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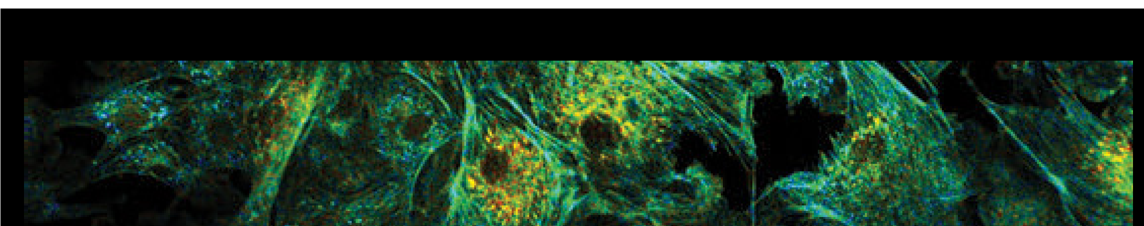
ТЕЗИСЫ ПОСТЕРНЫХ ДОКЛАДОВ

2-я Школа ADFLIM

для молодых ученых, аспирантов и студентов



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CHANGES IN ADULT HIPPOCAMPAL NEUROGENESIS IN RATS UNDER THE INFLUENCE OF STRESS

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Chronic stress is a widespread condition leading to various brain pathologies including depression and post-traumatic disorder. The exact way of long-term action of stressful impacts remains not completely understood. A possible mechanism may involve the changes in adult neurogenesis, caused by stress condition and leading to formation of abnormal neuronal circuits.

We applied two paradigms of the chronic stress: chronic unpredictable mild stress (CUS) and early-life inflammatory stress (ELIS). In CUS paradigm, the rats were subjected to a series of stressful events including food and/or water restriction, cage tilt, crowded housing, isolation, and inversion of the light-dark schedule. Stressors were changed twice a day and presented randomly during two months. After completion of CUS protocol, animals were assessed in behavioral tests and sacrificed for analysis of neurogenesis. In ELIS paradigm, the rat pups were injected with bacterial lipopolysaccharide on postnatal days 3 and 5, and behavior and neurogenesis were assessed later in adulthood, at the age of 3 months.

Two stress paradigms have led to opposing effects on neurogenesis. The proliferation of precursor cells after completion of stress, assessed by PCNA staining, was unaffected in both paradigms. However, the neuronal differentiation assessed by doublecortin staining was suppressed by ELIS and enhanced by CUS. On the other hand, the number of new neurons and astrocytes generated from the cells which were born during early-life inflammation was increased in the dentate gyrus of rats subjected to ELIS. Our results suggest the difference between the ways in which these two stress paradigms influence the process of postnatal neurogenesis in the hippocampus of rats.

The study was supported by RSF grant no. 14-25-00136

COVALENT LABELING OF CELL-SURFACE GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED RECEPTORS FR STUDY OF DIMERIZATION AND OLIGOMERIZATION IN LIVING CELLS BY FRET

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Many receptors in plasma membranes transmit signal from the outside to the inside of cells by clustering upon ligand binding. However, there is growing evidence that some receptors, such as receptor tyrosine kinases, G protein-coupled receptors, and glycosylphosphatidylinositol (GPI)-anchored receptors form dimers

and oligomers even ligand independently. Imaging protein-protein interactions by Förster resonance energy transfer (FRET) microscopy between genetically encoded receptors tagged with fluorescent proteins (FPs) is often used to elucidate the mechanism underlying the signal transduction and the role of receptor dimerization or clustering. Here we suggest a covalent labeling of cell-surface GPI-anchored receptors with synthetic fluorophores for these purposes instead of using FPs. This method has some advantages, especially for GPI-anchored receptors. First, it minimizes distortion of protein properties by fluorescent labeling since small tag (S6 peptide sized 12 amino acids flanked by one amino acid linkers) and fluorophores used in this case are much smaller than FPs (1.5 kDa vs 26 kDa). Second, using cell-impermeable fluorescent CoA substrate restricts labeling to surface-exposed proteins of living cells. Third, synthetic fluorescent dyes do not prone to dimerize unlike many FPs. Finally, it gives wider choice of optimal fluorophores and superior control of the donor:acceptor ratio.

In the present study, we have investigated lateral organization of a T-cadherin as example GPI-anchored receptor. The substituted phosphopantetheine groups of coenzyme A-linked Dyomics DY-547 or DY-647P1 dyes (CoA547 or CoA647) were covalently attached to a serine residue of S6 peptide within T-cadherin-S6 protein by a phosphopantetheinyl transferase on a surface of living cells. FRET was analyzed by laser scanning confocal microscopy by measuring either sensitized acceptor emission or acceptor photobleaching. We have found that the FRET signal is acceptor density-independent, suggesting the non-random dimer organization of T-cadherin molecules on the plasma membrane.

The work was supported by the Russian Science Foundation project № 14-24-00086.

THE HOMOLOGUE OF THE KUNITZ PEPTIDASE INHIBITOR PARTICIPATES IN THE INTERCELLULAR TRANSPORT OF MACROMOLECULES IN *NICOTIANA BENTHAMIANA* LEAVES

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Higher plants are constantly exposed to various biotic and abiotic stresses. Wherein, the same defense response genes have different transcriptional activity in leaves and roots. A typical example is the members of Kunitz peptidase inhibitors (KPI) gene family some of which are expressed only in roots but not in leaves others in both parts of plants. We have identified and isolated from the genome of Nicotiana benthamiana a gene NbKPILP encoding Kunitz peptidase inhibitor-like protein. We showed that NbKPILP lost the ability to inhibit serine peptidases but acquired the capacity to influence the intercellular transport of macromolecules in plant leaves providing protection from pathogens. To prove the new function of NbKPILP we have examined the state of intercellular transport in leaves using a

2xGFP (54 kDa) molecule. Normally, plasmodesmata are not able to pass molecules larger than 40 kDa. We used agroinfiltration technique to deliver 2xGFP-encoding plasmid together with NbKPILP-expressing construct into plant cells. Then we counted clusters of GFP-containing cells 24-30 hours post agroinjection using a fluorescent light microscope. NbKPILP overexpression leads to the increase of the intercellular transport activity: more than 50% of the fluorescent signals are distributed between 2-3-4-cell clusters in comparison with the control where the majority of clusters contain 1-2 cells. Another technique for estimation of NbKPILP role in the intercellular transport is the complementation test, when the function of the defective gene is compensated by another gene in trans. We used tobacco mosaic virus (TMV)–based vector encoding GFP to assess the ability of NbKPILP to complement the function of the TMV movement protein (MP) which is known to effectively increase the plasmodesmata size exclusion limit, performing "gating", and to participate in the transport of viral RNA. The mutation in MP resulted in failure of MP synthesis from TMV-GFP(mut) vector led in GFP accumulation only in single cells. The co-injection of the TMV-GFP(mut) and NbKPILP into plant leaves resulted in the complementation of the defective MP transport function: we observed the multicellular GFP-containing clusters. Thus, we have identified the ability of NbKPILP to complement the transport function of TMV MP. We concluded that the NbKPILP interacts with plasmodesmata and stimulates intercellular transport of macromolecules in the plant.

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SWITCHABLE ACCEPTORS OF RESONANCE ENERGY TRANSFER BASED ON LIPOCALIN BLC AND SYNTHETIC CHROMOPHORES

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Fluorescence Resonance Energy Transfer (FRET) between fluorophores is widely used in the study different biochemical pathways and protein-protein interactions inside living cells. This work is devoted to development of modulated acceptors based on synthetic chromophore-binding proteins. Reversible interaction of the chromophore with the ligand-binding "pocket" of the protein will make it possible to repeatedly measure the experimental and control points by adding or washing the chromophore.

As a model system, a number of indicators of caspase-3 activity consisting of fluorescent proteins (donors) and lipocalin mutants Blc binding synthetic chromophores (acceptors) were created. Caspase-3 cleaves the linker between donor and acceptor which contains a specific amino acid sequence. The transfer efficiency was determined from the change in fluorescence of the donor upon addition of chromophores and caspase-3. As a result we found donor-acceptor

combination with highest energy transfer efficiency (60% for NeonGreen). The efficiency of this donor-acceptor pair in the fusion protein with histone H2B was also determined under conditions of wide-field fluorescence microscopy and fluorescence lifetime imaging microscopy in the mammalian cell culture model (cell line HEK293T).

We suppose that systems of inductive energy transfer with a controlled acceptor qualitatively surpass traditional FRET pairs based on fluorescent proteins. Dynamic control of the acceptor, achieved by the addition or washing out the chromophore, allows for more accurate determination of the energy transfer efficiency.

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DESIGN OF Β7-EGFP AND CD63-TAGRFP FUSIONS FOR IMAGING OF PROTEASOME TRANSPORT IN LIVING CELLS VIA EXOSOMES

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The proteasome is a multisubunit protein complex that serves as a major pathway for intracellular protein degradation, playing an important role in various biological processes such as transcription, progression through the cell cycle, signal transduction, cell death, immune responses, metabolism, protein quality control and development. The proteasome is composed of a 20S core particle possessing proteolytic activity and one or two 19S regulatory complexes. Recent data showed the presence of proteasomes in extracellular space. Moreover, these extracellular proteasomes did not contain any regulatory particles and are represented exclusively by the 20S complex. However, the mechanism of transport and functions of these proteasomes are unclear. It is assume that the proteasome can be secreted by cells via extracellular vesicles called exosomes. Although exosomes differ from other extracellular vesicles by smaller size (30-100 nm), nevertheless, vesicles with a similar size can also bud out from cell membrane. However, exosomes were shown to differ clearly from other vesicles by enrichment of the transmembrane tetraspanin proteins CD63, CD9 and CD81. To address whether proteasomes are transported into extracellular space by exosomes, we generated a HeLa cell line, stably expressing exosomal marker CD63 fused with a TagRFP and subunit of 20S proteasome β7 fused with an EGFP. HeLa and other cell types harbouring the CD63-TagRFP and β7-EGFP constructs represents a valuable tool that should allow real-time observations of proteasomes transport via exosomes.

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WATER SOLUBLE RHENIUM(I) COMPLEXES FOR TWO-PHOTON IMAGING

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The luminescent metal complexes have been intensively investigated during the last two decades due to their attractive physical properties, which open new horizons for a number of technological applications, including luminescent analytical methods[1]. The novel water soluble rhenium(I) complexes were obtained following a protocol of one-step thermal substitution carried out in a pressure autoclave. One of the complex is intensely luminescent in aqueous solution at 298 K

(Φem = 20%, λem = 583 nm). Moreover, it demonstrates high two–photon absorption cross-section (σ = 300 GM, λex = 780 nm) and high intensity of two–photon induced emission. This feature in combination with very small toxicity and high stability allows for using this complex as an efficient nonlinear optical imaging agent in physiological medium.

1 K. K.-W. Lo, Acc. Chem. Res. 2015, 48, 2985.

REFRACTIVE PROPERTIES OF OXY- AND DEOXYHEMOGLOBIN MEASURED AT SEVERAL LASER WAVELENGTHS IN THE VISIBLE AND NIR

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Recently, most often in medicine in the diagnosis and therapy used non-invasive or minimally invasive optical methods, for example optical coherence tomography. Since blood determines the absorbing characteristics of most biological tissues in the visible and NIR, it is important to study thoroughly and accurately the optical properties, in particular refractometric properties, of blood and its main components, hemoglobin and albumin.

In the previous studies, the dispersion of the oxygenated and deoxygenated forms of hemoglobin was experimentally determined and correlated to their absorption properties [1-3].In this study, the refractive index of oxy- and deoxyhemoglobin solutions was measured for a bigger wavelength range in the visible and NIR at the specific laser wavelengths: 480, 486, 546, 589, 644, 656, 680, 800, 930, 1100, 1300 and 1550 nm. The measurements were implemented using a multi-wavelength Abbe refractometer Atago (Japan). Measurements were carried out for solutions of oxy- and deoxyhemoglobin with concentrations of 80, 120 and 160 g/l, which corresponds to a reduced, normal and elevated hemoglobin concentration in the blood. The measurement temperature was 23 °C.
Based on the results of the measurements, the specific increment of the refractive index of oxy and deoxyhemoglobin on the wavelength and the coefficients for the Sellmeier formula were calculated.Ellipsometric measurements of refractive properties hemoglobin were also done using a spectroscopic ellipsometers (SE, HORIBA Jobin Yvon Auto SE, France), which was carried out at an incidence angel of 70° with the wavelength range of 439–842 nm AUTO-SE. It was done for the laser with the wavelength 632.8 nm for fresh and dried drop of hemoglobin. Advantages and drawbacks of such measurements will be discussed.
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CYCLOMETALATED IRIDIUM(III) COMPLEXES: TUNING THE LUMINESCENT PROPERTIES FOR DEVELOPMENT DUAL-EMISSIVE PROBES

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Molecular oxygen is essential for maintaining vital processes in almost all organisms and cells. Intracellular oxygen status can change due to cellular activity and in the cases of pathological conditions. Determination of intracellular oxygen status and mapping of oxygen concentration in tissues can be done using luminescent microscopy and oxygen sensitive phosphorescent dyes. It is worth noting that oxygen concentration could be determined precisely from the ratiometric (phosphorescence vs fluorescence) measurements provided that additional independent fluorophore is introduced into the probe molecule. Herein we report on cyclometalated [Ir(C^N)2(NN)]+ complexes bearing the N^N diimine ligand functionalized with rhodamine and cyanine dyes. Emission properties (absorption, lexc, lem, sensitivity to oxygen) of the complexes obtained

were tuned by variations in structure and donor properties of the metalated C^N ligand.

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INTEGRATION OF MOUSE NEURONAL ACTIVITY AND BEHAVIOR: ACQUSITION AND AUTOMATICAL PROCESSING OF CALCIUM IMAGING DATA

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Interconnection between neuronal activity and behavior of living animals is the thing which neuroscientists traditionally are focused at. However, only recently, with development of in vivo calcium imaging technique, it has become possible to perform direct observations of the activity of big number of neurons simultaneously. Comparing to the "classic" method of electrode registration, the key advantage of this technique is the availability of explicit visualization of spiking neurons, their morphology and spatial distribution. However, optical in vivo imaging apparati are often bulky (e.g., multiphoton microscope) and requiring immobilization of animal, which restricts significantly the variety of cognitive tasks that can be presented to animal during imaging session. This problem can be solved by means of the novel technique: miniaturized 2 g weighted microscope, which can be mounted directly onto mouse's head is capable to detect calcium neuronal activity of freely moving animal. However, the detected signal contains a plenty of noise, so the isolation of distinct neurons and their temporal dynamics is a pretty difficult problem.

In the present study a set of programming solutions based on PCA/ICA algorithm is worked out, which makes possible the automatical analysis of such signal. In particular, these solutions can be used for:

-motion correction and registration of data from different imaging sessions;

-extraction of geometric places and temporal dynamics (traces) of active neurons;

-sorting of extracted traces and artifact filtering;

-detection of significant events of neuronal activity (calcium spikes);

-synchronization of neuronal activity data with video tracking;

-segmentation of animal behavior onto distinct behavioral acts;

-matching of neuronal spikes and behavioral acts, counting of spiking statistics;

-visualize obtained data with graphs and figures.

All listed above is now successfully used in the current research on spatial and

social memory formation in mice, where direct observations of neuronal activity in various cognitive tasks are used.

IMAGING TUMOR MICROSCOPIC VISCOSITY IN VIVO USING MOLECULAR ROTORS

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Viscosity is one of the key parameters that determine the morphological and physiological state of living cells. Changes in viscosity at the cellular level can be associated with carcinogenesis. The aim of the work was to develop methods for measuring tumor microviscosity in vivo using a molecular rotor based on BODIPY. Real-time viscosity measurement in living cells was made possible by the development of FLIM (Fluorescence Life-time Imaging Microscopy) and the appearance of fluorescent molecular rotors.

The study was performed on Balb/s mice with CT26 tumor. Fluorescent molecular rotors BODIPY1, solubilized with polymeric brushes, and water-soluble BODIPY2 were used in the work. For a fluorescence microscope with a time resolution, a multiphoton tomograph MPTflex (JenLab, Germany) was used. Fluorescence of the rotors was excited at a wavelength of 800 nm, fluorescence was detected in the range 409-680 nm. Microscopic images of the tumor were obtained in vivo during the first 1.5 h after injection, and also after 24 h.

It was shown that both rotors fluoresce quite brightly in the tumor tissue. It is found that the rotor BODIPY1 solubilized polymer brushes shows monoexponential decay in connective tumor tissue. The lifetime of the fluorescence of the rotor in the connective tissue was 2.24 ± 0.06 ns, which corresponds to a viscosity of 265 ± 16 cP. The molecular rotor BODIPY2 showed a monoexponential decay of fluorescence in tumor cells and was characterized by a fluorescence lifetime of 2.67 ± 0.06 ns, which corresponds to a viscosity of 386 ± 19 cP. It is important that the measurement of the viscosity of tumor tissue using molecular rotors based on BODIPY in real time is performed for the first time in the world.

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LASER SPECKLE CONTRAST IMAGING OF BLOOD MICROCIRCULATION IN VIVO

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The study of blood microcirculation is one of the urgent problems of the medicine. We present the results of calibration Laser Speckle Contrast Imaging (LSCI) system and experimental study of changes of microcirculation of blood flow in rats with diabetes under the influence of optical clearing agents (OCAs) by using LSCI. Laser speckle contrast techniques are based on the spatial and temporal statistics of the speckle pattern, calculating of contrast of time-averaged dynamic speckles in dependence on the exposure time at the registration of the speckle-modulated images.

In our research, we used 20 white laboratory rats weighing 300-500 g. We used alloxan induced animal model of diabetes. The results obtained at the study of blood microcirculation disorders in diabetes show that disease development in animals causes changes in the microcirculatory system response to OCAs.

INVESTIGATION OF MYOCARDIUM PERMEABILITY FOR GLYCEROL DURING DEVELOPMENT OF ALLOXAN INDUCED DIABETES

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The goal of the study is to quantify permeability of ex vivo myocardium in control and diabetic rats for glycerol by optical method. Tissue permeability for chemical agents can allow assessing the structure change of tissue, which can be used as a biomarker of tissue protein glycation degree. Degree of tissue glycation in available organs can be used to diagnose tissue condition of internal organs, thus predict the dangerous complications of diabetes mellitus.

Six months outbred white male rats with body mass of 500 g were used in the study. Three groups of animals were formed: control, 2 and 4 weeks after diabetes induction. In the study diabetes was induced by injection of a single dose of alloxan (Acros Organic, Belgium) mixed with saline to experimental animals as 10 mg of alloxan per 100 g rat body mass. The investigation was performed on thirty ex vivo myocardium samples (ten samples in each group) obtained by autopsy.

Tissue permeability for glycerol was estimated from kinetics of collimated transmittance of tissue samples measured during their optical clearing by aqueous 70%-glycerol solution (n=1.427). Each sample was fixed in the cuvette filled up with the glycerol solution using the plastic plate with a square aperture in the centre. The collimated transmittance kinetics of myocardium samples was measured in the spectral range of 500-900 nm using USB4000-Vis-NIR spectrometer (Ocean

Optics, USA) concurrently with administration of the glycerol solution. The measurements were performed at room temperature ~20°C.

The increase of collimated transmittance and its further saturation was observed for all myocardium samples, both non-diabetic and diabetic ones, during the immersion in 70%-glycerol solution. Rate of collimated transmittance kinetics were used for determination of permeability coefficient of diabetic and non-diabetic myocardium for glycerol. Decreasing of myocardium permeability for glycerol with diabetes developing was observed, which is associated with structural changes of myocardium tissue due to molecular glycation of tissue components.

Presented results allowed us to propose a method for early diagnostics of diabetes mellitus complications, in particular, assessment the degree of myocardial lesion evidence by the stage of skin glycation in the process of diabetes development and treatment. Optical clearing of skin and myocardium also can be applied during therapy, diagnostics or surgery to increase light penetration in tissues.

SIGNAL SWITCHING IN MESENCHYMAL STROMAL CELLS: NORADRENALINE DOWNREGULATES Β-ADRENERGIC RECEPTORS LEADING TO UPREGULATION OF Α1-RECEPTORS

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Sympathetic neurons are important component of mesenchymal stem cells (MSC) niche. Catecholamines regulate proliferation, differentiation and secretion of bone marrow mesenchymal stromal cells (MSCs) acting through β1- and β2-adrenergic receptors. However, the expression of adrenergic receptors on adipose-derived MSCs as well as their regulation on those cells remains poorly understood. We examined the mechanisms of regulation of MSC responsivity to noradrenaline. Using flow cytometry, we demonstrated that α1A adrenergic receptors isoform was the most abundant in adipose tissue-derived MSCs. Using calcium imaging in single cells, we demonstrated that only 6.9 ±0.8% of MSCs responded to noradrenaline by intracellular calcium release. Noradrenaline increases MSC sensitivity to catecholamines in a transitory mode. Within 6 hrs after incubation with noradrenaline the proportion of cells responding by Ca2+ release to the fresh noradrenaline addition has doubled but declined to the baseline after 24 hrs. Increased sensitivity was due to the elevated quantities of α1A adrenergic receptors on MSCs. Such elevation depended on the stimulation of β-adrenergic receptors and adenylate cyclase activation. These data for a first time clarify mechanisms of regulation of MSC sensitivity to noradrenaline.

РАЗРАБОТКА МЕТОДА УВЕЛИЧЕНИЯ ОПТИЧЕСКОЙ ГЛУБИНЫ ДЕТЕКТИРОВАНИЯ НАНОЧАСТИЦ В КОЖЕ ПРИ ОКТ - ВИЗУАЛИЗАЦИИ

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В результате многолетних исследований было установлено, что наночастицы могут быть использованы для доставки лекарственных препаратов через кожный барьер. Одним из возможных путей преодоления кожного барьера функционализированными наночастицами является их внедрение внутрь волосяных фолликул, окруженных густой сетью капилляров. Очевидно, что данная процедура требует соответствующего неинвазивного мониторинга. Однако для увеличения глубины детектирования частиц требуется применение методик, позволяющих уменьшить рассеяние окружающей биоткани. Целью работы является исследование возможности увеличения глубины детектирования наночастиц в волосяном фолликуле с помощью оптической когерентной томографии за счёт техники оптического просветления кожи.

В работе использовались наночастицы диоксида титана диаметром 25 нм, внедрённые в волосяные фолликулы лабораторных крыс с помощью ультразвука. В качестве иммерсионных агентов использовались полиэтиленгликоль 400 (ПЭГ-400) и смесь ПЭГ-400 и диметилсульфоксида (ДМСО), применяемого для увеличения проницаемости эпидермиса. Сравнение оптической глубины детектирования области локализации наночастиц в фолликулах при использовании в качестве иммерсионного агента ПЭГ-400 и смеси ПЭГ-400 и ДМСО показало, что в последнем случае глубина детектирования увеличилась более чем в 1.5 раза.

КОЛИЧЕСТВЕННАЯ ОЦЕНКА ЭКСПРЕССИИ РЕЦЕПТОРА TLR4 ПРИ РАБОТЕ С ИЗОБРАЖЕНИЯМИ, ПОЛУЧЕННЫМИ С КОНФОКАЛЬНОГО МИКРОСКОПА

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В настоящее время актуальной является проблема стандартизации методов при обработке изображений для получения количественных данных. Не смотря на избыточность высокотехнологичного оборудования и программного обеспечения, используемого при современных молекулярных исследованиях, именно корректность выбранных методов компьютерной морфометрии и строгое следование стандартизированным

протоколам работы с полученными изображениями позволяет обеспечить достоверность получаемых результаты. Конфокальная микроскопия является разновидностью флуоресцентной микроскопии с улучшенным разрешением вдоль оптической оси объектов, что позволяет получать более точные данные о положении флуоресцентной метки, а, следовательно, и об особенностях расположении искомого белка на исследуемом препарате. При определении уровня экспрессии рецептора TLR4 необходимо учитывать, что его локализация не является высокоспецифичной. В связи с этим необходимо дополнительно проводить зонирование препарата, чтобы получить количественные данные по каждому участку исследуемой ткани.С помощью конфокального лазерного сканирующего микроскопа LSM 710 фирмы Carl Zeiss (Центр коллективного пользования «Конфокальная микроскопия» ИФ РАН) делали снимки для оценки экспрессии рецептора TLR4 методом непрямого иммуногистохимического окрашивания. Встроенное программное обеспечение, поставляемое с системами визуализации как правило имеет широкий функционал, однако не всегда отвечает исследовательским задачам. Так, показатель интенсивности флюоресценции часто используется для дальнейшей статистической обработки данных, однако является нежелательным. В связи тем, что флуорохромы со временем выгорают, а в разных сериях эксперимента используются отличные друг от друга настройки микроскопа, необходимо разрабатывать методы обработки биологических изображений. Использование программ обработки изображений, таких как ImageJ, позволяет проводить точную оценку искомого антигена. В результате введения относительных величин представляется возможным сравнение данных, полученных в разных сериях эксперимента. Использование масок позволяет получать данные по интересующим областям препарата, исключая из получаемых данных остальное поле изображения. Для проведения морфометрии препараты срезов легких фотографировали с помощью конфокального лазерного сканирующего микроскопа LSM 710 фирмы Carl Zeiss (Центр коллективного пользования «Конфокальная микроскопия» ИФ РАН).

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2-я Школа ADFLIM

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