optical path, which significantly enhances the capabilities for differentiating biological species of different microstructure but maintains the compatibility between data obtained by the two versions of instruments.

In this report, we present a polarized light scattering method for differentiating organic and inorganic particles, particularly marine algae suspended in water. A proof-of-concept experimental setup is established which illuminates the suspended cells with polarized light and takes the SOP of the scattered light at 120°. The optical path follows the optical configuration of a scattering based commercial marine instrument (ECO-BB9 by WET Labs) well known in ocean color research. By varying the SOP of the illumination beam and analyze the SOP of the scatter light using linear discriminant analysis (LDA), we demonstrated that the new technique can effectively differentiate polystyrene microspheres, porous polystyrene microsphere, silicon dioxide microsphere, and a large number of different marine microalgae species. It can even detect the responses by some marine microalgae to their surrounding environments. Simulations based on the Mie theory and discrete dipole approximation confirm that the scattered polarizations are sensitive to the size, shape, submicron intracellular microstructure and the refractive indices of the particles. The experimental results based on such laboratory proof-of-concept system and the simulations prove the feasibility of new label-free techniques and instrumentations for marine observations, even for label-free flow cytometer for biomedical applications.

**INVITED B4:**

# Metabolic imaging, cells and proteins assessment in the human papillary dermis in vivo

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Most of the methods used in clinics to diagnose skin are invasive and require a biopsy for histological examination. In the last 10-15 years, the multiphoton tomography (MPT) with fluorescence lifetime imaging (FLIM) have advanced so much that it allow to obtain results comparable in sensitivity and specificity with histological analysis. MPT is based on non-linear optical effects, such as the optical harmonics generation, mainly the second harmonic generation (SHG), two-photon excited auto fluorescence (TPEAF) and coherent anti-Stokes Raman scattering (CARS). These effects are observed when ultrashort (usually femtosecond) laser pulses are used as a pump. The use of such pulses can increase the penetration depth of radiation into the skin. MPT is an imaging technique allowing not only to analyze the architecture of the tissue, but also to selectively detect certain types of molecules using their spectral or fluorescence lifetime-based signature, thus allowing to perform imaging of metabolic processes. However, MPT capabilities are currently limited to several specific applications, e.g. assessment of epidermal cells and collagen type I in the upper dermis, while a number of clinically relevant structures and processes remain not investigated. Recently, we suggested the MPT/FLIM based approach to assess blood vessels and structural proteins localization in the upper dermis. This stimulated further research of the area around superficial capillaries, which is metabolically active due to exchange of chemicals through the capillary wall. The first question we addressed was the manifestation of fluid retention, i.e. the edemateous syndrome, in the properties of perivascular zone for patients suffering from heart failure. It was shown that the increased amount of interstitial fluid controls the intercellular space and the size of viable epidermis. Next, migration and localization of cells in the vicinity of blood vessels in the papillary dermis was investigated, and the question about the possibility of mast cells imaging was addressed. Finally, transcapillary diffusion of model substances was studied in the papillary dermis, and the origin of two-photon and one-photon fluorescence of blood cells is discussed. The work was supported by the Russian Science Foundataion (grant №17-75-10215).

**INVITED B5:**

# Nanolayers – new possibilities in fiber-optic biosensors technology

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Fiber-optic sensors based on nanolayers or thin films, and their ability to perform measurements will be presented. In the last decade, fiber-optic sensors gained popularity as sensing devices. This was possible because of the design and the integration of new materials into fiber-optic technology. Nanolayers and thin films made from various materials such as: nanocrystalline diamond (NCD), boron-doped nanocrystalline diamond (B-NCD), zinc oxide (ZnO), titanium dioxide (TiO2), aluminum oxide (Al2O3) and boron nitride (BN) were successfully applied in the construction of fiber-optic sensors. Nanocrystalline diamond and boron-doped nanocrystalline diamond were synthesized by the Chemical Vapor Deposition (CVD) methods, while oxide and nitride based thin films were produced using Atomic Layer Deposition (ALD). These nanolayers and thin films were widely used in fiber-optic sensors technology as protective coatings, reflective layers and/or as sensing medium.

**INVITED B6:**

# Autofluorescence and diffuse-reflectance spectroscopy for skin cancer diagnosis

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Optical biopsy is a relatively new term used in clinical practice for description of spectroscopy approaches for detection of human tissues in vivo. Painless, instant diagnoses from optical biopsies will soon be a reality. Such forms of optical diagnoses are preferable to the removal of several cubic millimetres of tissue, as used in traditional biopsies – followed by delays while samples are sent for histological analysis. There is also possible that the optical biopsy apparatus will requires a learning curve of several practice attempts, compared to years of training needed for more conventional techniques. Most popular combination of spectroscopic modalities investigated to become an additional tool to the medical diagnostics instrumentation is based on fluorescence and diffuse-reflectance techniques.

Autofluorescence spectroscopy of human tissues is very attractive tool for early diagnosis of cancer due to its high sensitivity, easy-to-use methodology for measurements, lack of need for contrast agents' application on the tissue under investigation, possibilities for real time measurements and noninvasive tumour detection. However, no reliable and universal system for fluorescence detection of skin cancer appeared on the medical market up to nowadays. Problems for development of such diagnostic fluorescence system for skin cancer detection are related to the great variety of benign and malignant forms of skin pathologies, for example basal cell carcinoma lesions have more than 15 sub-types, squamous cell carcinoma lesions, have about 10 different subtypes, and all of them have variety of benign and dysplastic forms, as well as they are different, including by their fluorescence properties, on different stages on the lesion growth. Other very important disadvantage is the fact that under different excitation wavelengths – different endogenous fluorophores appear in the integral autofluorescence signal coming from the skin, which makes this kind of spectra uneasy for analysis and comparison with the fluorescence signals, detected from various pathological lesions. High pigmentation of skin lesions is also significant drawback for application of autofluorescence technique for discrimination of cutaneous pathologies, as the pigments re-absorb both the excitation and emission light, distorting significantly the spectra received. In case of melanin-pigmented malignant melanoma, which is the most severe cutaneous neoplasia, it is a crucial for the proper diagnosis using fluorescence mode of detection solely.

In such cases diffuse-reflectance technique, based on broad-band light irradiation of the skin surface and detection of the back-scattered light from the areas of interest could help and improve rapidly the diagnostic accuracy.

We will present an overview of the several year's research efforts and experimental results of detection of different types of cutaneous benign, dysplastic and malignant lesions detected from patients in vivo in University hospital "Tsaritsa Yoanna-ISUL" in Sofia, using both techniques. Discussion about the internal sources of the optical signals and specific spectral features would be expounded; as well the applicability of fluorescence and diffuse-reflectance techniques solely and in combination into the clinical diagnostic practice would be evaluated.

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**INVITED B7:**

# The Road to Real Time 3D Optical Coherence Tomography

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Optical coherence tomography (OCT) has attracted much attention after it was introduced in the 1990s. This cross-sectional imaging technique may provide depth-resolved information of bio-tissues with micron-scale resolution in a non-invasive manner and therefore has been widely used in clinic. Nowadays, there is an increasing need for real-time volumetric imaging in OCT-based clinical diagnosis. However, the slow data acquisition of early time-domain OCT systems in the range of ~1 kHz A-scan rate limited the imaging speed to only several frames per second. The introduction of frequency-domain or Fourier-domain (FD) detection techniques, categorized in two main classes as spectral-domain OCT (SD-OCT) and swept-source OCT (SS-OCT), has led to a dramatic increase in imaging speed and sensitivity.

However, to realize real time 3D optical coherence tomography imaging, there are still several challenges in optical source, detection scheme, data processing and etc., due to the acquisition and processing of massive data of >10 GB/s required by real time 3D display. It is implied that the road to real time 3D optical coherence tomography imaging may rely on the innovations of both the fast swept light sources and the data processing techniques including compressed sensing and optical computing. In this talk, I will discuss and demonstrate some new progress on the solutions related to these challenges:

A direct linear-in-wavenumber swept laser based on AOD filter for SS-OCT with 2MHz linear scan rate will be demonstrated. Because of avoiding data resampling and recalibration that are generally required in conventional FD-OCT, this new laser source enables ultrahigh imaging speed and is more favorable and promising for future applications.

Furthermore, I will talk about a novel all-optical swept-source based on the buffered optical time-stretch technique utilizing LCFBG as the dispersive medium that achieves linear-in-wavenumber tuning range of 40nm at an A-scan

rate of as high as 40MHz, which is, to the best of our knowledge, the highest speed swept-source at 1545nm for SS-OCT.

To process big data of > 10 Gb/second in real time 3D OCT imaging, we propose an all-optical Fourier transformation system for real-time massive data processing. In the so-called optical computing OCT, fast Fourier transformation (FFT) of A-scan signal is optically processed in real time before being detected by photoelectric detector. Therefore, the processing time for interpolation and FFT in traditional Fourier domain OCT can be dramatically eliminated. A processing rate of 10 mega-A-scans/second was experimentally achieved, which is, to our knowledge, the highest speed for OCT imaging.

**INVITED B8:**

# No Fourier Transform Optical Coherence Tomography

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We present here the advantages of a novel optical coherence tomography (OCT) technology, Master Slave (MS). The MS method radically changes the main building blocks of a spectral (Fourier)-domain OCT set-up. The signal conventionally provided by a Fourier Transform (FT) or equivalent is replaced by delivery of multiple signals, a signal for each optical path difference in the sample investigated. In this way, it is possible to: (i) directly access the information from selected depths; (ii) eliminate the process of resampling, required by the FT based conventional technology, with immediate consequences in improving the decay of sensitivity with depth, in achieving the expected axial resolution limit, in reduction of the time to display an *enface* OCT image and lower cost OCT assembly; (iii) tolerate the dispersion left unbalanced in the OCT interferometer. Applications in ophthalmology and Gabor microscopy will be presented using the MS OCT technology.

**INVITED B9:**

# Robust strain mapping in phase-sensitive optical coherence tomography: applications to compressional elastography and beyond

In the talk, recent results on the development of OCT-based strain mapping methods demonstrating high robustness to deformation-induced decorrelation noises, as well as other measurement noises will be reported and examples of their applications will be given.

The performed analysis of OCT-scan forming revealed the reasons of insufficient efficiency of correlational speckle tracking in OCT and made it possible to propose alternative ways of strain mapping using comparison of complex-valued (i.e., phase-resolved) OCT scans in the reference and deformed states. We call the proposed method "vector" since all intermediate signal transformations are performed with complex-valued amplitudes treated as vectors in the complex-valued plane and the sought phase-variation gradients proportional to strain are singled out at the very last stage. This method demonstrates very high robustness and intrinsically comprises amplitude-weighting procedures and suppression of especially strong phase errors close to Pi rad. In contrast to conventional approaches, the vector method obviates the necessity of error-prone phase unwrapping even for fairly high strains that cause multiple phase-wrapping within the scale over which the phase-gradient is estimated.

Several applications of the developed approach will be discussed. In particular, realization of compressional elastography will be demonstrated, including the use of reference silicone layers with pre-calibrated reference stiffness for quantification of unknown stiffness in the studied tissues. Obtaining of nonlinear stress-strain curves for various tissues in fairly large strain ranges (up to tens per cent) will be also shown. In comparison with conventional usage of simple contrast in stiffness (even quantified), examples of much clearer discrimination of tissues in various states based on the reconstructed complete stress-strain curves will be demonstrated.

Other applications of the developed approach to monitor "instantaneous" and time-dependent cumulative strains in procedures of laser reshaping of collagenous tissues will be presented. Such techniques open interesting prospects for non-surgical, biologically non-destructive modification of geometrical shape of cartilagionous samples (e.g. in preparation of implants) or thermomechanical cornea shape modification due to moderate heating by IR laser irradiation. The relevant examples will be demonstrated. Some other applications of the developed technique will also be discussed (e.g., long-term monitoring of tiny slowly-varying tissue strains).

**INVITED B10:**

# High speed OCT for *in-vivo* cellular resolution imaging of the structure, function and blood perfusion of biological tissue

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***Abstract*— Diseases alter the morphology, function and blood vasculature of biological tissue. We have developed high speed, OCT technology with 1-μm axial resolution in biological tissue, capable of *in-vivo*, non-contact, volumetric imaging of the cellular structure of biological tissue. The same technology can also be used to map and evaluate simultaneously the blo0od perfusion and functional response of the tissue to external stimulation.**

Diseases cause changes in the morphology, physiology and vasculature of biological tissue. Vascular changes such as alterations in the blood flow and neovascularization, as well as functional changes such as impaired cellular function, can also occur at the early stages of disease development and in many cases precede any large-scale morphological changes. Since treatment is most effective when diseases are diagnosed at their earliest stages of development, it is essential to develop non-invasive imaging technologies that can image the biological tissue at cellular resolution. Acquisition of large-scale images at cellular resolution that are not significantly affected by tissue motion, require very high image acquisition rates. Furthermore, multimodality imaging technologies that can image the tissue structure, vasculature and probe its physiological state synchronously, can improve significantly the disease diagnostics process by providing complementary information, while reducing significantly the cost and time for patient evaluation. In this invited presentation, we review state-of-the-art, high speed, ultrahigh-resolution optical coherence tomography (UHR-OCT) and its applications for in-vivo, non-contact imaging the structure, function and vasculature of different types of biological tissues.

Optical coherence tomography (OCT) is a biomedical imaging modality capable of generating cross-sectional and volumetric images of biological tissue both *ex-vivo* and *in-vivo* with micrometer scale resolution and up to several millimeters penetration depth. Over the past 25 years advancements in light sources and high speed and large pixel-number cameras led to the development of spectral domain OCT (SD-OCT) and full-field OCT (FF-OCT) systems with an axial resolution ~1 μm, and acquisition rates up to 70,000 A-scans/s. By using novel lasers and high speed cameras, our research group has developed UHR-OCT technology that offers axial resolution in the range of 0.95 μm to 1.5 μm in biological tissue, with image acquisition rates in the range of 34 fps to 2,500 fps, capable of in-vivo, non-invasive, volumetric imaging of the cellular structure of biological tissue. Furthermore, we have developed image acquisition and processing algorithms to allow for non-invasive imaging of the tissue's vasculature and measurement of the blood flow. Last, though not least, we have developed a multimodality UHR-OCT technology capable of simultaneous imaging of the retinal structure, blood perfusion and functional response to visual stimulation.

## method

The UHR-OCT technology was applied to a number of animal and clinical studies of ocular pathology. One group of clinical studies focused on *in-vivo,* non-contact, volumetric imaging of the cellular structure of the human cornea and corneo-sclera limbus in healthy humans and subjects with different stages of Keratoconus and Limbal Stem Cell Dystrophy. Another group of studies focused on an animal model of Glaucoma, where the multimodality UHR-OCT technology was able to measure for the first time and correlate changes in the retinal structure, functional response to visual stimulation and blood flow changes in healthy animals and animals with induced Glaucoma. A third line of studies imaged morphological and vascular changes in brain tumors in an animal model. All of these studies were approved by the University of Waterloo Research Ethics Committee and complied with the tenets of the Declaration of Helsinki and the ARVO directive for use of animals for ophthalmic research.

## Conclusion

We demonstrate the ability of high speed UHR-OCT technology to image non-invasively the structure of biological tissue at cellular level, as well as provide simultaneously information about changes I the tissue morphology, vasculature and physiological state. We also discuss the benefits of this technology for biomedical and clinical applications

**INVITED B11:**

# Optical clearing skull window for cortical neural and vascular imaging

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Intravital optical imaging provides a significant tool for investigating cortical neural, vascular structure and function. However, its imaging contrast and depth are limited by the turbid skull. Skull windows based on craniotomy or skull-thinning surgery have been developed, but they cannot circumvent various problem, such as inflammatory response, the complexities in surgical procedures, etc. Tissue optical clearing shows a great potential for solving this problem. In this presentation, we developed an easy-to-handle, switchable, and safe optical clearing skull window with topical application of skull optical clearing agents, rather than performing a craniotomy. Combined with two-photon microscopy, this optical clearing skull window not only allowed us to repeatedly image neurons at synaptic resolution, but also to image microglia, and microvasculature and function of mice. We applied it to study the plasticity of dendritic spines in critical periods and to visualize dendrites and microglia after laser ablation. Combined with integrated system of laser speckle contrast imaging and hyperspectral imaging, the cortical hemodynamics could be monitored. This window could be repeatedly established without inducing observable inflammation and metabolic toxicity. Thus, it has the potential for use in basic research on the physiological and pathologic processes of cortical vessels.

**INVITED B12:**

# *In vivo* optical coherence tomography of mouse reproductive processes

**Irina V. Larina**

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The Fallopian tube (oviduct) serves as the site for oocytes transport, fertilization, and early embryonic development. While research has shed light on the cellular and molecular mechanisms mediating these events, much of these data are derived from static histological analysis, low-resolution visualizations, and studies of invertebrate models (e.g. sea urchin). Therefore, any conclusions regarding mammalian fertilization, which takes place deep inside the body, are extrapolated and do not necessarily represent the native state.

To overcome this technical limitation, we developed a 3D optical imaging approach combining optical coherence tomography (OCT) with an intravital dorsal imaging window, which allows for prolonged, functional, and quantitative analysis of the mouse oviduct *in vivo*. These methods provide information about transferring of oocytes/embryos, the contraction of the oviduct muscle, distribution of the frequency of cilia beat, as well as sperm behavior in the ampulla, revealing never-before-seen dynamic events. We observed movement patterns of oocytes and embryos which question current believes in reproductive community. Regarding sperm, volumetric imaging in the oviduct revealed novel sperm behaviors relative to the wall of the oviduct, suggesting a role for cilia dynamics in the regulation of sperm movements. We are now using these methods to elucidate the specific roles of the oviduct contraction and cilia beat in the gametes transport and fertilization.

**INVITED B13:**

# Dynamic range improvement and contrast enhancement in swept source optical coherence tomography

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Swept source optical coherence tomography (SSOCT) is an effective imaging technology that is capable of high-resolution cross-sectional imaging in biological tissues. Integrated with fiber-optic endoscopic scanning probes, SSOCT is especially attractive in internal organ imaging. However, endoscopic SSOCT imaging is usually compromised by the dynamic range of an OCT system due to the limited bit-depth of an analog to digital converter (ADC) used in the system. In the common case of a strong reflection from interstitial fluid that is covered on the surface of organs such as airway and digestive tract, the OCT imaging quality will be degraded by bright lines generated by the Fourier transform of saturated interference signals that exceed the input range of the ADC. Endoscopic SSOCT imaging also suffers from reduced contrast with depth as the incident light attenuates exponentially in turbid and highly scattering tissues and a logarithmic transform is required to display images of weak signals.

To improve the dynamic range and enhance the contrast in an endoscopic SSOCT system, we demonstrate a cost-effective design by using a frequency splitter in the detection circuits. The interference signal was divided by the splitter into two channels. The low frequency strong signal that is from the tissue surface was detected by one channel with a low level to keep the signal within the measurement range of the ADC and the high frequency weak signal was detected by the other channel with a high level to image deep tissues with a high contrast. By integrating two channels with a frequency combiner, high contrast imaging in both surface and deep areas will be obtained. It was demonstrated that this technique can be used to improve the dynamic range of the system by more than 10 dB and enhance imaging contrast especially in deep tissue areas.

**INVITED B13:**

# An electrochemical biosensor for sensitive detection of microRNAs based on target-recycled non-enzymatic amplification

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In this study, a simple, economic, and label-free electrochemical biosensor was developed for highly sensitive and selective miR-21 detection based on $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ electrochemical signal, which relies on DNA structures conjugating with carboxyl multi-wall carbon nanotubes (MWCNTs-COOH)-modified glassy carbon electrode (GCE). High sensitivity and specificity of the biosensor were achieved by taking advantage of the target-recycled non-enzymatic amplification strategy (TRNEAS), which relies on sequence-specific hairpin strand displacement and does not require the addition of environment-susceptible enzymes. Besides, the SP-DNA strictly binds to the cMWCNTs/GCE via amide bonds, which ensure good electrochemical results and excellent stability for target miRNA detection. The developed miR-21 electrochemical biosensor exhibited a broad linear dynamic range of 0.1 fmol to 5 pmol and a detection limit of 56.7 amol for miR-21 detection in vitro. The relative miR-21 expression levels were particularly detected by our developed miR-21 biosensor and were highly consistent with the stem-loop RT-PCR results. In summary, our developed electrochemical biosensor exhibits great potential for further application in biomedical research and early clinical diagnosis.

Keywords: MicroRNA, Electrochemical biosensor, TRNEAS, High sensitivity

**INVITED B15:**

# A microfluidic assaying device for RBC aggregating force with analysis of backscattering light

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The aggregation of red blood cells (RBC) is a reversible dynamic phenomenon that has a strong effect on blood microcirculation. For past decades, RBC aggregation had been measured with various devices and methods but the results were quantified in a relative value such as AI and M, which are relative or arbitrary units. Our previous study introduced a critical shear stress measured by RheoScan, which is defined as a minimum stress to disaggregate RBCs and is known as hematocrit-independent index. Another research with optical tweezers confirmed that the CSS is the minimum shear stress to aggregate between RBCs. Therefore, the CSS was proved to be an index to represent RBC aggregation having an absolute dimensional unit such as stress. According to clinical data, CSS for healthy people yield 150 ~ 300 mPa and that for cardiovascular patients yield higher than 350 mPa. Therefore, it is strongly required to accumulate clinical data for further application of CSS as a diagnostic index of hemorheology.

**INVITED B16:**

# Computational Imaging and Sensing Systems

**Maxim Batalin and Aydogan Ozcan**
*Electrical and Computer Engineering, UCLA*

Our research focuses on the use of computation/algorithms to create optical microscopy, sensing, and diagnostic techniques, significantly improving existing tools for probing micro- and nano-objects while also simplifying the designs of these analysis tools. In this presentation, we will introduce a set of computational microscopes which use lens-free on-chip imaging to replace traditional lenses with holographic reconstruction algorithms. Basically, 3D images of specimens are reconstructed from their "shadows" providing considerably improved field-of-view (FOV) and depth-of-field, thus enabling large sample volumes to be rapidly imaged, even at nanoscale. These computational microscopes routinely generate >1–2 billion pixels (giga-pixels), where even single viruses can be detected with a FOV that is >100 fold wider than other techniques. At the heart of this leapfrog performance lie self-assembled liquid nano-lenses that are computationally imaged on a chip. These self-assembled nano-lenses are stable for >1 hour at room temperature, and are composed of a biocompatible buffer that prevents nano-particle aggregation while also acting as a spatial "phase mask." The field-of-view of these computational microscopes is equal to the active-area of the sensor-array, easily reaching, for example, >20 $mm^2$ or >10 $cm^2$ by employing state-of-the-art CMOS or CCD imaging chips, respectively.

In addition to this remarkable increase in throughput, another major benefit of this technology is that it lends itself to field-portable and cost-effective designs which easily integrate with smartphones to conduct giga-pixel tele-pathology and microscopy even in resource-poor and remote settings where traditional techniques are difficult to implement and sustain, thus opening the door to various telemedicine applications in global health. Some other examples of these smartphone-based biomedical tools that I will describe include imaging flow cytometers, immunochromatographic diagnostic test readers, bacteria/pathogen sensors, blood analyzers for complete blood count, and allergen detectors. Through the development of similar computational imagers, we will also report the discovery of new 3D swimming patterns observed in human and animal sperm. One of this newly discovered and extremely rare motion is in the form of "chiral ribbons" where the planar swings of the sperm head occur on an osculating plane creating in some cases a helical ribbon and in some others a twisted ribbon. Shedding light onto the statistics and biophysics of various micro-swimmers' 3D motion, these results provide an important example of how biomedical imaging significantly benefits from emerging computational algorithms/theories, revolutionizing existing tools for observing various micro- and nano-scale phenomena in innovative, high-throughput, and yet cost-effective ways.

**INVITED B17:**

# Title: Fiber-based methods for deep brain Calcium recording in behaving mice

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Dysfunctions of many nuclei in the brain (striatum, thalamus, amygdala etc.) are associated with highly prev alent mental diseases such as Parkinson's disease and Alzheimer's disease. It is of prime importance to unco ver the fundamental behavioral functions and mechanisms of these nuclei that underlie conditions of both he alth and disease in model animals. Optical calcium imaging is a powerful tool to record neural activity indica ted by calcium transients both *in vitro* and *in vivo*, but its imaging depth is restricted within 1 mm due to the high scattering and absorption of biological tissues. Deep brain function research requires modification of exi sted optical detection method. Relaying the deep brain calcium signals to the surface with optical fibers is an efficient approach to extend the *in vivo* optical detection methods. Here, we implemented two optical fiber ba sed methods for deep brain calcium signal measurements in behaving mice: a multichannel fiber photometry and a GRIN lens based confocal microscope.

**Biography**

**Professor** Ling Fu is a Professor in Wuhan National Lab for Optoelectronics in Huazhong University of Science and Technology. Her research interest is developing real-time nonlinear optical imaging approaches to monitor how molecules work and cells interact in their natural environment. Her research focuses on the multicolor multiphoton microscopy for *in vivo* immunology, and confocal/multiphoton microendoscopy for neuron imaging. Dr. Ling Fu serves as an assistant editor in Journal of Innovative Optical Health Sciences, and an editorial board member of Scientific Reports. She is an International Council member of Optical Society of America (OSA), one of program chairs for 100th Annual Meeting of OSA (2016).

**INVITED B18:**

# State-of-the-art Clinical Multiphoton Tomography (MPT)

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**Keywords:** Two-photon imaging, multiphoton, skin, malignant melanoma, breast cancer, fluorescence lifetime Imaging, Second Harmonic Generation, psoriasis, dermatitis, brain tumor, cornea transplant, CARS, autofluorescence

Multiphoton tomography (MPT) provides label-free optical tissue biopsies with subcellular resolution within seconds. MPT is based on two-photon autofluorescence (AF), second harmonic generation (SHG), fluorescence lifetime imaging (FLIM), and Raman spectroscopy (CARS) using NIR femtosecond laser. CE-certified clinical multiphoton tomographs (DermaInspect, MPTflex) have been used in clinics and research institutions of cosmetic and pharmaceutical companies in Europe, Australia, Asia, and the USA. Clinical MPT studies include (i) early detection of skin cancer, (ii) imaging of patients with psoriasis and dermatitis, (iii) wound healing studies, (iv) detection of pigmentation disorders, (v) skin modifications after long-term space flights, (vi) quality checks of corneas for transplantation, (vii) crosslinking studies, (viii) in vivo imaging of brain tumor borders during surgery, and (ix) early detection of alterations induced by radiation therapy of breast cancer.

**ORAL B1:**

# Cross-polarization optical coherence tomography for tissue type differentiation and blood vessels detection during brain cancer surgery

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An introduction of innovative minimally invasive methods like multimodal (polarization-sensitive and blood vessels sensitive) optical coherence tomography (OCT) for intrasurgical guidance appears to be a critical challenge for the modern neurooncology. This study aimed to develop the new approaches to intraoperative express diagnostics of taken/removed brain cancer tissues and prevention of intracranial hemorrhages by blood vessel detection based on cross-polarization (CP) OCT. Preclinical study was presented on Wistar rat's brains with inoculated glioma model (3 different gliomas, n=15). Clinical demonstrations of the developed approaches realization were presented intraoperatively for human gliomas of different degree of malignancy. The spectral domain CP OCT device that approved for clinical use and provides two images: in co- and cross-polarizations, was used in the study. As a result, CP OCT allowed differentiating tissue types (tumor core, normal and infiltrated white and gray matter), gliomas margins and visualizing of the blood vessels by visual criteria. However, CP OCT signal quantification methods increased the sensitivity/specificity for the tissue type differentiation and blood vessels detection. The potential of CP OCT as an effective instrument for *in vivo* OCT-guided brain cancer surgery was demonstrated. The CP OCT method has the further prospect of intraoperative application for a number of the most complex clinical problems in neurosurgery. The study was supported by the Russian President grant for young scientists No MK-6634.2018.7 and RFBR project No. 18-29-01049_mk.

**ORAL B2:**

# The study of the lymphatic system dynamics by using optical coherence tomography and gold nanorod contrasting of the deep cervical lymph nodes

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The lymphatic system plays the key role in the fluid drainage in the peripheral blood circulation. Despite discovery of the meningeal lymphatic vessels, the role of this lymphatic system in brain cleansing remains unknown and approaches for monitoring and quantifying of its dynamics are not yet developed. One of the ways to provide such measuring technology is to use optical coherence tomography (OCT) which is based on principles of a low-coherence interferometry and widely used for studying scattering layered tissues. OCT method works on ballistic photons that have experienced a single scattering at the interface between two media with different refractive indexes. Multiple scattering significantly reduces the penetration depth and degrades the signal-to-noise ratio, however highly scattering contrast agents such as gold nanoparticles may help significantly to monitor functioning of brain fluid drainage system within the deep lymph nodes.

In our studies, we used the commercial OCT Thorlabs GANYMEDE and gold nanorods as a contrast agent for visualization of lymphatic vessel dynamics. Two distinct accumulation dynamics were clearly visible during the experiment, within the first ten minutes there was a significant increase in the average OCT signal and the second stage was a slow growth with a lower increment.

In summary, our results clearly show that the meningeal lymphatics is a pathway for brain fluid drainage and clearing. The gold nanorods are good contrast agents for dynamic optical imaging of the meningeal lymphatic system in the near infrared range.

The research was supported by grant of Russian Science Foundation № 17-75-20069.

**ORAL B3:**

# Remote optical sensing in otolaryngology

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## 1. Introduction

Vocal cords paralysis (VCP) is heavily depended on visual inspection of the vocal cords or subjective examination of the patient voice by an expert. However, there are several conditions in which visual inspection is not possible even for an expert. These cases include for example overhanging epiglottis or severe gag reflex. Furthermore, sometimes subjective judgment can be not accurate and detect small tumors with respect to examination with high signal to noise ratio (SNR) objective tool [1, 2].

The most common acoustic measurement system consists only a microphone and measures several acoustic parameters to detect VCP. The major parameters for VCP detection are as follows: fluctuations per second, extent of fluctuation, jitter ratio and maximum phonation duration. The main disadvantage of this method is the ability to extract the acoustic data from a single spatial spot.

In this paper we present a simple remote photonic technique for detection of the vocal cords vibrations from a specific spot (i.e. the presented technique behaves as a 'directional microphone') using a laser and a camera. The main advantage of this approach is the ability to diagnose tumors or VCP from a specific location. Furthermore, using this approach, new parameters can be evaluated for the diagnostic of VCP or tumors. The technique is based on temporal tracking of back-reflected secondary speckle patterns generated while illuminating different spots of the throat with respect to the vocal cords location.

## 2. Operation principle and optical setup

The optical setups (Fig. 1) consisted of: (a) an eye safe 532nm laser and a camera (Baler acA1920-25um, monochrome). The camera captures the speckle images at 150 frames per second (fps). The distance from the laser to the subject's throat was approximately 90 cm. First, the speckles were extracted to frames. Later, to calculate the movement, the 2-D position of the peak correlation versus time was obtained.

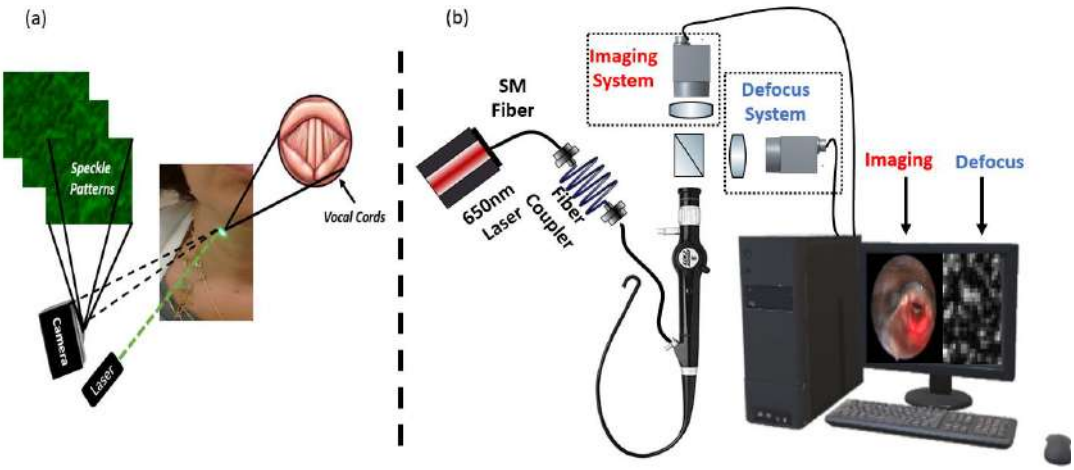


Fig. 1. Schematic sketch of the (a) external configuration and the (b) internal configuration

**ORAL B4:**

# *In vivo* imaging of leukocytes and platelets in humans using fluorescence microscopy

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Under normal conditions, the number of leukocytes and platelets in the blood is several orders of magnitude lower than the concentration of erythrocytes in the blood, but these cells have important functions such as immune response and blood clots formation. Quantification of the number of leukocytes of different subpopulations (neutrophils, eosinophils, etc.), as well as determination of number of activated and non-activated platelets per volume is an important clinical test that can reveal abnormalities in functional state of the patient. Leukocytes and thrombocytes counting typically is carried out *in vitro* using special staining and fluorescent labels using flow-cytometry or conventional wide-field microscopy. Recent studies show that leukocyte counting *in vivo* can be performed by monitoring the circulation of blood in microvessels [1]. Also, unlike red blood cells, leukocytes have relatively high autofluorescence signal [2]. Thus, the classification of blood cells can be performed *in vivo* using autofluorecence properties of white blood cells. Here we show how fluorescent properties of different subpopulation of leukocytes and activated and non-activated platelets differ *in vitro* using single cell fluorescence microscopy. Based on these results, we suggests the method for detecting fluorescent response from leukocytes in the microcapillary vessels of the human papillary dermis *in vivo*. Our results show to which extent autofluorescence of white blood cells and platelets can be used to observe and classify them in the blood flow both *in vivo* and *in vitro*. We believe that these results can be applied in the sphere of *in vitro* clinical tests and in the non-invasive monitoring of leukocytes and platelets levels in the blood.

The work was supported by the Russian Science Foundation (grant №17-75-10215).

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**ORAL B5:**

# Doppler Imaging of tympanic membrane vibrations

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The tympanic membrane plays a key role in the human hearing by translating air pressure waves into bone vibrations, and its function and dynamics are directly linked to various pathologies and hearing disorders. Current methods for imaging tympanic membrane dynamics, including stroboscopic holography and Doppler OCT would be challenging for *in-vivo* applications due to high system complexity or the need for point-by-point scanning. Here, we demonstrate *in-vivo* imaging of the tympanic membrane dynamics of a human volunteer using interferometric spectrally encoded endoscopy (iSEE). Briefly, in iSEE, spectral interference between a reference signal and the reflectance along a spectrally encoded transverse line is captured by a high speed (20 kHz) spectrometer. Using single-axis scanning across the membrane provides a two-dimensional interferometric data that is later analyzed using specialized software. The imaging probe includes a single optical fiber, optics for light delivery and scanning of the tympanic membrane, and a dedicated port for transmitting the excitation acoustic signals comprised of multiple single-frequency stimuli. Measuring the full vibration patterns of the tympanic membrane would help scientists to study signal transduction into the middle ear and observe the three-dimensional acoustic motion of the membrane. From a clinical perspective, the study could be used for developing a compact system that could be incorporated into conventional clinical otoscopes for providing functional information noninvasively with unprecedented resolution and sensitivity.

**ORAL B6:**

# Differential diagnosis of melanocytic lesions by in vivo label-free multimodal optical imaging

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Melanomas account for 90% of the deaths associated with cutaneous tumors. Even small tumors may have a tendency to metastasis and thus lead to a relatively unfavorable prognosis. The differential diagnosis of melanoma involves other pigmented melanocytic lesions (dysplastic, benign melanocytic naevi, Spitz naevi and lentigo simplex). The using of label-free multimodal imaging including optical coherence tomography microangiography (OCT MA), multiphoton fluorescence tomography (MPT) and fluorescence lifetime imaging (FLIM) may improve diagnostic value of *in vivo* differentiation of benign and malignant melanocytic lesions. Forty pigmented lesions were studied. Melanoma had thick and tortuous vessels in comparison with benign nevi. Dysplastic naevus contained dense uniform microvascular networks consisted of thin curved vessels. MPT images of lentigo simplex were characterized by lentiginous hyperplasia (pleomorphic melanocytes with dense distribution along the basal layer). Both mild cytological atypia (enlarged nuclei) and enlarged intercellular distance were identified in dysplastic naevus. The presence of melanocytes as well as dendritic structures in all layers of the epidermis was showed in MPT images of melanoma. Large intercellular distance and poorly defined keratinocyte cell borders were detected in the stratum granulosum and stratum spinosum. MPT images of Spitz nevus had the same features like melanoma. Analysis of cells metabolic state by FLIM revealed that melanoma had the smallest values of τm and a2, that corresponding to switching to glycolysis. The value of a2 slightly increased for Spitz nevus and lentigo simplex. This is due to predominance of oxidative phosphorylation. Joint using of OCT MA, MPT and FLIM showed potential to discriminate in vivo benign and malignant melanocytic lesions.

**ORAL B7:**

# Research on neurovascular coupling of mouse cerebral edema model by using photoelectric method

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## 1. BACKGROUND

Brain edema is an important clinical complication of intracerebral hemorrhage. During the past decades various studies have focused on the water content, intracranial pressure (ICP) or cerebral bl ood flow. However, the variation of the electrophysiological signal induced by blood-brain barrier (B BB) remains poorly understood. Therefore, we take photoelectric properties, including electrophysiolo gical signals, laser speckle contrast image (LSCI) and near-infrared spectroscopy parameters, to study the neurovascular coupling on cerebral edema model.

## 2. MATERIALS AND METHODS

Male Institute of Cancer Research (ICR) mice (25 - 30 g) were purchased from the Animal Exp eriment Center. All animal experimental procedures were conducted in accordance with the guideline under Institution Animal Care and Use Committee-approved protocols at Nanjing University of Aer onautics and Astronautics. Then 20 mice were randomly assigned to two groups. Many studies foun d that lipopolysaccharide (LPS) could induce inflammation and BBB disruption in mice [1-3]. The l ow dose LPS was used to make the brain edema model. The group I received LPS (5 g/kg, body weight, i.p.) and the group II consumed only saline (5 g/kg, body weight, i.p.). For each group, ele ctrophysiological signals, speckle images and near-infrared spectroscopy parameters were collected at 90 minutes after injection.

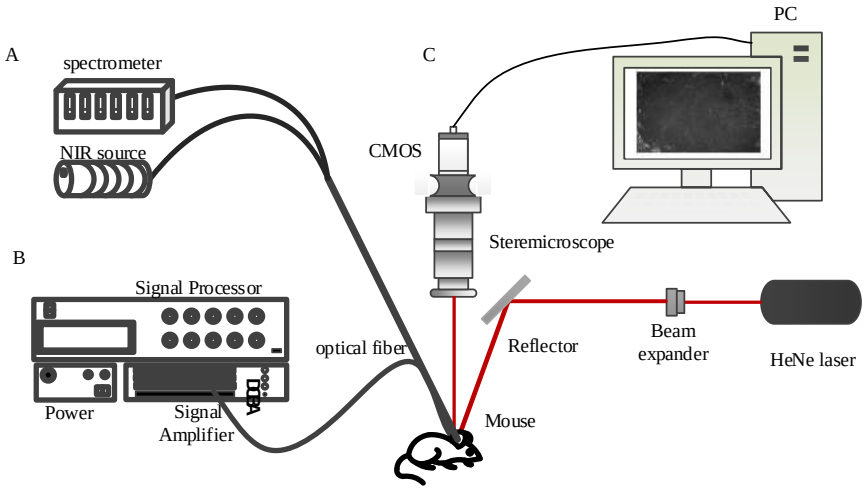


Fig.1 Schematic of the system for recording photoelectric signals. (A) near-infrared spectroscopy system

(B) Multichannel neural data acquisition system; (C) The component of the LSCI system.

Oxygen Saturation ($SO_2$) can be calculated by near-infrared spectrum collected by optical fibers probe (Fig.1A). In Fig.1B, 8-channel Local Field Potentials (LFPs) were recorded simultaneously fro m the hippocampus of mice by Cerebus Acquisition System (Cyberkinetics, Utah, USA). Subsequentl y, the LSCI system was performed and the schematic of the system for recording relative cerebral b lood flow (rCBF) is shown in Fig. 1C.

## 3. RESULTS

As shown in Fig. 2 (A) and (D), the normalized $SO_2$ in the LPS group gradually increases and reach es a plateau in the beginning 40 minutes after LPS injection. After 60 minutes, the normalized $SO_2$ decreases. Similarly, the time-frequency spectrum of LFP rise immediately after LPS injection in the beginning 40 minutes and then keep decreasing in Fig. 2 (B) and (E). LSCI mages from left to ri ght in Fig. 2(C) and (F) obtained from pre to 160 min with the 20 min interval. Notability the rC BF value remarkably reaches a peak at 40 min. The linear relationship between LFPs with Normali zed $SO_2$ and LFPs with rCBF is shown in Fig. 3.

## 4. DISCUSSION

The present study was designed to explore $SO_2$ and rCBF responses to LPS-induced neural acti vity. It is consistent with the previous studies [4] and the changes in blood flow may be caused by a direct neuroinflammatory response of LPS on cerebral vessel [5]. Generally, brain edema is associ ated with BBB disruption and cell toxicity. The neurovascular unit is composed by neurons, astrocyt es, and endothelial cells of BBB. So BBB disruption maybe the incentive of neural activity abnorm al [6]. Our results demonstrated that hemodynamic parameters and neural signal has an uncertain pr oportional relation, which is consistent with the neurovascular unit model.

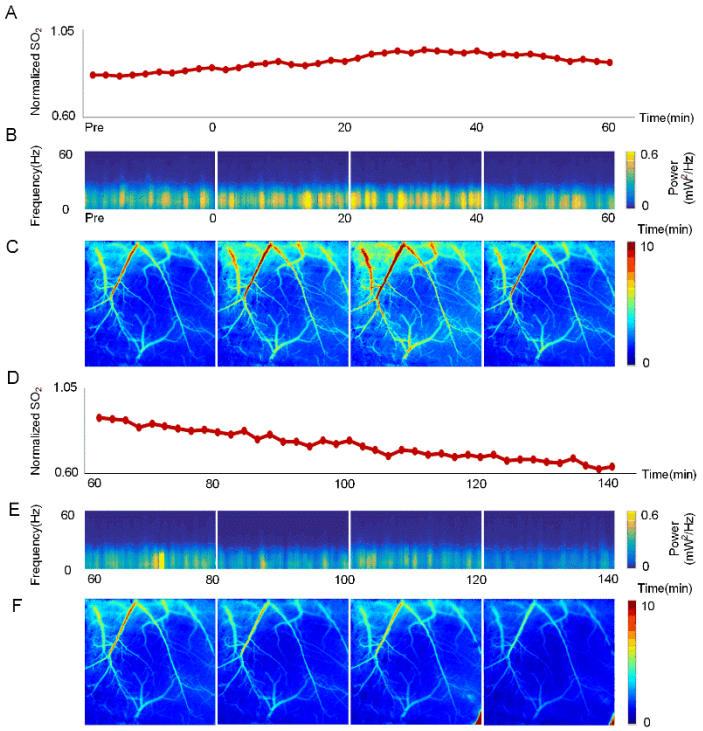


Fig.2 The variation of normalised oxygen saturation (A) and (D) pre- and post LPS injection for 160 minute. Time–frequency analysis (B) and (E) pre- and post LPS injection for 160 minute. The relative CBF image of LPS administration group (C) and (F).

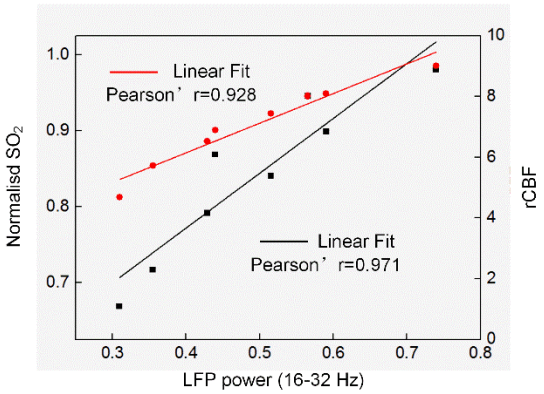


Fig. 3. Spearman correlations including regression lines for $SO_2$ and LFP low frequency band power (red point), rCBF and LFP low frequency band power (black point).

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**ORAL B8:**

# Challenges in using time-resolved fluorescence anisotropy for the size determination of carbon dots

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In this work, we exploited time-resolved fluorescence depolarization imaging (r-FLIM) to determine the averages size of carbon dots (CDs). The characteristic rotational correlation time connected to the size of the particle through classical Stokes–Einstein–Debye equation. The r-FLIM technique applied in this study achieves picoseconds time resolution, which approximately corresponds to the particle size determination at sub-nanometer precision. The depolarization time constants of the heteroatom-doped CDs in aqueous solution are found ca. 315 ps. The calculated diameter of the carbon dots is about 1.4 nm, which is smaller than the actual size of the carbon dots (5 nm). It has been recently reported that the proton transfer between water molecules and CDs might contribute to the faster depolarization process of CDs in aqueous solution. The discrepancy between the measured and calculated size of the CDs, therefore, can be attributed to the interaction between CDs and polar solvent molecules. Further studies required to ensure the reliability of r-FLIM as nanoparticle metrology standards of CDs size determination.

**ORAL B9:**

# Low-frequency laser Raman and FTIR spectroscopy in the study of structural elements of protein molecules

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In the framework of the protein-machine concept, a protein molecule is represented as a mecha nical structure consisting of fragments with different eleasticities and mobilities including active site an d supporting structures. In accordance with the concept, protein functioning is directly related to relativ e motions of molecular fragments. Domains of tertiary structure or highly ordered alpha-helical and bet a-sheet elements may serve as such fragments. Estimations show that oscillation frequency of such larg e fragments belong to the low-frequency (terahertz) range. Spectral characteristics that depend on the sp atial structure can be used to determine possible transformations in the molecular structure and, hence, changes of protein functioning.

Low-frequency laser Raman and FTIR spectroscopic techniques are used to study protein molec ules. Several proteins with dominant helical elements in the structure and poly-L-lysine prepared in diff erent conformations serve as objects under study.

Based on the results obtained in the fingerprint spectral range, we interpret spectral differences observed for the objects under study in the low-frequency range and analyze possible relations to the el ements of the secondary and tertiary structure that can be used to characterize protein functioning. Spec tral characteristics of proteins with similar secondary and different tertiary structures are studied. It is s hown that the low-frequency FTIR spectra of fibrillar proteins noticeably differ from the spectra of glo bular proteins. Spectral changes resulting from denaturation of superhelical protein (collagen) are analyz ed. A possibility of the study of structural hierarchy of protein molecules using low-frequency vibration al spectroscopy is discussed.

# LASER RAMAN AND INFRARED DIAGNOSTICS OF ENZYMATIC REACTIONS

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## ABSTRACT

Determination of chemical reaction rates is one of the main tasks in many fields of biochemistry. Practical aspects of such a problem are related to the comparison of the parameters of different reactions or a single reaction under different conditions. For this purpose, the measurement conditions must be exactly reproduced. Sufficient results can be obtained in arbitrary units. However, a comprehensive analysis of a reaction necessitates exact characterization of the corresponding physicochemical processes. In particular, it is expedient to determine the effect of reagent mixing, experimental configuration, type of substrate in enzymatic reaction, and time delay of the reaction initiation relative to the beginning of measurement on kinetic characteristics of reactions.

Laser Raman spectroscopy is a useful tool in the study of the above problems. Raman spectroscopy provides extensive information, permitting the study of molecular structure and the determination of reaction products. The method is applicable for compounds in any aggregate state and for any pH and temperature. Raman spectra have a well-known fingerprint region that can be used to identify a particular compound. Analysis of the spectra in this region allows determination of structural features of the components of enzymatic reactions, in particular, proteins, lipids, and carbohydrates. Thus, Raman spectroscopy can be used to determine chemical reaction rates using specific spectral features typical of reactions components and products.

We compare laser Raman and ATR FTIR spectroscopy in the study of the kinetics of enzymatic chemical reactions under various experimental conditions. The differences in reaction rates obtained for the same reaction using Raman and ATR FTIR spectroscopy are discussed. We consider the possibility of specific interaction of the components of reaction solution with the surface of ATR crystal. Time-dependent spectral changes are also observed and discussed.

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**ORAL B11:**

# LASER IR AND THZ SPECTROSCOPY ANALYSIS OF HUMAN SKIN AND FOOD SURFACE VOLATILE ORGANIC COMPOUNDS

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Volatile Organic Compounds (VOCs) name a wide range of chemical molecules with low molecular weight which are able to be emitted by a bio object under study. VOCs emitted by human beings include mainly saturated hydrocarbons (ethane, pentane, aldehydes), unsaturated hydrocarbons (isoprene), oxygen containing (acetone), sulphur containing (ethyl, mercaptane, dimethylsulfide) and nitrogen containing (dimethylamine, ammonia). The most commonly identified VOCs are isoprene, acetone, ethanol, methanol, other alcohols and alkanes. Their monitoring provides a good ability for express examination of a human state, including medical diagnosis. It could be performed in breath air, skin evaporation, various biofluids, including, urine, saliva, blood.

Gas chromatography is the "gold standard" among the instrumental methods of profiling VOCs, but it too complicated for being used in routine practice. Devices consisting of a set of chemical sensors, each of which corresponds to a particular substance or group of substances (so called "e-nose" technology) hold great promise for monitoring VOCs. The "e-nose" drawbacks are low selectivity and fast degradation.

High resolution gas phase molecular spectroscopy is very attractive instrumental method for VOCs profile analysis with increasing performances in term of sensitivity, selectivity, robustness and acquisition time. To realize a potential of this approach it is necessary to develop the experimental and methodical bases of rovibrational spectroscopy of complex biological origin gas mixtures analysis and to create special data bases which contain information about spectral characteristics of target VOCs. These aspects are planned to be discussed. The work was carried out under partial financial support of the Russian Fund of Basic Research (grant No.17-00-00186).

**ORAL B12:**

# Application of Fluorescence Lifetime Imaging in Skin Cancer Diagnosis

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**Abstract:** Skin cancer is the most common type of cancer that usually caused by ultraviolet radiation. It threatens seriously to patients' health for their ability to invade nearby tissue or spread to other parts of the body. The traditional method for skin cancer detection is using biopsy for histopathological validation, but it is invasive and non-quantitative. Recent development of advanced optical techniques, such as fluorescence lifetime imaging (FLIM), has enabled significant improvement in skin cancer diagnosis. Fluorescence lifetime of fluorophores is sensitive to the changes in their surrounding microenvironment. The structural and functional information of the samples can be quantitatively obtained by fluorescence lifetime measurement and imaging, which make FLIM evolve into an important tool in biomedicine. In this paper, we firstly reviewed the recent research progresses in the diagnosis based on FLIM of three different skin cancers, including malignant melanoma, basal cell carcinoma and squamous cell carcinoma. Then, our study on skin cancer by using time-correlated single-photon counting-based fluorescence lifetime imaging microscopy (TCSPC-FLIM) was elaborated. The fluorescence lifetime images of several skin cancer tissues and normal skin were mapped and the results we obtained agreed well with those reported by others. Finally, the potential development of FLIM in clinical applications and the challenges that may be faced in the future were prospected.

**Keywords:** fluorescence lifetime imaging; skin cancer, malignant melanoma (MM); basal cell carcinoma (BCC); squamous cell carcinoma (SCC)

**ORAL B13:**

# Quantitative phase microscopy of highly dynamic cells using flipping interferometer with doubled field of view as phase microscopy

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We lately proposed an external label-free off-axis interferometric module, for quantitative phase microscopy (QPM) of dynamics cells, with doubling the sample field of view (FOV), based on using flipping interferometry (FI) and interferometry with doubled imaging area (IDIA) [1]. In contrast to previous designs, this design does not require spatial filtering (no pinhole and lenses) to create the reference beam externally. Flipping interferometry (FI) is an external nearly common-path interferometric module that uses half of the optical beam as the reference beam for the other half and does not requires pinhole alignment. QPM is a useful technique for imaging transparent cells by capturing the quantitative phase profile, which takes into account the cell refractive index and physical thickness, enables to extract the optical path delay of the cell and obtain the cell quantitative topographic map with great contrast and without the need for external contrast agents. In off-axis holography, we induce interference with small angle between the sample and reference beams, which enables capturing the complex wavefront of the sample within a single camera exposure. In the spatial frequency domain, there is a full separation across a single axis between the auto-correlation terms, or the sample and reference beam intensities, and each of the cross-correlation terms, containing the complex wavefront of the sample. Multiplex two off-axis holograms with orthogonal interference fringe orientations into a single multiplexed hologram allows compressing more information on the other axes as well; thus recoding more information with the same number of camera pixels. Each of these holograms contains different area of the imaged sample FOV.
This power spectrum contains two off-axis cross-correlation complex conjugate pairs, each encodes a different FOV of the sample. By digitally cropping one cross-correlation element from each pair and applying an inverse Fourier transform to each of them separately, we obtain the complex wavefront of each of the recorded FOV, and can extract the optical path delay map.

Our compact module can be connected to the output of an inverted microscope and double the interferometric FOV without the need for pinhole alignment due to using a retro-reflector to flip half of the FOV on top of the other one. The proposed module is expected to enable imaging samples that are larger than a single camera FOV and thus cannot be imaged at once. Furthermore, by increase the camera frame rate, it is possible to obtain phase imaging of dynamic objects, such as cells flowing between two FOVs, without the need to shift the camera to track the cell during flow, which is very useful for imaging flow cytometry, where imaging throughput is critical.

**Reference:**

1. “Flipping interferometry with doubled imaging area”, submitted, 2018

**ORAL B14:**

# Raman microscopy for non-invasive determination of the skin barrier function *in vivo*

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The maintaining of the skin barrier is an important function of the human stratum corneum (SC), the outer layer of the skin, for the regulation of the water balance and the reduction of penetration of xenobiotics through the skin. The SC consists of keratin-rich corneocytes, embedded in a matrix containing intercellular lipids and water. The skin barrier function is mainly related to the lateral organisation of intercellular lipids in the SC, which was so far measured in *in vitro* experiments. We introduced confocal Raman microscopy for the *in vivo* non-invasive application in human skin. It was shown that the lamellar and lateral packing order of intercellular lipids, determined by analysis of the $(I_{1060}+I_{1130})/I_{1080}$ and $I_{2880}/I_{2850}$ peak ratios, is non-homogeneously distributed in the human SC, showing a prominent maximum in the depth approx. 20–40% of the SC thickness (1). The same depths was shown to have a maximum of the hydrogen bonding states of water molecules, which was determined as a ratio of weakly/strongly bound water types (2). These results were achieved by Gaussian deconvolution of the water related Raman band in the high-wavenumber region into Gaussian bands, whose intensity is sensitive to the concentration of tightly, strongly, weakly bound and free water in the SC. Application of the developed methods to porcine skin *ex vivo* show that porcine SC is characterized by a higher hexagonal lateral packing order of intercellular lipids (in 10–50% of the SC thickness) and by lower hydrogen bonding states of water (in 10–30% of the SC thickness). Therefore, porcine SC is characterized by a lower skin barrier function in comparison to human SC *in vivo* (3). These findings should be taken into consideration when applying porcine skin as an *ex vivo* model of human skin *in vivo* in dermatologic penetration studies. The introduced method opens wide the application of Raman microscopy in dermatology in relation to *in vivo* non-invasive investigation of the SC.

**Keywords:** Stratum corneum, corneocytes, intercellular lipids, water, moisturizing, skin model, penetration

**References:**

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**ORAL B15:**

# Polarization imaging for dynamic monitoring of tissue clearing

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Polarization optical imaging is an effective way to evaluate the microstructure characteristics of tissues. It can be applied to the monitoring of dynamic tissue processes such as optical clearing, to analyze and explain the microscopic phenomena in the tissues.

In this paper, we employed the saturated sucrose solution as the agent to penetrate the beef and pork skeletal muscles, and we observe the tissue change with the clearing time by Mueller matrix imaging. Experimental results show that tissue scattering will be significantly reduced with the clearing process, in agreement with the increased value of the diagonal elements of the Mueller matrix closely related with the depolarization phenomena. By monitoring the dynamic change of a single matrix element $M_{44}$, we found the obvious oscillation of this Mueller element with clearing, implying that some clearing induced changes of tissue microstructures can also cause the increasing of the depolarization items.

By the combination with experimental and Monte Carlo simulations based on the sphere-cylinder birefringence tissue scattering model (SCBM), it can be seen that the refractive index matching and birefringence effects induced by the saturated sucrose solution can explain most of Mueller matrix change. For the interesting oscillation phenomenon of the $M_{44}$ element, it is most probably due to the decrease of tissue thickness and more orderly fiber orientation caused by clearing, according to the comparison of three kinds of modeling of fiber diameter, fiber hydrolysis and fiber orientation, respectively. Both experimental and simulation results have shown that it is a feasible and promising research attempt to understand and evaluate the tissue clearing by polarization imaging.