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LALS

Laser Applications in Life Sciences

Posters

POSTER 1:

Red blood cells microrheologic and size distribution parameters in vascular diseases: evaluation by laser techniques

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Erythrocyte reversible aggregation and deformability parameters as well as size distribution of the cells are the major characteristics of blood microcirculation. Alteration of these properties lead to changes of blood viscosity and, as a consequence, to changes in blood flow through capillaries. This can lead to significant impairment of blood function, which increases a risk of occurrence of vascular concomitant diseases, and even the mortality especially in the case of cardiovascular pathologies. In this work, complex studies of the factors determining the capillary blood flow in patients suffering from socially significant diseases as arterial hypertension and diabetes mellitus were conducted by optical methods.

Light scattering laser aggregometer and diffractometer RheoScan AnD-300 (Rheomeditech, Korea) was used to conduct in vitro measurements of aggregation and deformability characteristics of the cells on ensembles of erythrocytes. The essence of laser diffractometry is in obtaining and subsequent analysis of the diffraction pattern (DP) from a highly diluted suspension of RBCs at rest and under shear flow [1]. This method allows for estimating the mean characteristics of the cell ensembles because the diffraction patterns are formed by thousands of the cells illuminated by the laser beam. We improved the method of laser diffractometry to evaluate statistical characteristics of the cell ensembles: the mean values and dispersion of red blood cells in sizes. Our approach is based on registering DP from the blood smears and calculation of DP visibility, which is related to the characteristics of the cells ensembles. Laser aggregometry technique allows to register the kinetics of RBC spontaneous aggregation (time dependence of light intensity forward scattered from a sample of whole blood at rest) and shear-induced disaggregation (shear stress dependence of light intensity backscattered from a sample of whole blood under shear flow). The following parameters are measured: the characteristic time of aggregates formation, aggregation index as well as hydrodynamic strength of the aggregates [2]. Home-made double-channeled optical tweezers (OT) were used for measuring the aggregation speed and interaction forces during erythrocyte doublet formation on cellular level [3]. OT are formed by two single-mode Nd:YAG lasers and a water-immersion objective with high numerical aperture. OT allow for freely manipulating the individual cells with a sharply focused laser beam. All in vitro measurements were performed with EDTA-stabilized human blood samples drawn from patients with arterial hypertension (AH) and diabetes mellitus (DM) and practically healthy volunteers – control.

The experimentally obtained results show that the microcirculation gets impaired due to the alterations of the blood microrheology properties in case of AH and DM. It was shown that in AH-patients, the ability of erythrocytes to deform is slightly reduced while the aggregation speed and forces of the cells

interaction are significantly increased relative to the control group. DM is characterized by enhanced aggregation: the characteristic time of aggregates formation is dramatically increased in whole blood of patients with DM relative to the control group.

This work was supported by the RFBR grants № 17-29-03507-ofi_m and 17-02-01200.

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POSTER 2:

Remote Optical Evaluation of Facial Nerve Degeneration

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Bell's palsy is an idiopathic unilateral facial paralysis. The prognosis of Bell's palsy depends tightly in the facial nerve maximal degeneration. The functionality of the nerve is determined with respect to the healthy side of the patient's face. Timing has a crucial role in Bell's palsy recuperation. Although most of the patient's will recover without any treatment, there are some indications for poor prognosis that may indicate for the necessary treatment. Among antiviral and corticosteroid therapy, the extreme treatment is a decompression surgery. A reasonable selection of surgical candidates can only be achieved by a quantitative evaluation of facial nerve fibers degeneration. This kind of an assessment is preformed nowadays by using electroneuronography. The remote evaluation of the facial nerve degeneration is executed by observation of the secondary speckle patterns that are created by illuminating the patient face with a laser beam in some key spots (according to the seventh cranial nerve anatomy). We ask the patient to present a list of face expression and via the analysis of the generated secondary speckle and comparison between the results accepted in two sides of the face - we can evaluate the nerve degeneration.

POSTER 3:

Tissues viability and blood flow sensing based on a new nanophotonics method

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Extracting optical parameters of turbid medium (e.g. tissue) by light reflectance signals is of great interest and has many applications in the medical world, life science, material analysis and biomedical optics. The reemitted light from an irradiated tissue is affected by the light's interaction with the tissue components and contains the information about the tissue structure and physiological state. In this research we present a novel noninvasive nanophotonics technique, i.e., iterative multi-plane optical property extraction (IMOPE) based on reflectance measurements. The reflectance based IMOPE was applied for tissue viability examination, detection of gold nanorods (GNRs) within the blood circulation as well as blood flow detection using the GNRs presence within the blood vessels. The basics of the IMOPE combine a simple experimental setup for recording light intensity images with an iterative Gerchberg–Saxton (G-S) algorithm for reconstructing the reflected light phase and computing its standard deviation (STD). Changes in tissue composition affect its optical properties which results in changes in the light phase that can be measured by its STD. This work presents reflectance based IMOPE tissue viability examination, producing a decrease in the computed STD for older tissues, as well as investigating their organic material absorption capability. Finally, differentiation of the femoral vein from adjacent tissues using GNRs and the detection of their presence within blood circulation and tissues are also presented with high sensitivity (better than computed tomography) to low quantities of GNRs (<3 mg).

POSTER 4:

Optical configuration of skin hydration detection by temporal analysis of skin speckle patterns

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A novel technique for measuring the amount of moisture in human skin is presented. Our non-contact optical method provides a quantitative measure of skin moisture for the purpose of comparison between various cosmetic products scientifically and also to identify skin pathologies. The approach is based on temporal tracking of back-reflected secondary speckle patterns generated while illuminating the skin area with a laser and applying periodic vibration to the surface via a controlled vibration source. Analyzing the 2-D time varying speckle patterns in response to the applied periodic vibration provides information about the amount of moisture in the corneum, the upper skin layer. Using the presented method, the skin hydration condition can be extracted. The measurements taken by this novel method will be compared to measurements by the Corneometer, a reference device for measuring skin hydration, which provides an assessment of the amount of moisture in the skin during contact. The measurements also will be compared to LASCA analysis. The standard deviation of the pixel shifts in each video was calculated. In order to test the nature of skin moisture changes over time after applying a moisturizer, experiments were performed on 4 fingers of the same person under the same conditions (fig.1). Similar results can be seen in all fingers. The standard deviation in pixels shifts is greater after applying a moisturizer than without a moisturizer, and for two hours is maintained in the same range. Further experiments are being conducted and additional parameters are currently being measured.

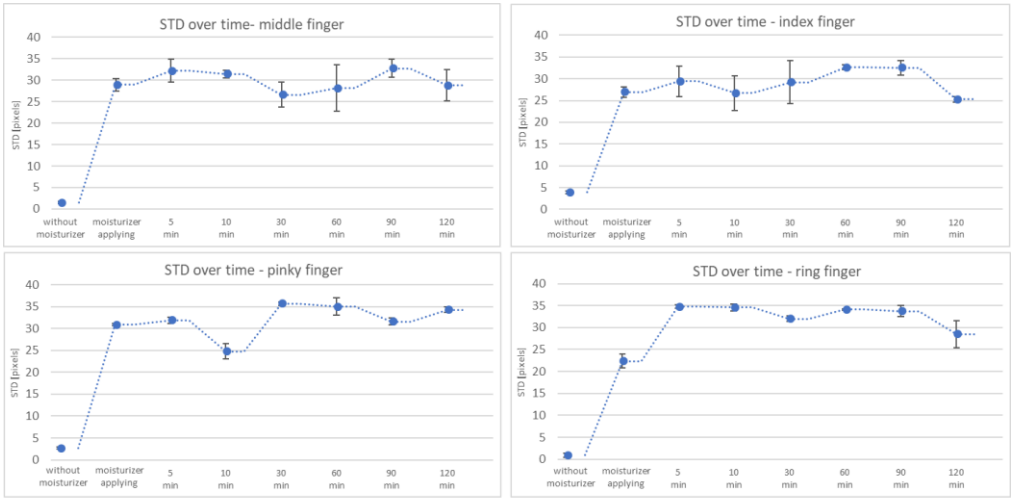

Fig1. Mean of standard deviation from three experiments on 4 fingers of the same person under the same conditions are presented in the following graphs. First, a control experiment was conducted without a moisturizer, then a moisturizer was applied and the condition was examined at 6-time intervals over two hours. The test sites were approximately 1(height) X 1(width) cm^2 each. 0.20 mL of AQUEOUS CREAM moisturizer was glove-rubbed into the test site until completely absorbed.

POSTER 5:

High Resolution Imaging Inside and Behind the Biological Scattering Medium by Focusing Light Using a Non-Invasive Optical Wavefront Shaping Technique

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Optical wavefront shaping is one of the most effective techniques in focusing light inside a scattering medium. Unfortunately, most of these techniques require direct access to the scattering medium or need to know the scattering properties of the medium beforehand. Through our scheme we develop a novel concept in which both the illumination and the detection is on the same side of the inspected object. We model the scattering medium being a biological tissue as a Matrix having mathematical properties matched to the physical and biological aspects of the sample. In our adaptive optics scheme, we aim to estimate the scattering function and thus to encode the wavefront of the illuminating laser light source using DMD (Deformable Mirror Device) with an inverse scattering function of the scattering medium, such that after passing its scattering function a focused beam is obtained. The estimation of the scattering is done by illuminating the medium and in parallel collecting the back reflected light and analyzing its wavefront via off-axis holography (phase and amplitude). We optimize the pattern to be displayed on the DMD using Particle Swarm Algorithm. Hence, we have a map of all the distributions to be displayed in the DMD for focusing inside scattering medium per pixel. Thus, after applying the right beam shaping an imaging of objects behind scattering medium as biological tissue can be made possible. As first proof of concept we experimentally show that we obtain a focused spot behind a biological scattering medium (a tissue) in amplitude modulation scheme using a DMD.

POSTER 6:

The microbiological analyzer based on coherent fluctuation nephelometry

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Coherent fluctuation nephelometry (CFN) is a modern testing tool for rapid testing of susceptibility to antibiotics in clinical cultures and microflora of urine without the isolation of bacteria. The method is practically insensitive to the quality of the cuvette, which allows to design microbiological analyzers with a high degree of usability, allowing to determine the growth of microbes, starting from 10^3-10^4 cfu/ml.

More than 650 urine specimens were examined and the effectiveness of the CFN analyzer for pre-sampling urine for analysis was demonstrated. The method allows to identify the majority of negative samples, reducing the total number of urine tests by 80%.

More than 250 studies of sensitivity to antibiotics on the CFN analyzer have been carried out. The efficacy is shown for the rapid determination of the sensitivity of both pure cultures and microflora of urine without obtaining bacterial isolates. The using of CFN analyzer in tandem with a mass-spectrometric method of typing microorganisms will allow a complete urinalysis to be performed in one working day.

In the future, the CFN analyzer will allow screening of various human biological fluids, and will also be used in a wide range of microbiological tasks, for example, to accelerate and standardize biomedical research

POSTER 7:

The study of the antitumor immunity mechanism induced by anti-CTLA-4 therapy on the B16 melanoma model in FoxP3EGFP transgenic mice

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Immunotherapy with antibodies against inhibitors of lymphocyte activation control points is an effective and promising approach to cancer therapy. Despite the rapid spread in clinical practice, many details of induced anti-tumor immunity are unknown. Understanding these details will increase the effectiveness of therapy and understand the criteria for selecting a target for therapy. Since the study of the fundamental mechanisms in patients is difficult, mouse models are often used. However there is no understanding of the factors of the therapy effectiveness. In the current study, we have used mouse model of subcutaneous melanoma B16F0 in transgenic C57BL/6-FoxP3GFP mice. For this model, we have developed anti-CTLA-4 therapy scheme, that provides suppression of tumor growth in 40% of animals and allows to extract four CTLA-4-positive TILs subsets (CD4 T-, CD8 T-, regulatory T- and B-cells). Prerequisite for this scheme is the ability to isolate a sufficient number of TILs in each subset (more than 2000 cells) both from animals without therapeutic effect and from those with reduced tumor progression. The data showed complex nature of the immune response in the immunotherapy of melanoma B16 and the important role of CD4 + and B-cells in therapy. We plan to study this answer in more detail by genomic analysis of lymphocyte subpopulations.

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POSTER 8:

Partial Aperture Imaging with a Single Camera Shot

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Abstract: We present a partial aperture imaging method based on the recently developed incoherent imaging process termed interferenceless coded aperture correlation holography. The observed 3D scene is captured by a single camera shot and reconstructed by a non-linear technique.

Summary

Interferenceless coded aperture correlation holography (I-COACH) is a motionless, non-scanning, incoherent 3D imaging technique [1]. In I-COACH, a pseudorandom coded phase mask (CPM) is used to modulate the object wave before its intensity signature is recorded. The imaging process starts with a training stage in which the light diffracted from a point object is modulated by a CPM and a library of point spread holograms (PSHs) for multiple axial locations are stored. A multi-point object placed within the pre-defined axial range is modulated by the same CPM, and the intensity pattern called object hologram (OH) is recorded. The various axial planes of the object are reconstructed by correlating the OH with the corresponding PSHs. In order to remove the bias and background noise generated during cross-correlation between OH and PSHs, multiple intensity patterns are recorded using different CPMs and the intensity patterns are projected into the complex space before the cross-correlation. Further, an averaging technique has been implemented for improving the signal to noise ratio (SNR).

Recently, we have shown that I-COACH can retain its lateral resolution capabilities even when most of the light is blocked besides an annular narrow pass along the original aperture [2]. Conventional direct imaging with the same annular aperture fails to preserve the same resolving power, yielding blurred images of the scene. A possible application for the partial aperture configuration has been envisioned with orbiting satellites whose orbital path forms the annular aperture. An aperture ratio (between areas of the ring and the clear circle limited by the ring) of less than 1% is sufficient in the case of I-COACH to reconstruct 3D images of the object with the same lateral resolution as the full clear aperture. However, the averaging technique over images captured with multiple independent CPMs is needed for maintaining a desirable SNR. A total of 60 camera shots were required for every new object in addition to the preliminary training stage involving an additional of 60n camera shots with the point object, where n is the number of the axial positions in the object space.

A non-linear reconstruction technique with an improved SNR has been developed lately for 3D imaging by I-COACH with a single camera shot [3,4]. In this study, we implement the non-linear reconstruction procedure in partial aperture imaging to reduce the number of camera shots much below the above-mentioned number. In this case, the aperture configuration includes only eight equally spaced isolated sub-apertures along the orbit. The proposed configuration offers a partial aperture I-COACH with smaller area than [2] and with an improved time resolution due to the use of a single camera shot. Experimental results of the proposed method are compared with the results of direct lens-based imaging.

Funding

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POSTER 9:

Fiber-optic Fabry-Pérot sensor with nanodiamond film for determination of hemoglobin level

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This work presents an interferometric fiber-optic sensor of hemoglobin concentrations with nitrogen-doped nanodiamond film. The sensor was designed as a Fabry-Pérot interferometer working in a reflective mode. The nanodiamond film was used in its construction as a mirror. The Microwave Plasma Enhanced Chemical Vapor Deposition (MW PE CVD) System was used to deposit the nanodiamond film on silicon substrate. Its surface and structure investigation was performed which indicated that it can be used as a reflective layer.

Diamond is known for its outstanding mechanical and chemical properties [1]. The application of nanodiamond material introduces many advantages into the sensor in comparison to conventional silver mirrors, for instance better chemical and mechanical resistance, smaller sample volume needed to perform measurements, opportunity to perform biomedical measurements [2].

The experimental setup consisted of a superluminescent diode with a central wavelength of λ = 1550 nm, an optical spectrum analyser (OSA), a telecommunication 2 x 1 coupler, commercially available single mode optical fibers and a set of micromechanical elements for positioning and stabilizing the measurement head. The Fabry-Pérot cavity was formed between the fiber tip/sample boundary and sample/nanodiamond boundary.

The measurements performed with the use of the developed sensor shown that it works with linear characteristics. The value of coefficient of correlation is equal to R^2 = 0.988. This means that the adopted mathematical model is very well fitted to the data. Detailed presentation of this sensor was described by Majchrowicz et al. [3].

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POSTER 10:

Imaging of Brain Vasculature by Laser Speckle Contrast Imaging under the Broken Ergodicity Conditions

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Laser Speckle Contrast Imaging (LSCI) technique is a powerful cost-effective diagnostic method extensively used in various biomedical applications for non-invasive real-time monitoring of blood flow and blood microcirculation [1-4]. The majority of biological tissues are the highly heterogeneous media composing mixture of static and dynamic structural inclusions. The presence of static areas exhibit non-ergodic features providing systematic uncertainty in the quantitative interpretation of laser light fluctuations during dynamic scattering. In fact, the LSCI and other dynamic light scattering techniques are widely used for imaging of blood flow in biological tissues, and the issues associated with non-ergodicity are typically ignored. In current report, based on the recently developed a simple phenomenological approach [5,6], we introduce a justification for application of laser speckle contrast imaging technique for imaging of blood flow under the breaking ergodicity conditions. Implementation of the approach is presented for the transcranial visualization of brain vasculature and quantitative assessment of blood flow in variety of vascular bed *in vivo* (Fig.1).

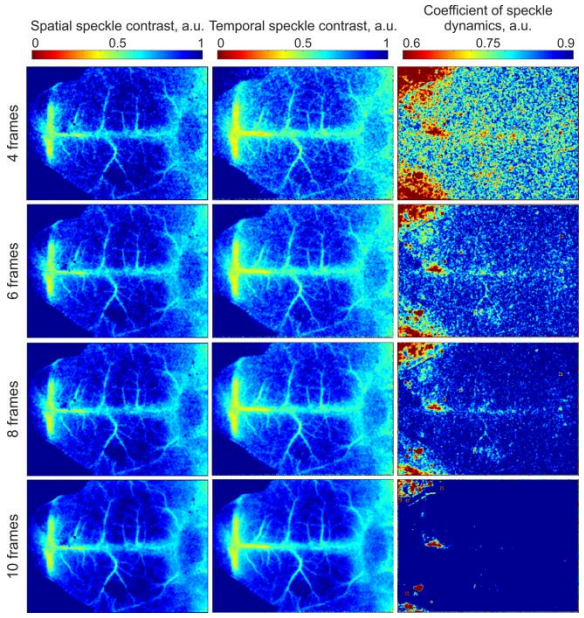

Fig.1 Images of brain vasculature obtained by LSCI technique at different ergodicity conditions, presented as spatial and temporal speckle contrasts and the difference between them.

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POSTER 11:

Frequency domain fluorescence lifetime imaging microscopy system for detecting inflammatory cells

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Abstract— Objective: Inflammation of the meninges is a source of severe morbidity and therefore is an important health concern worldwide. The conventional clinical microbiology approaches used today to identify pathogens suffer from several drawbacks and frequently provide false results. This research describes a fast method to detect the presence of pathogens using the frequency domain (FD) fluorescence lifetime (FLT) imaging microscopy (FLIM) system. *Methods:* The study included 43 individuals divided into 4 groups: 9 diagnosed with diverse types of bacteria; 16 diagnosed with diverse types of viruses; 5 healthy samples served as a control; and 12 samples were negative to any pathogen, although presenting related symptoms. All samples contained leukocytes that were extracted from the cerebrospinal fluid (CSF) and were subjected to nuclear staining by 4′, 6-diamidino-2-phenylindole (DAPI) and FLT analyses based on phase and amplitude crossing point (CRPO). *Results:* Using notched boxplots, we found differences in 95% probability between the first three groups through different notch ranges (NR). Pathogen samples presented a longer median FLT (3.28ns with NR of 3.24-3.32ns in bacteria and 3.18ns with NR of 3.16-3.21ns in viruses) compared to the control median FLT (2.65ns with NR of 2.63-2.67ns). Furthermore, we found that the undetected forth group was divided into two types: a relatively normal median FLT (2.72ns with NR of 2.68-2.76ns) and a prolonged FLT (3.22ns with NR of 3.17-3.27ns). *Conclusion:* FLT measurements can differentiate between control and pathogen by the CRPO method. *Significance:* The FD-FLIM system can provide a high throughput diagnostic technique that does not require a physician.

POSTER 12:

Generation of reactive oxygen species during autofluorescence photobleaching in-vitro.

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This study aims at development of a non-invasive time-resolved autofluorescence methodology for estimation of singlet oxygen generation during endogenous photosensitization. Photosensitization is a process wherein optical acceptor (photosensitizer) absorbs light to create an excited electronic state, followed by non-radiative energy transfer from the excited state sensitizer to the oxygen. We assume that tissue/cells endogenous fluorophores act as acceptors of optical radiation leading to production of singlet oxygen trough the photosensitization process. To confirm proposed hypothesis the *in-vitro* mouse melanoma cells *B16F10* with externally added of SOSG (singlet oxygen fluorescence sensor) were irradiated by continuous laser irradiation (470 nm, power density 30 mW/cm^2) within 20 minutes [1]. During the continuous laser irradiation the fluorescence spectral characteristics (decay distribution, intensity and spectra) of SOSG and in-vitro cells were recorded and analyzed. Two groups of mouse melanoma cells were included to the study: 1) transfected cells with GFP were used in order to estimate singlet oxygen generation caused by GFP photosensitization and 2) non-transfected cells for the study of generation of singlet oxygen during autofluorescence photobleaching. Fluorescence time-resolved spectral characteristics obtained during the 20 minutes of continuous 470 nm laser irradiation will be presented followed with assumptions and conclusions.

This work was supported by European Regional Development Fund project: “Time-resolved autofluorescence methodology for non-invasive skin cancer diagnostics” (No. 1.1.1.2/16/I/001, agreement No. 1.1.1.2/VIAA/1/16/014).

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POSTER 13:

Interferometric Phase Microscopy of Biological Cells using New Phase Unwrapping Strategies

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Imaging biological cells in vitro is useful for both medical diagnosis and biological research. However, isolated cells in vitro are mostly transparent, and therefore cannot be imaged with sufficient contrast via standard bright-field microscopy. Quantitative interferometric phase microscopy enables imaging of isolated cells in vitro without using cell staining, by measuring how much the light is delayed when passing through the sample. However, the measured phase is inherently encoded inside a complex exponent, such that the phase obtained is wrapped, meaning that it is a modulo 2π function of the actual phase, where the quotient is unknown. As long as the local spatial gradient in the true phase profile does not exceed an absolute value of π radians, so that there is no aliasing, the 2-D phase profile can be found in a satisfactory manner by various phase unwrapping algorithms, which are typically based either on path following methods or minimum norm methods. Nevertheless, these methods might fail when the phase gradients are steep, such that they exceed π radians in all boundaries of the object. We propose two different approaches to tackle this problem. The first approach is using the information present in additional dimensions; In Ref. [1], we suggested to use the angular dimension, in order to earn information helpful for phase unwrapping of optically thick objects that cannot be reconstructed by conventional 2-D phase unwrapping algorithms but are thin enough at certain viewing angle. In this approach, we reconstruct the object phase for the angular view from which it is optically thick by utilizing multiple interferometric projections acquired from consecutive angles in small angular increments, containing at least one viewing angle where the object is optically thin. This approach is specifically useful for tomographic phase microscopy, where multiple angular phase maps are acquired for 3-D refractive-index map reconstruction, enabling the reconstruction of quantitative phase projections even from the thick angular views. This concept can be further generalized to acquiring this angular information over time, enabling incorporating temporal and angular phase unwrapping in order to properly unwrap the phase maps. Therefore, in Ref. [2], we used four dimensions: two spatial dimensions of the projection (x and y), illumination angle, and time. This algorithm is suitable even for time points where the second condition of a thin angular view is not met, given that it is met at least once during the recording of the dynamic object, and that the time or angular steps between acquisitions are sufficiently small. The second approach is using state-of-the-art deep learning tools for phase unwrapping. Artificial neural networks have the potential to yield a robust solution to the phase unwrapping problem, since they can learn the characteristics of the input data, and if trained properly, use this information to unwrap the phase more accurately based on the characteristic gradients while ignoring noise. We explored two inherently different deep-learning concepts for approaching the 2-D phase unwrapping problem; either observing it as an inverse problem, where the output image is composed of continuous values, or as a semantic segmentation problem, where the output

image is composed of integers. We then compared the two methods, to determine the favorable deep-leaning approach for 2- D phase unwrapping when imaging biological cells in vitro.

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POSTER 14:

Fabry-Perot Interferometric optical fiber sensors with diamond structures

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The paper presents a Fabry-Perot Interferometric optical fiber sensor with diamond structures. Its performance was investigated for three kinds of diamond films [1,2]. Thin diamond films have been used due to their outstanding properties such as: biocompatibility, great chemical stability, remarkable hardness, high thermal conductivity, optical transparency. In addition, diamond is a promising material to build various biocompatible and chemically stable optical biosensors. Thanks to the above properties, diamond can operate in harsh environmental conditions.

The detection of the measured signal of a Fabry-Pérot interferometer was performed using an optical spectrum analyzer (Ando AQ6319). The metrological properties have been examined with the use of two broadband light sources (SLD type S1550-G-I-10 with central wavelength λ_0 = 1560 nm, $\Delta\lambda_{FWHM}$ = 45 nm, produced by SUPERLUM and SLD type S1300-G-I-20 with central wavelength λ_0 = 1290 nm, $\Delta\lambda_{FWHM}$ = 50 nm, produced by SUPERLUM). All devices were connected with a single-mode commercially available, 2x1 telecommunications coupler (SMF-28 fiber).

The achieved results show that diamond film provides a good visibility of the interference signal [2]. It could be also used for creating small and immune to electromagnetic interferences sensors for biomedical measurements, for instance of hemoglobin level.

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POSTER 15:

Photodynamic diagnostics of stomach cancer

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Gastric cancer is the main cause of death among patients with gastrointestinal tract neoplasia . This study was aimed at developing of improved approach for early diagnosis of stomach cancer using delta-aminolevulinic acid/ protoporphyrin IX (5-ALA/PpIX) fluorescence detection. The fluoresc ent detection of lesions was made on experimental laboratorial animals – on adult rats, using our or iginal model of gastric adenocarcinoma induced by combination of two carcinogenic factors, such as stress (overpopulation) that is common for modern society and nitrites that are often used as food s upplements. To induce malignant alterations in animal stomach mucosa, the rats underwent chronic s ocial stress (overpopulation) during 9 months with the daily using of toluidine (2g/kg) in food and n itrites (2g/l) in water. The control group included healthy rats (n=10) living in standard conditions. I n the end of this period, 5-ALA was applied intravenously 2 hours before the spectroscopic observa tion of gastrointestinal tract in a dose of 20 mg/kg. A LED source on 405 nm (AFS-405, 25mW, P olironik Inc., Russia) was used for excitation. The emission of endogenous autofluorescence (in the r egion 420-630 nm) and exogenous 5-ALA/PpIX fluorescence (630-710 nm) was detected using micr ospectrometer USB4000 (OceanOptics Inc., USA) coupled to a fiber probe. Each suspicious site wit h increased level of fluorescence max at 635 nm and the surrounding mucosa, which did not presen t exogenous fluorescence, were measured from 7 to 10 different points and spectra were averaged T he both parts of the tissues investigated were examined afterwards histologically and the results of microscopic examinations were used as a gold standard for evaluation of the sensitivity and specifici ty of the fluorescence technique for diagnosis and discrimination of neoplastic alterations.

The spectral measurements were made on normal mucosa and suspicious sites. The signal detected fr om tumour sites has consists of mucosal autofluorescence, fluorescence from exogenous fluorophore protopo rphyrin IX and re-absorption from the haemoglobin of the vascular network of the tissue. Normal mucosa has significant autofl uorescence, related mainly to the emission of co-enzymes and proteins in these tissues. The tumour area has reduced autofluorescence signal and well pronou nced signal at the red spectral region with two specific maxima at 635 nm and 704 nm, typical for 5-ALA/PpIX. Inflammatory areas observed showed intermediate levels of the fluorescence at 635-70 4 nm, related to 5-ALA/PpIX accumulated in these tissues due to the changed metabolism of the ce lls. However, the spectral intensity at 635 nm in inflammatory areas was about three times and low er that the signal obtained from cancerous sites, which allow differentiation between benign and mal ignant changes. Fluorescence emission intensity at 635 nm for normal and cancerous mucosa was fo und to be more than 10 times higher for the malicious tissues vs. healthy ones. Observed difference

s in the exogenous fluorescence intensity levels for normal, inflammatory and neoplastic mucosa cou ld be used for fluorescence mapping of the suspicious lesions and for diagnostic differentiation durin g endoscopic observations of stomach neoplasia.

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POSTER 16:

MRI contrast media as optical clearing agents: perspectives for tissue multimodal imaging

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We have discovered that MRI contrast media can be successfully used as optical clearing agents for enhancing optical imaging via various spectral and coherent-domain methods. This application clears the path for a fundamentally new approach in multimodal imaging, since synchronization in time and space occurs for enhancement of optical and MRI image contrast simultaneously because the same agent enables both modalities.

In this study, *ex vivo* and *in vitro* mouse skin samples were used for spectral and OCT measurements. For both measuring approaches, we tested Gadovist, Magnevist, and Dotarem as MRI contrast agents whereas saline served as a control.

Collimated transmittance spectra in the wavelength range of 500-900 nm were measured for *ex vivo* skin samples concurrently during the course of sample immersion by using a multichannel spectrometer USB4000-Vis-NIR (Ocean Optics, USA). Samples were immersed in MRI contrast solutions and thickness and weight were measured before and after spectral measurements. We determined that the application of

MRI contrast agent solutions lead to the increase of skin transmittance in the 500-900 nm spectral range. The best result was obtained for Gadovist, which increased the collimated light transmittance at 700-900 nm approximately 6 times in 10 min and 11 times during one hour after applying the solution with no noticeable change the geometric and weight characteristics of mouse skin samples with a thickness of 0.28 mm. At a wavelength of 500 nm the efficiency of optical clearing by Gadovist reached 10 times in 10 min. The application of Magnevist resulted in only a three-fold increase of the transmittance at these wavelengths and Dotarem provided no more than-two-fold.

The optical coherence (OCT) measurements were performed for *in vitro* Nu/Nu mice (Animal Breeding Center, Pushchino, Russia) skin samples. In that case, the average skin thickness was 0.36 mm. Each intact skin sample was photographed with a digital camera and imaged (scanned) by a Spectral Radar OCT System OCP930SR 022 (Thorlabs Inc., USA) at a wavelength of 930 nm. The samples were then placed in the test solution. The OCT skin images were recorded after 30 and 60 min of exposure to the solution for each sample. At immersion optical clearing by topical application of MRI agents, OCT images also demonstrated an increase of light beam probing depth that reached the subcutaneous fat layer. In general, much more contrast OCT images of skin were obtained.

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POSTER 17:

Raman spectroscopy of prebiotic compounds obtained after proton irradiation of formamide used as precursor of meteorite-catalyzed synthesis

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Formamide (NH2CHO) is becoming one of the most intensively studied precursors for prebiotic syntheses of compounds potentially relevant for the origin of life. [1] It is discussed as a possible parential molecule for synthesis of prebiotic molecules under proton irradiation, mimicking open space conditions. Different mineral content of meteorites allow variety of possible catalytic actions that would force such synthesis. The products of these processes could be detected using highly sensitive techniques for investigation of trace quantities of compounds, such as Raman spectroscopy method.

A liquid formamide had been irradiated by high-energy proton beams in the presence of powdered meteorites from several different sources with variable properties and mineral content, and the products of the catalyzed resulting syntheses were analyzed by Raman spectroscopy technique, which is an extremely sensitive spectral tool for detection of organic matter. The meteorites tested were representative of the four major classes: iron, stony iron, chondrites, and achondrites. In the irradiated samples were observed Raman signals from amino acids, carboxylic acids, and sugars, which correspond to some earlier investigations, when mass-spectrometry approach was applied [2].

This investigation is a part at answering the central question of the problem of the production of the prebiotic compounds underlying the formation of the living systems. Research is focused on the development of unified theoretical and experimental approaches taking into account the influence of the following factors: energy, evolution, protometabolic, and the primordial environment — on the origin of life. We are looking for a sequence of processes that can lead to the formation of a complete, chemically active prebiotic system. Raman spectroscopy could be used as a fast and non-destructive complementary tool for evaluation of

synthesized prebiotic molecules in such “metheorite+proton irradiation + formamide” model system. The obtained results could contribute to the general understanding of the origin of life.

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