BP 23: Bioimaging and Biopspectroscopy II

Time: Wednesday 15:00–17:15 Location: H 2013

BP 23.1 Wed 15:00 H 2013

Label-free, real-time monitoring of cytosolic composition and dynamics using digital holographic microscopy — •Daniel Midtvedt, Erik Olsén, Gavin Jeffries, and Fredrik Höök — Chalmers University of Technology

Cells continuously adapt their biophysical properties during their Life cycle as well as in response to changes in the environment. However, quantifying these biophysical changes on single cell level has only recently become possible. In this work we use a digital holographic microscope in combination with a millifluidic chip to study the response of microorganisms and mammalian cells to external stimuli. We demonstrate a label-free quantification of changes in both intracellular osmolarity and macromolecular concentration, on single cell level, in response to a change in medium osmolarity. This platform allows to disentangle cellular water fluxes from production and accumulation of higher molecular weight compounds, which suggests an applicability of this platform in studying a broad range of cellular processes.

BP 23.2 Wed 15:15 H 2013

Structure and Dynamics of the Trypanosoma brucei Plasma Membrane — Marius Glogger, •Marie Schwebs, Markus Engstler, and Susanne Fenz — Universität Würzburg, Biocenter: Cell and Developmental Biology, Würzburg, Germany

African trypanosomes are the causative agents of sleeping sickness in human and Nagana in livestock. In the bloodstream of their host, they exhibit a dense coat of variant surface glycoproteins (VSG). Fluidity of the VSG coat is a fundamental for parasite survival. However, the diffusion behavior of the VSGs is also limited by the physical properties of their lipid matrix. We have recently introduced super-resolution imaging of intrinsically fast moving flagellates based on cyto-compatible hydrogel embedding [Glogger et al. JPD: Appl Phys 17]. Building on this work we employ leaflet-specific membrane probes and single-molecule fluorescence microscopy to elucidate the structure and dynamics of the plasma membrane and VSG coat in living trypanosomes. Using expressed lipid-anchored eYFP as a probe for the inner membrane leaflet, we found specific domains where the probe accumulates or appears diluted rather than being homogenously distributed. We hypothesize that this structuring of the membrane is associated with the underlying microtubule cytoskeleton. The next steps include employment of a more stable fluorescent label to resolve dynamic interaction of single probes with the observed domains. Moreover, we aim to track fluorescently labeled lipids in the outer leaflet to gain insight in inter-leaflet coupling in vivo, and we plan a two-color experiment to simultaneously investigate membrane and VSG dynamics.

BP 23.3 Wed 15:30 H 2013

Enhanced fluorescence resonance energy transfer in G protein-coupled receptor probes by nano-coated microscopy coverslips — •Benjamin Schreiber[1], Michael Kauk[1,2,4], Hannah S Heil[1], Martin Kamp[3], Sven Höfling[3], Carsten Hoffmann[1,2,4], and Katrin G Heinze[1] — [1]Rudolf Virchow Zentrum, Universität Würzburg — [2]Institut für Pharmakologie und Toxikologie, Universität Würzburg — [3]Technische Physik, Universität Würzburg — [4]Institut für Molekulare Zellbiologie, Universität Jena

For probing biomolecular interactions in a live-cell setting the distance depending Fluorescence resonance energy transfer (FRET) is often the method of choice. G-protein-coupled receptors mediates cellular responses and communication across cellular membranes, and is the largest known class of molecular targets with proven therapeutic value. The design of FRET probes is crucial to ensure unhampered functionality and binding kinetics of the molecular complex. Thus, such FRET probes usually require labeling compromises with limited FRET efficiencies. Here, we present an approach to optimize the energy transfer without changing the design of the FRET probe. We show that gold coated glass cover slips allow reinforcing the otherwise forbidden donor-acceptor energy transfer by virtual optimization of the dipole orientation. We show resulting enhanced FRET on our nano-coatings for the ligand driven activation of M1 muscarinic acetylcholine receptors labeled with a CFP-FlAsH pair. We believe that our techniques has particular potential for pharmaceutical drug screening.

BP 23.4 Wed 15:45 H 2013

Characterisation of Metabolic Dynamics by Fluorescence Lifetime Imaging Microscopy of NAD(P)H — •André Weber[1], Yury Prokazov[1], Marcus Hauser[2], and Werner Zuschratter[1] — [1]Leibniz-Institut für Neurobiologie Magdeburg, Germany — [2]Institut für Biometrie und Medizinische Informatik, Otto-von-Guericke- Universität Magdeburg, Germany

The energy metabolism of eucaryotic cells can show complex dynamics, i.e. glycolytic oscillations. Monitoring this intrinsic behaviour by fluorescence microscopy is influence the metabolism, which is sensitive to excitation light intensities, especially in UV range.

We show a low light imaging approach using a single photon counting position sensitive detector working with laser intensities below 3mW/cm^2 and a time resolution below 90ps. For excitation of intracellular NAD(P)H a 8 MHz pulsed frequency-tripled Nd:vanadate laser tuned at 355 nm was used. The analysis of the complex fluorescence decay of NAD(P)H in intact yeast cells revealed 4 molecular species with characteristic fluorescence lifetimes showing individual behaviour and glycolytic oscillations as response to glucose addition. Laser intensities higher 3mW/cm^2 did not lead to long lasting glycolytic oscillations.

BP 23.5 Wed 16:00 H 2013

Cardiac Cells and Heart Tissue Studied by X-ray Imaging and Scanning X-ray Diffraction — •Jan-David Nicolas[1], Marten Bernhardt[1], Susanne Schlick[2], Malte Tiburcy[2], Wolfram-Hubertus Zimmermann[2], Amara Khan[3], Frauke Alves[3], Karl Toischer[4], and Tim Salditt[1] — [1]Institut für Röntgenphysik, Friedrich-Hund-Platz 1, 37077 Göttingen — [2]Institut für Pharmakologie und Toxikologie, Robert-Koch-Str. 40, 37075 Göttingen — [3]Max-Planck-Institut für Experimentelle Medizin, Hermann-Rein-Straße 3, 37075 Göttingen — [4]Klinik für Kardiologie und Pneumologie, Institut für Pharmakologie und Toxologie, Robert-Koch-Str. 40, 37075 Göttingen

We have applied scanning x-ray diffraction on cardiac cells and tissue to characterize the cytoskeletal architecture and to localize potential structural defects due to disease. The method typically involves raster-scanning of a microfocused x-ray beam and collecting scattering patterns at each scan point. The collected data can then be analyzed in view of structural parameters such as the interfilament spacing or orientation of the myosin and actin filaments that are in almost crystalline arrangement in the sarcomere. We will show what signals can be extracted from cells and tissue and how nanoscale architecture develops with differentiation. Since many cardiomyopathies rely on the structural integrity of the sarcomere, the contractile unit of cardiac muscle cells, the present study can be easily extended to characterize cells and tissue from a diseased heart.

Invited Talk BP 23.6 Wed 16:15 H 2013

Illuminating physical cues for the early embryogenesis of a simple model organism — •Matthias Weiss — Experimental Physics I, University of Bayreuth, Germany

Embryogenesis is a remarkably robust, but still poorly understood self-organization phenomenon that is governed by a variety of biochemical and physical cues. Due to its simplicity, the small roundworm Caenorhabditis elegans is a superb model organism to study the role of physics during early developmental stages. Using single plane illumination microscopy (SPIM), we have explored how physical cues determine the cell arrangement during the early embryogenesis of C. elegans. In particular, we have studied the coupling of cellular volumes and cell cycle times, the nature of asymmetric cell divisions, and the mechanically driven cell arrangement process [1]. Going beyond mere imaging, we also have used pixel-wise fluorescence correlation spectroscopy (SPIM-FCS) to spatiotemporally quantify the diffusion of proteins in individual cells of the embryo in cytoplasm and on membranes [2]. As a result, we were able to monitor the emergence of intracellular diffusion and concentration gradients prior to the first cell division, which define the anterior- posterior body axis already in the single-cell state.

[1] Biophys. J. 105, 1805 (2013); Phys. Rev. Lett. 117, 188101 (2016); Sci. Rep. 7, 9369 (2017).

[2] J. Phys. D 49, 044002 (2016).

BP 23.7 Wed 16:45 H 2013

Non-equilibrium forces drive the anomalous diffusion of vital cell constituents — •Lorenz Stadler, Konstantin Speckner, and Matthias Weiss — Experimentalphysik 1, Universität Bayreuth, Universitätsstr. 30, 95447 Bayreuth

Diffusion is the basic mode of motion for supra-molecular assemblies in living cells, often with an anomalous scaling of the mean square displacement, with $ text $. Considerable effort has been invested to uncover the underlying types of subdiffusive processes, yet often with the tacit assumption that the involved random forces are mostly of thermal origin. Contrary to this simple assumption, our single-particle tracking data on the subdiffusive motion of telomeres in the nucleus of mammalian cells reveals an important role of cytoskeleton-associated non-equilibrium forces [1]. In line with this finding, we also have found strong non-equilibrium contributions in the trajectories of membrane domains in the cells' Endoplasmic Reticulum (so-called 'ER exit sites'). Due to the complex topology of the ER, exit sites not only are shaken by the cytoskeleton-driven motion of the entire ER network but they also show an (activated) subordinated motion on individual ER tubules. Altogether, our data show that even subdiffusive motion patterns in cells may not represent simple thermal transport process but rather are non-equilibrium events.

[1] Stadler & Weiss, New J. Phys. (in press) 2017

BP 23.8 Wed 17:00 H 2013

Investigating Transient Peptide-Membrane Interactions with TIR-FCS — •Philipp Blumhardt[1,3], Jonas Mücksch[1,3], Henri G. Franquelim[1], Maximilian T. Strauss[1,2], Philipp Glock[1], Johannes Stein[1], Ralf Jungmann[1,2], and Petra Schwille[1] — [1]Max Planck Institute for Biochemistry, Martinsried, Germany — [2]Ludwig-Maximilians-Universität, Munich, Germany — [3]authors contributed equally

The accurate determination of binding rates to membranes or membrane-bound proteins is of key relevance for quantitative biology. Despite the existence of multiple methods to characterize surface interactions, there are still many experimental challenges regarding simplicity of use and general applicability. We developed a simple and versatile single-molecule fluorescence approach for the accurate determination of binding rates to surfaces or surface-bound receptors. Our approach combines Fluorescence Correlation Spectroscopy (FCS) with Total Internal Reflection (TIR) Fluorescence microscopy and a camera-based detection. This combination not only yields a high surface selectivity, but also resolves association and dissociation rates over a wide time range. Previously, we quantified the transient hybridization of single-stranded DNA to the complementary handles of immobilized DNA origamis. We varied the nucleotide overlap, yielding different transient binding times in the range of milliseconds to tens of seconds. Here, we present our latest results on the transfer of this assay to the otherwise challenging quantification of transient interactions between protein segments and lipid bilayers.