BP 5: Focus Session: Super Resolution Microscopy and Dynamics of Supramolecular Complexes

organized by Jonas Ries (EMBL Heidelberg) and Ulrich Schwarz (Heidelberg University)

Time: Monday 15:00–17:30 Location: H15

Invited Talk BP 5.1 Mon 15:00 H15

The functional nano-architecture of axonal actin — •Christophe Leterrier — Aix Marseille Université, CNRS, INP UMR7051, NeuroCyto, Marseille, France

The intricate arborization and molecular identity of axons is maintained for decades, but must also continuously adapt to changes in the environment and modulate the activity of neurons. Axons fulfill these paradoxical demands thanks to a unique cytoskeletal organization that ensures the coordinated transport, anchoring and assembly of axonal components. In our lab, we use super-resolution microscopy to delineate and map the nanoscale architecture of actin-based structures within the axon: the periodic actin/spectrin submembrane scaffold, intra-axonal hotspots and trails, and presynaptic actin assemblies. We are exploring their molecular organization and functions by combining versatile labeling approaches, correlative live-cell/super-resolution/electron microscopy and quantitative analysis that allow for high-content, nanoscale interrogation of the axonal architecture.

BP 5.2 Mon 15:30 H15

Photon-stream-based aberration correction for STED microscopy — •Debadrita Ghosh, Claudia Geisler, and Alexander Egner — Institute for Nanophotonics, Goettingen, Germany

Stimulated emission depletion (STED) microscopy is the most prominent super-resolution fluorescence microscopy method and achieves a resolution far beyond the diffraction limit. However, like all these methods, it is adversely affected by sample-induced aberrations, which can degrade the achievable resolution and image quality significantly. These aberrations are caused by wavefront distortions due to refractive index variations, for example within thick biological specimens. This challenge can be addressed by using adaptive optics (AO) in a feedback-controlled manner such that the wavefront distortions are compensated and the image quality is restored. Typically, the feedback loop uses image features which necessitates repeated acquisitions of the same field-of-view. This approach, therefore, is slow and prone to unwanted photo-bleaching. Here, we present an AO correction scheme that does not rely on image features, but exploits the dependence of the fluorescence lifetime on the local STED intensity. In principle, our photon-stream-based metric can be evaluated on a single image pixel, which makes it photon-budget-friendly and allows to correct aberrations rapidly in parallel with image acquisition. We successfully utilized this new metric for automated and continuous aberration correction in biological samples, making imaging fast and easy even for users without expert knowledge.

BP 5.3 Mon 15:45 H15

Cytoskeletal organization of red blood cells during malaria infections investigated with super-resolution microscopy and pair cross-correlation analysis — •Pintu Patra[1], Cecilia P Sanchez[2], Michael Lanzer[2], and Ulrich S Schwarz[1] — [1]Institute for Theoretical Physics & BioQuant, Heidelberg University, Germany — [2]Center of Infectious Diseases, Parasitology, University Hospital Heidelberg, Germany

Measuring the distance between molecules is key to understanding the molecular organization of biological systems. The pair cross-correlation (PCC) function computed from two-color super-resolution microscopy images provides a measure of co-localization between differently labeled molecules. Here, we theoretically compute the PCC-function between two molecules by using 2D Gaussian distribution as the effective point spread function for single molecules. By fitting this function to simulated data based on experimentally measured images, one can estimate both small and large separation distances. We apply this method to malaria-infected red blood cells and demonstrate that the knob-associated histidine-rich protein (KAHRP), which is used by the parasite to remodel the spectrin-actin network from a distance, relocalizes from the ankyrin bridges to the actin-based junctional complexes during the 48 hours course of the infection.

15 min. break

BP 5.4 Mon 16:15 H15

Superresolution microscopy for structural cell biology — •Jonas Ries — EMBL Heidelberg

Superresolution microscopy, such as single-molecule localization microscopy (SMLM), is becoming a key technique for structural cell biology, ideally complementing electron microscopy. I will discuss projects in my group in which we push SMLM towards nanometer resolution in 3D and multicolor with the aim to investigate the structure and dynamics of molecular machines in cells. I will show how these technologies allowed us to gain mechanistic insights into the machinery that drives endocytosis. Endocytosis is an essential cellular function by which cells take up molecules from the environment. We were able to reconstruct the dynamics of this process from thousands of snapshots taken in fixed cells. I will conclude with first results illustrating the potential of MINFLUX to image dynamic structural changes of protein machines in the living cell with nanometer resolution. Specifically, I will show how we resolved the precise stepping motion of the motor protein kinesin in living cells.

BP 5.5 Mon 16:45 H15

Spatiotemporal SARS-CoV-2 binding dynamics investigated with 100 Hz ROCS microscopy and thermal fluctuation analysis — •Dominik Huber and Alexander Rohrbach — Laboratory for Bio- and Nanophotonics, Department of Microsystems Engineering - IMTEK, University of Freiburg, 79110 Freiburg, Germany

The emergence of the new severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) in recent years has caused tremendous interest in investigating the interactions between viruses and cells. Especially the imaging and tracking of viruses are of great importance to see and understand the binding and uptake of viruses into cells and to be able to intervene in these processes. Due to their small size and fast movement imaging virus dynamics is a very challenging task, which has so far been achieved with different fluorescence methods, being limited by photo bleaching and imaging speed.

In our research we apply Rotating Coherent Scattering (ROCS) microscopy in order to visualize the diffusion of SARS-CoV-2 virus like particles (VLPs) close to A549 lung epithelial cells. ROCS microscopy allows for label-free imaging with more than 100 Hz temporal at 150 nm spatial resolution. The high contrast image stacks enable single particle tracking and characterization of the binding process and strength of VLPs with SARS-CoV-2 spike proteins to A549 cells using thermal fluctuation analysis.

BP 5.6 Mon 17:00 H15

Transient Optoplasmonic Detection of Single Proteins on the Nanosecond Time Scale — •Martin D. Baaske[1,2], Nasrin Asgari[1], Deep Punj[1], and Michel Orrit[1] — [1]Leiden University, Leiden, Netherlands — [2]Johannes Gutenberg-University, Mainz, Germany

Label-free optical detection schemes commonly rely on specific chemical interactions between receptor and target molecule in order to facilitate analyte recognition. Here I present our first steps on a novel pathway to fingerprint proteins via analysis of their motion, i.e., physical properties such as stokes radius and polarizability rather than chemical interactions. We show that via a polarization selective technique and careful optimization of a confocal microscope single gold nanorods, which are commonly used as labels, can be transformed into high-speed nanoscale sensors. We perform photothermal spectroscopy on single gold nanorods and use it as a means to probe their sensitivity to refractive index changes with respect to the experimental parameters * in turn allowing us to optimize the later on a rod-to-rod basis. This enables the detection single protein molecules traversing plasmonic near fields with previously time resolutions on the nanosecond scale.

BP 5.7 Mon 17:15 H15

Modeling the assembly and invagination of clathrin lattices at the cell membrane — •Felix Frey[1,2] and Ulrich S. Schwarz[3] — [1]Department of Bionanoscience, Delft University of Technology,

Delft, the Netherlands — [2]Institute of Science and Technology Austria, Klosterneuburg, Austria — [3]Institute for Theoretical Physics and BioQuant-Center, Heidelberg University, Heidelberg, Germany

Biological cells constantly relay material and information across their plasma membranes. For particles with sizes between 50 and 200 nm, clathrin-mediated endocytosis (CME) is the main uptake route. In CME clathrin triskelia assemble at the cell membrane and form a clathrin lattice. After initially growing flat the lattice starts to curve before it reaches its final size [1]. However, how this flat-to-curved transition proceeds in detail is still elusive, because theoretically several pathways can be envisioned [2]. When confronted with conventional imaging data, a microscopic model for the growth of clathrin lattices indeed suggests some level of plasticity as required for bending [3]. Recently we have combined mathematical modeling with 3D superresolution microscopy to determine the dynamics of membrane invagination. We find that membrane curvature is generated cooperatively between the triskelia of the clathrin lattice [4].

[1] D. Bucher, F. Frey et al., Nat. Commun. 9, 1109 (2018). [2] F. Frey and U.S. Schwarz, Soft Matter 16, 10723 (2020). [3] F. Frey et al., New J. of Phys. 22, 073043 (2020). [4] M. Mund et al., bioRxiv, doi:10.1101/2021.10.12.463947 (2022).