BP 2: Bioimaging and Spectroscopy I

Time: Monday 9:30–12:45 Location: HÜL 386

Invited Talk BP 2.1 Mon 9:30 HÜL 386

Visualization and Manipulation of the Invisible — •Heinrich Leonhardt — Ludwig Maximilians University Munich, Biocenter, Martinsried, Germany

Fluorescence light microscopy allows multicolor visualization of cellular components with high specificity, but its utility has until recently been constrained by the intrinsic limit of spatial resolution and the lack of specific detection tools. To circumvent these limitations, we applied three-dimensional structured illumination microscopy (3D-SIM, Science, 320, 1332-6) and high-throughput STED microscopy in combination with automated image analysis. For detection of cellular structures, we have generated fluorescent, antigen-binding proteins, termed chromobodies, by combining epitope-recognizing fragments with fluorescent proteins (Nature Methods, 3, 887-9). These chromobodies can be expressed in living cells and used to target or trace epitopes in subcellular compartments providing an optical readout for novel high content analyses and functional studies (Nature Struct. Mol. Biol., 17, 133-139). These antigen-binding fragments can also be recombinantly produced, chemically functionalized and directly used for super-resolution microscopy (Science, 331, 1616-20). To study the dynamics of genome organization we have repurposed prokaryotic DNA binding proteins (TALEs and CRISPR/Cas) for the detection of specific DNA sequences in living cells (NAR 42, e38 and Nucleus, 5, 163-172). This combination of detection tools and microscopy techniques provides new insights into the structure and function of mammalian cells.

BP 2.2 Mon 10:00 HÜL 386

(3+1)D SIM + quantitative analysis for ophthalmologic research — •Florian Schock[1,2,3], Gerrit Best[1,3], Nil Celik[4], Yangyi Wang[2,3], Alena Bakulina[5], Saadettin Sel[4], Udo Birk[1,3], Rainer Heintzmann[6,7], Jürgen Hesser[5], Stefan Dithmar[4,8], and Christoph Cremer[1,2,3] — [1]Institute of Molecular Biology, University of Mainz — [2]Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg — [3]Kirchhoff Institute for Physics, University of Heidelberg — [4]Department of Ophthalmology, University-Hospital Heidelberg — [5]Experimenatal Radiation Oncology, University Medical Center Mannheim, University of Heidelberg — [6]Institute of Physical Chemistry and Abbe Center of Photonics, University of Jena — [7]Leibniz Institute of Photonic Technology — [8]Department of Ophthalmology, HELIOS HSK, Wiesbaden, Germany

While super-resolution-microscopy has become widely available, its application in clinical context is still mostly restricted to cultivated cells. We present the application of (3+1)D(3 excitation/emission spectra) Structured Illumination Microscopy to clinical research on extracted tissues as well as quantitative analysis of over 300 human RPE cells and their granules (intracellular particles) regarding connections to age related macular degeneration. Autofluorescencence behaviour is a characteristic hallmark for several chorioretinal conditions, so SIM offers potential for further clinical imaging at illumination powers that allow application to living patients (ongoing clinical study).

BP 2.3 Mon 10:15 HÜL 386

Live-cell super-resolution imaging of intrinsically fast moving flagellates — Marius Glogger[1], Simone Stichler[2], Ines Subota[1], Sarah Bertlein[2], Marie-Christin Spindler[1], Jörg Tessmar[2], Jürgen Groll[2], Markus Engstler[1], and •Susanne Fenz[1] — [1]Biocenter: Cell and Developmental Biology, University of Würzburg, Würzburg, Germany — [2]Department of Functional Materials in Medicine and Dentistry, University of Würzburg, Würzburg, Germany

Recent developments in super-resolution microscopy make it possible to resolve structures in biological cells at a spatial resolution of a few nm. However, the optimal structural resolution requires repeated illumination cycles and is thus limited to chemically fixed cells. For live cell applications substantial improvement over classical Abbe-limited imaging can already be obtained in adherent or slow moving cells. Nonetheless, a large group of cells are fast moving and thus could not yet be addressed with live cell super-resolution microscopy. These include flagellate pathogens like African trypanosomes. Here, we present an embedding method based on an in situ forming cytocompatible UV-crosslinked hydrogel. The fast cross-linking hydrogel immobilizes trypanosomes efficiently to allow microscopy on the nanoscale. We characterized both the trypanosomes and the hydrogel with respect to their autofluorescence properties and found them suitable for single-molecule fluorescence microscopy (SMFM). As a proof of principle, SMFM was applied to super-resolve a structure inside the living trypanosome. We present an image of a flagellar axoneme component.

BP 2.4 Mon 10:30 HÜL 386

Coordinate-targeted fluorescence nanoscopy with multiple off-states — •Johann Georg Danzl[1,2], Sven Sidenstein[2], Carola Gregor[2], Nicolai Urban[2], Peter Ilgen[2], Stefan Jakobs[2], and Stefan Hell[2] — [1]Institute of Science and Technology Austria, 3400 Klosterneuburg, Austria — [2]Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany

Far-field optical nanoscopy techniques "super-resolve" features residing closer than the diffraction-limit by transiently preparing fluorophores in distinguishable (typically on- and off-) states and reading them out sequentially. In coordinate-targeted superresolution modalities, such as stimulated emission depletion (STED) microscopy, this state difference is created by patterns of light, driving for instance all molecules to the off-state except for those residing at intensity minima. For high resolution, strong spatial confinement of the on-state is required. However, this also subjects fluorophores at intensity maxima to excess light intensities and state cycling. In addition, as spatial confinement of the on-state is increased, state contrast between designated on- and off-regions has to be improved, too. We show that driving fluorophores to a second off-state enables protection of fluorophores and superior state contrast. In a realization that we dubbed "protected STED", we used reversibly switchable fluorescent proteins as labels and employed both STED and reversible photoswitching as off-transitions. This directly translated into reduced bleaching and enhanced resolution in live-cell nanoscopy (J. G. Danzl, S. C. Sidenstein et al., Nature Photonics 10, 122 (2016)).

BP 2.5 Mon 10:45 HÜL 386

Exploring protein diffusion landscapes in living embryos with SPIM-FCS — •Philipp Struntz, Dirk Hofmann, and Matthias Weiss — University of Bayreuth, Experimental Physics I, Germany

Macromolecule diffusion in the complex and dynamic environment of living organisms often features spatial variations that report on the cells' secret life, e.g. during embryogenesis. To explore these spatial heterogeneities one needs to quantify the local diffusion characteristics in extended regions of the sample in a multiplexed fashion. To obtain diffusion maps with high spatiotemporal resolution we have combined single plane illumination microscopy (SPIM) and fluorescence correlation spectroscopy (FCS). By refining a custom-made SPIM setup that was originally designed for long-term in-vivo imaging of early embryos of the small nematode Caenorhabditis elegans [1], we were able to acquire pixel-wise FCS curves on spatially extended regions within the embryo. We demonstrate the capabilities of SPIM-FCS by determining the diffusion maps of the peripheral membrane protein PLC1δ1 in the cytoplasm and on the plasma membrane during early stages of embryogenesis [2]. In a next step, we have focused on time-resolved diffusion maps of the protein PIE-1, for which we see the formation of a mobility gradient along the anterior-posterior axis before the first, asymmetric cell division. Our data hence show that SPIM-FCS can be used to explore intracellular transport phenomena even in fragile developmental model organisms.

[1] R. Fickentscher, P. Struntz & M. Weiss, PRL 117, (2016).
[2] P. Struntz & M. Weiss, J. Phys. D 49, 044002 (2016).

30 min break

BP 2.6 Mon 11:30 HÜL 386

Disentangling the effects of viscosity and refractive index mismatch in single-focus FCS — •Jonas Mücksch, Petra Schwille, and Eugene P. Petrov — Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

Fluorescence Correlation Spectroscopy (FCS) is a powerful tool to assess molecular mobilities in various settings ranging from bulk solutions to model lipid membranes, to living cells. The media in which FCS measurements are performed, frequently differ from water with respect to their viscosity and their refractive index. On the other hand, it has

been shown that the refractive index mismatch can severely affect the results of FCS measurements [1]. Here, we explore under which conditions it is possible to disentangle the effects of the solution viscosity and refractive index mismatch on FCS measurements carried out using a standard confocal microscope-based setup and thus employ this experimental technique for reliable determination of diffusion coefficients in various media.

[1] J. Enderlein, I. Gregor, D. Patra, T. Dertinger, and U. B. Kaupp, ChemPhysChem **6**, 2324 (2005).

BP 2.7 Mon 11:45 HÜL 386

Investigating DNA binding kinetics by camera-based total internal reflection fluorescence correlation spectroscopy (TIR-FCS) — Jonas Mücksch[1,3], •Philipp Blumhardt[1,3], Maximilian Strauss[1,2], Ralf Jungmann[1,2], and Petra Schwille[1] — [1]Max Planck Institute of Biochemistry, Martinsried, Germany — [2]Ludwig Maximilian University, Munich, Germany — [3]equal contribution

Fluorescence correlation spectroscopy (FCS) has been extensively used to study the kinetics of various *in vitro* and *in vivo* systems on a molecular level. The vast majority of FCS studies is performed using confocal setups, which feature well-defined detection volumes but suffer from low surface selectivity. Combining FCS with total internal reflection fluorescence (TIRF) illumination drastically enhances the spatial selectivity and enables the investigation of reversible binding of fluorescently labeled ligands to surface-confined receptors. So far, this potential to observe and quantify surface binding using TIR-FCS has been used only to minor extent. Here, we present a versatile optical setup for exploring surface-binding kinetics with TIRF illumination and point- (APD) or camera-based (EMCCD) fluorescence detection. In a first application, our camera-based assay facilitated the investigation of the transient hybridization of fluorescently labeled single-stranded DNA to the complementary handles of a surface-immobilized DNA origami scaffold. We varied the nucleotide overlap, yielding different binding times in the range of milliseconds to seconds. Using this highly tunable system, we systematically explored the parameter space accessible to EMCCD-based TIR-FCS.

BP 2.8 Mon 12:00 HÜL 386

A scanning ion conductance microscope (SICM) for large-range imaging — •Nicolas Schierbaum, Martin Hack, and Tilman E. Schäffer — Institute of Applied Physics, University of Tübingen, Auf der Morgenstelle 10, 72076 Tübingen, Germany

The scanning ion conductance microscope (SICM) is a high-resolution imaging technique using an electrolyte-filled nanopipette as probe, allowing non-invasive, contact-free topography imaging of soft and fragile biological samples in physiological conditions. The scan range of previously described SICM setups is limited to 200 μm in lateral and 25 μm in vertical direction. We present a SICM setup with a maximum scan range of 25 mm × 25 mm in lateral and 0.25 mm in vertical direction. The SICM is combined with an optical phase contrast microscope and is equipped with a heated sample stage for live cell imaging. We demonstrate the high versatility of the setup by imaging samples at different length scales: from macroscopic structures such as fingerprints or cell layers to microscopic structures such as small cell protrusions. The large scan range facilitates selecting a region of interest with subsequent high-resolution imaging. We applied the setup to the study of wound healing by time-lapse imaging a live epithelial cell monolayer over 20 hours, demonstrating the long-term imaging stability of the setup.

BP 2.9 Mon 12:15 HÜL 386

Mapping surface charge density of lipid bilayers by quantitative surface conductivity microscopy — Lasse H. Klausen[1], •Thomas Fuhs[1,2], and Mingdong Dong[1] — [1]Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark — [2]School of Chemical Engineering and Technology, Harbin Institute of Technology, Harbin, PR China

Local surface charge density of lipid membranes influences membrane-protein interactions leading to distinct functions in all living cells, and it is a vital parameter in understanding membrane-binding mechanisms, liposome design and drug delivery. Despite the significance, no method has so far been capable of mapping surface charge densities under physiologically relevant conditions. Here, we use a scanning nanopipette setup (scanning ion-conductance microscope) combined with a novel algorithm to investigate the surface conductivity near supported lipid bilayers, and we present a new approach, quantitative surface conductivity microscopy (QSCM), capable of mapping surface charge density with high-quantitative precision and nanoscale resolution. The method is validated through an extensive theoretical analysis of the ionic current at the nanopipette tip, and we demonstrate the capacity of QSCM by mapping the surface charge density of model cationic, anionic and zwitterionic lipids with results accurately matching theoretical values.

BP 2.10 Mon 12:30 HÜL 386

High Resolution Imaging of Cellular Signaling Using Fluorescent Nanosensor Arrays — •Sebastian Kruss — Institut für Physikalische Chemie, Universität Göttingen

Intercellular communication via chemical signaling proceeds with both spatial and temporal components, but analytical tools, such as microfabricated electrodes, have been limited to just a few probes per cell. In this work, we use a non-photobleaching fluorescent nanosensor array based on single walled carbon nanotubes (SWCNT) rendered selective to the neurotransmitter dopamine to study its release from neuroprogenitor cells at a resolution exceeding 20,000 sensors/cell. This allows the spatial and temporal dynamics of dopamine release, following stimulation, to be measured at exceedingly high spatiotemporal resolution. We observe localized, unlabeled release sites of dopamine spanning 100 ms to seconds that correlate with negative membrane curvature, and not predominately the positive curvature associated with the tips of cellular protrusions as intuitively expected. The results illustrate how directionality of chemical signaling is shaped by membrane morphology, and highlight the advantages of nanosensor arrays that can provide high spatial and temporal resolution of chemical signaling.