1732-Pos Board B624
Understanding High Definition Immunoassays (HDIA): Studies of Binding Kinetics on Microparticles
Qiaoqiao Ruan, Susan Gayda, Joseph P. Skinner, Sergey Y. Tetin.
Abbott Lab, Abbott Park, IL, USA.
Fluorescence imaging provides both spatial and temporal information about target molecules in biological systems. We have proposed to use imaging as a detection technique in microparticle based immunoassays. It has advantage over traditional approaches which measure only total signal but do not make use of the spatial or temporal information embedded in the system. The method can be readily adapted for quick assay prototyping and high throughput screening on any conventional fluorescence imaging system.
In microparticle immunoassays, analytes are captured with antibody coated microparticles and subsequently detected using second antibody labeled with a reporter group. In HDIA, fluorescence images of the microparticles are examined pixel-by-pixel to extract binding information only from the microparticles, thus minimizing irrelevant signals from solution and vessel surfaces. Our model systems include sandwich based Troponin and Bcl-xl (B-cell lymphoma-extra large) protein assays, as well as homogeneous competitive Methotrexate immunoassay.
Performance of the HDIA is dominated by binding kinetics of the microparticles. Depending on the microparticle number, concentration of the binding sites on microparticles, sample volume, sample concentration and the geometry of the reaction vessel, the binding kinetics can be either reaction limited or diffusion limited. We will present the effects of these factors on the binding kinetics of HDIA.

1733-Pos Board B625
EPSP Amplitudes and Dynamics in Dendritic Spines using Voltage-Sensitive Dyes
Erika A. Hoyos-Ramirez, Corey Acker, Ping Yan, Leslie Loew.
University of Connecticut Health Center, Farmington, CT, USA.
Dendritic spines are the initial site of the processing of information carried by excitatory network activity. Spine morphology often includes a narrow neck, which is known to isolate the biochemical signaling components within the spine from the dendritic branch. To what degree spines are isolated electrically and how local excitatory postsynaptic potentials (EPSPs) behave is still not fully understood. It is known, however, that the larger the electrical isolation, the larger the amplitude of membrane potential changes, which may have a significant impact on signaling mechanisms within the spine responsible for important processes such as synaptic plasticity. We are addressing this question using 2-photon imaging of voltage sensitive dyes along with synaptic activation via glutamate uncaging in acute brain slices. using a custom microscope we can control the positions of both a recording laser (fixed at the center of a spine) and the uncaging pulse directed at the desired uncaging position just off the spine. The amplitudes of the optical signals in the spine are calibrated using backpropagating action potential waveforms, which, as previously described, is consistent across spines of different sizes and shapes. Uncaging-evoked EPSP amplitudes are typically 10mV-20mV, which are highly attenuated upon reaching the soma, where they typically appear 20 fold smaller in amplitude. Additionally, EPSP dynamics within the spine are typically much faster in the spine, with half-widths around 5-10ms, compared to the same EPSPs measured at the soma, which are typically 50-100ms. The observed attenuation of EPSP amplitudes and increase in duration were used to fit a biophysical, NEURON-based model, in order to explore the role of the spine geometry. NIH grants R01 EB001963 and P41 GM103313

1734-Pos Board B626
Calcium Imaging and Optical Manipulation of Neuronal Activity in Axolotl using a LED-Based Microscope
Stephan Direnberger[1], Roberto Banchi[1], Christian Seebacher[2], Felix Felmy[1], Hans Straka[1], Lars Kunz[1].
[1]Ludwig-Maximilians-University, Department of Biology II, Division of Neurobiology, Martinsried, Germany, [2]Ludwig-Maximilians-University, Department of Biology I, BioImaging Centre, Martinsried, Germany.
Here we introduce a novel fluorescence microscope concept which expands the application of functional fluorescence imaging and further enables optical manipulation of biological samples. Taking advantage of the LED technique allows a space saving housing of multiple light sources and illumination at various wavelengths. Furthermore, the microscope provides an optical separation into two independently controllable excitation pathways enabling a simultaneous illumination of different regions which are adjustable in size. Image detection is realized via a super resolution high speed camera that allows high resolution image capturing (2560x2160) at rates up to 100 Hz. Moreover, we implemented a voice-coil driven high NA objective to ensure z-movement at maximum speed and precision. The multiple LED arrangement and the separation of excitation pathways therefore facilitate the usage of multiple imaging approaches at the same time.
To test the performance of the microscope we used *in vitro* whole head preparations of *Ambystoma mexicanum* tadpoles. This preparation allows studying neuronal systems with all sensory pathways intact and can be maintained up to one week. Thus, we were able to use calcium imaging to record sensory evoked neuronal responses of central vestibular neurons elicited by electric stimulation of specific semicircular canals. Further, to quantify the glutamate uncaging efficiency we calculated the required light intensity and duration to optically evoke action potentials by patching those neurons. Finally, we could show that by means of spatially separating calcium imaging and glutamate uncaging we were able to manipulate ipsilateral semicircular canal evoked calcium responses by optically activating contralateral inhibitory/excitatory pathways.

1735-Pos Board B627
Optimization of Mitochondrial and Cytosolic pH Determination in Madin Darby Kidney Cells using CLSM Images
M. vandeVen[1], C. Balut[2], Sz. Baron[3], I. Smets[4], P. Steels[1], M. Ameloot[1].
[1]Hasselt University, Diepenbeek, Belgium, [2]Pittsburgh University, Pittsburgh, PA, USA, [3]Weizmann Institute of Science, Rehovot, Israel, [4]Provinciale Hogeschool Limburg, Diepenbeek, Belgium.
Reactive Oxygen Species (ROS) production and impairment of mitochondrial functioning is a central research topic. A recent review for Multiple Sclerosis[1]. Investigation of mitochondrial functioning in living cells under normal physiological conditions and metabolic inhibition provides biologically relevant information using confocal cell imaging[2,3] Cytosolic calcium clearance does not necessarily lead to mitochondrial dysfunction as long as the mitochondrial matrix pH stayed acidified compared to the cytosol.[2] Studying intra-mitochondrial versus cytosolic electrolyte homeostasis in living cells under metabolic stress might be crucial to understand the conditions where mitochondria become detrimental by producing ROS. Caveats are presented to properly obtain information about mitochondrial and cellular pH, structure and properties of the mitochondrial network under normal conditions and metabolic inhibition, by using optimized protocols[4]. The method critically depends on availability and selection of nuclear areas with lowest background fluorescence contribution while keeping experiment conditions rigorously similar. We present the influence of factors such as cell type, plating number, age of the cells, attachment surface properties, cell confluency, loading protocol of the dyes, background area selection on the values obtained. Optimization of parameters such as objective choice, detector voltage stability, fluorescence intensity saturation, illumination power and bleaching, imaging depth, point spread function (PSF) and reduction of cell motility are elucidated. Confluent Madin Darby Canine Kidney (MDCK) cells were loaded with mitotracker green and pH indicator SNARF. Images were collected with a Zeiss LSM 510 Meta CLSM. Data were analyzed with ImageJ[4], and Matlab.
1. Lassmann, H., and van Horssen, J., FEBS Lett. 2011
2. Smets, I. et al., Am. J. Physiol. Renal Physiol., 2004
3. Baron, Sz. et al., JASN , 2005
4. vandeVen, M, & Balut, C. et al. Methods Mol Biol. 2010

1736-Pos Board B628
Application of FTIR Imaging on Healthy (Donor Age Effect) and Disease (Beta Thalassemia Major) States
Ceren Aksoy[1], Duygu Uckan[2], **Feride Severcan**[3].
[1]Department of Biotechnology, Middle East Technical University, Ankara, Turkey, [2]Department of Pediatric Hematology, Bone Marrow Transplantation Unit, Hacettepe University, Ankara, Turkey, [3]Department of Biological Sciences, Middle East Technical University, Ankara, Turkey.
Recent researches have displayed the significant role of stem cells in tissue renewal and homeostasis with their unique capacity to develope different cell types. Mesenchymal stem cells (MSCs) which are non-hematopoietic, stromal cells found primarily in bone marrow (BM) in addition to many human tissues. Bone marrow mesenchymal stem cells (BM-MSCs) can differentiate into a variety of non-hematopoietic tissues and maintain healthy hematopoiesis by providing supportive cellular microenvironment in BM. Stem cell studies hold enormous potential for development of new therapies. Therefore; investigation of stem cells in normal developmental and physiological states as well as in pathological conditions may lead to understanding of disease pathogenesis and development of new cellular therapies. The present study focused on the investigation of donor age effect on healthy human BM-MSCs and the characterization of beta thalassemia major (β-TM) disease by using a novel, rapid and non-destructive technique, Fourier transform infrared microspectroscopy (FTIRM). Aging process of healthy BM-MSCs is significant because of their important role in tissue regeneration and repairment. Characterization of

β-TM-induced structural and functional variations in BM-MSCs important because it may provide basic understanding of hematopoetic stem cell (HSC)-MSC interactions in such a pathological bone marrow microenvironment. In this scope, firstly, BM-MSCs were characterized in terms of their morphological, immunophenotypical and differentiation properties. Then, variation in the macromolecular concentrations in between studied groups was obtained visually. The spectral results reflected that there were significant changes in the concentrations of lipids, proteins, glycogen and nucleic acids in children and adolescent group BM-MSCs when compared with the infants, early and mid adults. In β-TM disease study, the differences in chemical maps belonging to different macromolecules clearly indicated the succesfull differentiation of healthy control, pre- and post-transplant BM-MSCs by FTIRM.

1737-Pos Board B629

Characterization of Sodium Butyrate Induced Differentiation in Colon Cancer Cells by Fourier Transform Infrared Spectroscopy and Microscopy

Nihal Simsek Ozek, Aslı Sade, Sreeparna Banerjee, Feride Severcan.
Department of Biological Sciences, Middle East Technical University, Ankara, Turkey.

Colon cancer is a major cause of morbidity and mortality throughout the world.The pathogenesis of colon cancer with respect to loss of cellular differentiation has not been clearly understood. To understand this loss in colon cancer and to differentiate the cancer cells, sodium butyrate (NaB), one of the differentiation inducer, is commonly used. NaB is produced in the colonic lumen as a consequence of bacterial fermentation of complex carbohydrates and it alters colon cancer cell morphology and reduces the cell growth and motility. Although, the differentiation process of colon cancers, which are stimulated with NaB, has been resolved at genetic level, the underlying mechanisms regarding structural and functional alterations regulating the differentiation of colon cancer cells have not been well characterized. Therefore, in this study, it was aimed to explore the NaB-induced macromolecular structural and functional changes in differentiation of colon cancer cells by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and FTIR microspectroscopy. These changes were determined from the spectral analysis of control and NaB treated colon cancer cell spectra and their chemical maps. Based on the spectral differences, cluster analysis was applied to discriminate the groups. The spectral analysis indicated the differences in saturated and unsaturated lipids, protein and nucleic acid content between the control and NaB treated cancer cells. The variation in membrane fluidity and lipid order was also determined in the NaB treated cells. Moreover, the successful discrimination between control and treated cells was obtained. The results of this study not only shed light on better understanding the loss of differentiation mechanisms in colon cancers but also strongly support the power of ATR-FTIR spectroscopy and FTIR microscopy as novel, simple, reagent-free methods for the identification of differentiated cancer cells.

1738-Pos Board B630

Optical Method to Asses Ex-Vivo the Extent of Atherosclerosis in Mouse Aortas

Susana A. Sanchez[1], Nerea Méndez-Barbero[2], Antonio M. Santos-Beneit[1], Vanesa Esteban Vázquez[2], Luis J. Jiménez-Borreguero[3], Miguel R. Campanero[4], Juan M. Redondo[2].
[1]Microscopy and Dynamic Imaging Unit. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, [2]Department of Vascular Biology and Inflamation. CNIC, Madrid, Spain, [3]Department of Atherotrombosis and Imaging, CNIC and Hospital de la Princesa, Madrid, Spain, [4]Instituto de Investigaciones Biomedicas Alberto Sols, CSIC-UAM, Madrid, Spain.

The main cause of Coronary Artery disease, the first cause of death in most industrialized countries, is Atherosclerosis. Atherosclerosis is a chronic inflammatory disease in the walls of arteries, promoted by low density lipoproteins (LDL) without adequate removal of cholesterol. Over the years, the response of the body to the continuous buildup of cholesterol is the formation of a plaque (atheroma) that can obstruct blood flow.

Many therapeutic strategies focus on reducing the size of the atheroma and the preclinical studies require methods to measure lesion extension in an accurate, reliable manner. One of the most used methods in mice is called *en face.* This technique consists of dissecting the aorta, opening it longitudinally to expose the luminal side, and staining it with dyes to reveal lipid-laden plaques. Photographs of the labeled arteries are taken after the staining and the area occupied by the stained structures (atheroma) is determined by using image-processing software. The pitfall of this method is the lack of tri-dimensionality that may lead to misestimation damage extension.

Here we show a fast and relatively simple solution for the lack of tri-dimensionality of the *en face* method by tri-dimensionally imaging the dissected aortas with a femtosecond pulsed infrared (IR) laser. We used mice prone to develop Atherosclerosis (*Apoe*$^{-/-}$) having different diets and age and compared the extension of the atheromic damage using *en face* and our optical method. Our results show the advantages of using volume for assessing atherosclerotic damage quantification and also the potential of the method in the characterization of single atheroma and atherosclerotic damage in preclinical studies.

1739-Pos Board B631

Spatial Organization of RNA Polymerase II Revealed by Super-Resolution Imaging of Mammalian Cell Nucleus

Ziqing Zhao, Rahul Roy, J. Christof M. Gebhardt, David M. Suter, Alec R. Chapman, X. Sunney Xie.
Harvard University, Cambridge, MA, USA.

Super-resolution microscopy based on single-molecule centroid determination has been widely applied to biology in recent years. However, quantitative imaging of mammalian nucleus has been challenging due to the lack of 3D optical sectioning methods for normal-size cells as well as the inability to accurately count the absolute copy numbers of biomolecules in highly dense structures. Here we report a Reflected Light Sheet Super-Resolution Microscopy (RLS-SRM) that allows counting of protein and RNA molecules inside a mammalian nucleus with single-copy accuracy, at 25-nm lateral and 50-nm axial resolutions. Applying RLS-SRM to probe the organization of mammalian transcription by RNA polymerase II (RNAP II), we observed that RNAP II distribution exhibit a punctate pattern with discrete foci, consistent with previous studies. Spatio-temporal clustering analysis showed that the average number of RNAP II molecules in each of the foci is one, and no statistical evidence is found for clustering of RNAP II molecules within 250 nm from each other, arguing against the hypothesis of 'transcription factories'. Two-color imaging revealed that 20% of the bound RNAP II is poised while 80% is actively transcribing. Using single-molecule FISH, we also imaged the distribution of U2 spliceosomal snRNA inside the nucleus, ~25% of which is found to colocalize with transcribing RNAP II, suggestive of co-transcriptional splicing. This study provides a way to quantitatively image and count key biomolecular species inside the mammalian nucleus with unprecedented level of detail.

1740-Pos Board B632

Potential and Limitation of Microparticle-Based Immunoassays: A Thermodynamic and Kinetic Study

Susan Gayda, Qiaoqiao Ruan, Joseph P. Skinner, Sergey Y. Tetin.
Abbott Diagnositics, Abbott Park, IL, USA.

The fundamental significance of immunoassays in diagnostics and research drives the ongoing quest for improving assay formats, sensitivity and instrument technology. Early on, the advantage of immobilizing antibodies to a surface to allow for separation of bound and unbound reagent was realized in the immunometric (sandwich) assay format. Today, this approach is established in most immunoassays as performed using ELISA (enzyme linked immunosorbent assay), GCSPR (grating-couples surface plasmon resonance) and microparticle-based chemiluminescence. However, antibody immobilization to a surface hampers reaction kinetics and imposes mass transport limitation and sterical hindrance. Theoretical considerations indicate that these effects can be minimized by using a spherical surface underlining why recently developed ultra-high-sensitivity techniques rely on the utilization of microparticles. In the present study, we investigate the potential and limitation of microparticle-based immunoassays in respect to impact of mass transport and surface immobilization on the intrinsic thermodynamic and kinetic properties of antibody-analyte interaction.

1741-Pos Board B633

Vibrational Spectroscopy, Microscopy and Imaging Probes Cutaneous Wound Healing and Artificial Skin Structure

Guo Yu[1], Carol Flach[1], Marjana Tomic-Canic[2], Olivera Stojadinovic[2], Richard Mendelsohn[1].
[1]Rutgers University, Newark, NJ, USA, [2]University of Miami Miller School of Medicine, Miami, FL, USA.

Wound healing in human skin consists of a complex series of spatially and temporally organized events to prevent infection and restore barrier function. Multiple biological pathways are activated after an injury. We used a human skin model to study re-epithelialization of excisional wounds. Infrared (IR) microscopic imaging at ~ 10 micron spatial resolution revealed a population of disordered lipids in the migrating epithelial tongue (MET) which has not been previously reported. Particular spectral features allowed us to elucidate lipid structure/organization during the healing process (from day 0 to day 6 post-wounding). Although the role of lipids in the MET is currently unclear, this finding may ultimately aid in the development of improved therapeutic agents for wound care.