BP 21: Bioimaging

Time: Thursday 9:30–13:00 Location: BAR Schö

BP 21.1 Thu 9:30 BAR Schö

Imaging DNA-Origami with Low Energy Electron Holography — •Moritz Edte[1], Hannah Ochner[1], Luigi Malavolti[1], and Klaus Kern[1,2] — [1]Max-Planck-Institute for Solid State Research, Stuttgart, Germany — [2]École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Our low-energy electron holography (LEEH) approach makes use of a coherent low-energy electron wave (energies in the range of 50 to 150 eV) for imaging biomolecules with high contrast [1]. Hence, the method allows true single-molecule imaging of large three-dimensional molecules and can map conformational variability of flexible molecules [2]. I will show LEEH measurements of individual DNA-Origami molecules on single layer graphene, demonstrating that LEEH is capable of imaging this class of molecules without introducing perceptible structural changes on the time scale of the measurements and on the spatial scale defined by our current resolution limit.

[1] J.-N. Longchamp, et al., PNAS, 2017, 114, 1474-1479. [2] H. Ochner, et al., PNAS, 2021, 118, e2112651118

BP 21.2 Thu 9:45 BAR Schö

Spectral and nanolocal discrimination of crosslinked from single fibrillar actin using mid-IR photoinduced force microscopy (PiF-IR) — Jesvin Joseph[1,2], Dijo Moonnukandathil Joseph[1,2], Lukas Spantzel[2,3], Katharina Reglinski[1,2], Christoph Krafft[1,2], Christian Eggeling[1,2], Rainer Heintzmann[1,2], Michael Börsch[2,3], and •Daniela Täuber[1,2] — [1]Leibniz Institute of Photonic Technology, Jena — [2]Friedrich Schiller University Jena — [3]Jena University Hospital, Jena, Germany

Fibrillar actin is one of the major structural components in cells. Consequently, pathogenic alterations in cell functionality may be revealed by monitoring the re-arrangement of F-actin. However, discriminating protein aggregation in the range below 10 nm is challenging even by high resolution fluorescence microscopy. This gap can be addressed by recently developed mid-IR photo-induced force microscopy (PiF-IR). PiF-IR spectra obtained from fibrillar and monomeric actin match the corresponding FTIR spectra. The high spectral resolution of PiF-IR provides simplified access to IR spectroscopic signatures from secondary protein structure. The intensity of bands at 1655 cm-1 and 1685 cm-1 associated to α-helices and intermolecular β-sheets, respectively, varied within the scan image. Furthermore, PiF-IR hyperspectra obtained form single fibrillar actin appear more homogeneous than those from cross-linked F-actin. These first results are very promising for using PiF-IR to discriminate F-actin structures to study pathogenic alterations in cells and tissue ex vivo.

BP 21.3 Thu 10:00 BAR Schö

Cryo-EM samples of gas-phase purified protein assemblies using native electrospray ion-beam deposition (ES-IBD) — •Tim Esser[1], Lukas Eriksson[2], Paul Fremdling[2], and Stephan Rauschenbach[2] — [1]Thermo Fisher Scientific, 1 Boundary Park, Hemel Hempstead, HP2 7GE, UK — [2]Department of Chemistry, University of Oxford, Oxford OX1 3TF, UK

Combining native mass spectrometry (MS) with cryo electron microscopy (cryo-EM) allows to correlate information on homogeneity, stoichiometry, shape, and interactions of native protein complexes, complementary to high-resolution protein structures. Cryo-EM samples are conventionally made by coating TEM grids with a protein-containing solution, blotting, and plunge freezing in liquid ethane, quenching proteins in their native state, embedded in ultra-thin films of vitreous ice. Reliable sample preparation remains a major challenge, in particular for heterogeneous samples. Here we demonstrate mass-selective cryo-EM sample preparation via native electrospray ion-beam deposition (ES-IBD), as a direct link between native MS and cryo-EM. Protein complexes are brought into the gas phase, mass-selected, and deposited on TEM grids with thin carbon films at defined temperature. By controlling interactions in solution, gas-phase, and on the surface, we probe protein conformations between native solution-phase and native-like gas-phase structures. We show sub-nanometer EM density maps obtained using native ES-IBD and discuss its potential to extend the scope of cryo-EM in structural biology.

BP 21.4 Thu 10:15 BAR Schö

CRISPR activation screen to improve the optical properties of living tissues — •Susan Wagner, Venkat Krishnaswamy, Kaushikaram Subramanian, Heike Petzold, Benjamin Seelbinder, Rico Barsacchi, and Moritz Kreysing — Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Today, optical microscopes deliver unprecedented resolution allowing discoveries down to the molecular level. Nevertheless, optical access of living biological samples by microscopes is usually restricted to the outer most surface owing to tissue-induced light scattering.

Using directed evolution, we successfully improved the optical properties of mammalian cells. We aim to fully understand this optical plasticity of cells, and genetically clear living mammalian tissues by targeting responsible genes. We are conducting a genome-wide CRISPR activation screen to find genes which confer transparency. This contribution seeks to report on progress, challenges, and opportunities of this technology for biophotonics research, and more generally its potential towards a system level understanding of cellular biophysics.

As a next step, we are investigating how improved optical properties of individual cells influence the optical properties of 3D cell clusters, such as spheroids, using interspersed external fluorescent microspheres to quantify unbiased imaging quality.

Understanding the full potential of the optical plasticity of a tissue bears the potential of providing us with a broad toolkit, so that different genetic strategies can be applied depending on the specific nature of the various biological samples.

BP 21.5 Thu 10:30 BAR Schö

Optical characterization of biological material across scales using interferometric microscopy — •Daniel Midtvedt — Department of Physics, University of Gothenburg

The physicochemical properties, such as size, mass, and composition, of biological matter generally impact its function. However, quantitative characterization of these parameters across the many length-, time-, and mass scales relevant for biology is challenging. Interferometric imaging techniques, such as holographic imaging and interferometric scattering microscopy (iSCAT), provide quantitative measurements of the light scattering properties of biological objects, and are promising techniques for achieving such characterization. However, although iSCAT provides exquisite sensitivity, quantitatively relating the measured signal to physicochemical properties is straightforward only for small (Rayleigh) scatterers. The holographic signal, on the other hand, can be quantified across arbitrary length scale, but due to poor signal-to-noise ratio it is typically restricted to particles larger than the illuminating wavelength. In this talk, I will showcase some of our recent results combining interferometric imaging techniques with deep learning, enabling pushing the limit of mass quantification in holographic imaging toward the Rayleigh limit, thereby bridging a gap in optical characterization of small particles. With our technique, we are able to characterize biological matter across four orders of magnitude in length, four order of magnitude in time, and ten orders of magnitude in mass. I will highlight the key steps that have enabled us to make this development, and discuss its potential impact on life science research.

BP 21.6 Thu 10:45 BAR Schö

Adaptive optics in Confocal microscopy and Fluorescence correlation spectroscopy — •Julius Trautmann, Philipp Kellner, and Christian Eggeling — Institute for Applied Optics and Biophysics, Friedrich-Schiller University Jena, Philosophenweg 7, 07743 Jena

For more than three decades adaptive optics have been widely used in astronomical applications, but only in recent years it has established itself as an important feature for high resolution microscopy. The ability to correct for optical aberrations can be useful for any kind of setup but it proved particularly useful when imaging samples with inhomogeneous refractive index structures such as cells and especially cell tissue.

The most established adaptive optic elements include deformable mirrors (DMs) and spatial light modulators (SLMs) which can dynamically correct for aberrations.

This talk will cover the basic idea of including a deformable mirror (DM) in a confocal microscope within a fluorescence correlation spec-

troscopy (FCS) setup. A comparison of placing the deformable mirror in the excitation or detection beam path will take place.

15 min. break

Invited Talk BP 21.7 Thu 11:15 BAR Schö

Visualizing the inner life of microbes — •Ulrike Endesfelder — Institute for Microbiology and Biotechnology, Bonn University, Germany

Microbes, as unicellular organisms, are crucial model systems for the study of cellular mechanisms and functions. With the advent of modern fluorescence microscopy techniques, we can now visualize the inner workings of microbes at the molecular level, e.g. the dynamics of single molecules and the molecular architecture of sub-cellular structures. By quantifying the molecular characteristics of microbes in vivo, we thus can create detailed, spatially and temporally resolved maps of their molecular makeup, allowing us to understand the dynamic heterogeneity and sub-populations at the sub-cellular level. In this talk, I will discuss the potential of single-molecule biophysical approaches for microbiology, using examples from our own research and outlining our future visions.

BP 21.8 Thu 11:45 BAR Schö

A Minimal Model of CD95 Signal Initiation Revealed by Advanced Molecular-Sensitive Imaging — •Nina Bartels[1], Nicolaas TM van der Voort[2], Claus AM Seidel[2], and Cornelia Monzel[1] — [1]Experimental Medical Physics, Heinrich-Heine University, Düsseldorf, Germany — [2]Molecular Physical Chemistry, Heinrich-Heine University, Düsseldorf, Germany

The spatio-temporal organization and dynamic interactions of receptors in the plasma membrane are fundamental for our mechanistic understanding of cell signal initiation. A paradigm of a cell signal initiation process is the ligand-induced oligomerization of TNF (tumor necrosis factor) receptor CD95 in the signaling pathway for apoptosis. Here, we scrutinize proposed CD95 oligomerization models in the cell plasma membrane by applying a molecular sensitive imaging toolkit with up to nanometric resolution including time-resolved FRET spectroscopy, confocal Photobleaching Step Analysis, STED microscopy, and FCS. Covering a wide range of parameters, CD95 interactions are probed over the whole dynamic range from μs to hours, molecular to cellular scales, and with particular focus on quantifying molecular concentrations. Our multiscale study reveals a minimal oligomerization model to trigger apoptosis efficiently, where only ∼8-17% CD95 monomers assemble into dimers/trimers after ligand binding. Further, we highlight the importance of combining complementary techniques for a full understanding of transient and potentially localized processes such as a cell signal initiation.

see also https://doi.org/10.1101/2022.11.29.518370

BP 21.9 Thu 12:00 BAR Schö

Investigating human lung tissue by propagation-based phase-contrast X-ray tomography — •Jakob Reichmann[1], Stijn Verleden[2], Mark Kühnel[3], Jan-Christopher Kamp[3], Lavinia Neubert[3], Jan-Hendrik Müller[1], Thanh Quynh Bui[1], Danny Jonigk[3], and Tim Salditt[1] — [1]Institute for X-ray Physics, University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen — [2]Anatomy and Research Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium — [3]Institute of Pathology, Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover

The lung is a perfect example of how the function of an organ is enabled by its three-dimensional structure, here formed by intricate and intertwined networks of ventilation and vasculature. In this work we study the structure of lung tissues over multiple scales down to the sub-cellular level by phase-contrast computed tomography (PCCT). We show how human lung tissues with their largely air-filled compartments and small vessels can be imaged non-destructively with a scalable, isotropic resolution and quantitative density. Three-dimensional reconstructions with varied voxel sizes down to 130nm are obtained by advanced phase retrieval and tomographic reconstruction, shedding light on the three-dimensional cytoarchitecture. Morphometric parameters are extracted by automated image processing, and used to quantify the degree of pathological alterations. This offers unique potential to extend histology and pathohistology to study e.g. SARS-CoV-2 infected tissue or other lung degrading diseases such as COPD or cystic fibrosis, as we show with first applications of the method.

BP 21.10 Thu 12:15 BAR Schö

Dynamic allometry of nuclei and cell size in early embryos of a model organism — Rolf Fickentscher[1], Tomoko Ozawa[2], Akatsuki Kimura[2], and •Matthias Weiss[1] — [1]Experimental Physics I, University of Bayreuth, Germany — [2]Cell Architecture Laboratory, National Institute of Genetics, Mishima, Japan

Allometric relations between two observables are a widespread phenomenon in biology. The volume of nuclei, for example, has frequently been reported to be linearly related to the cell volume, but also conflicting, sublinear power-law correlations have been reported. Given that nuclei are vital organelles that harbor and maintain the cells' DNA, an understanding of the allometric scaling of nuclear volumes, which eventually defines the concentration and accessibility of chromatin, is of high interest. Using the model organism Caenorhabditis elegans, we show here that the allometry between cell and nucleus volumes is a dynamically adapting phenomenon with an asymptotic linear scaling. The adaption rate of the nucleus volume also scales with cell size. Our experimental data are well captured by a simple and supposedly generic model based on a diffusion-limited liberation of chromatin sites which drives decompaction and hence nucleus growth. Extrapolating our results to the general case of growing and proliferating cells suggests an isometric scaling of cell and nucleus volumes as the generic case.

BP 21.11 Thu 12:30 BAR Schö

Mechanical and electrophysiological recordings of neural organoids — Elijah Shelton[1], Paulina Wysmolek[2], Filippo Kiessler[1], Achim Brinkop[1], Sebastian Willenberg[1], Michael Frischmann[1], and •Friedhelm Serwane[1,2,3] — [1]Faculty of Physics & CeNS, LMU Munich, Germany — [2]MPI for Medical Research, Heidelberg, Germany — [3]Munich Cluster for Systems Neurology, Munich, Germany

Stem-cell derived organoids have made the exploration of neuronal network function accessible in vitro and are now allowing disease modelling. Both biochemical and mechanical signals, such as the elastic modulus, modulate the underlying behaviour of neurons to connect to networks. My group is developing tools for the mechanical and electrophysiological characterization of neuronal organoids. I will present a minimal-complexity setup for 3D imaging of their network activity (Wysmolek et al., Sci Rep 12, 20420, 2022). To extract Ca-signals we combine a lightsheet microscope as an add-on to a standard inverted microscope with computational tools. We created a 3D connectivity map by imaging spontaneous activity. As a next step, we apply statistical models to characterize the network behaviour. Changes in the tissue mechanical properties are one biophysical hallmark of tumour formation *in vivo*. We map the mechanical properties of tumor-forming cerebral organoids using ferrofluid droplets as mechanical actuators. Our measurements performed in neural organoids could inform researchers about the interaction between mechanics and function in the central nervous system.

BP 21.12 Thu 12:45 BAR Schö

Multi-scale X-Ray phase contrast tomography from the whole cochlea to single cells — •Jannis Justus Schaeper[1], Christoph Kampshoff[2], Bettina Wolf[2], Daniel Keppeler[2], Tobias Moser[2], and Tim Salditt[1] — [1]Institut für Röntgenphysik, Georg-August-Universität Göttingen — [2]InnerEarLab, Universitätsmedizin Göttingen

The cochlea is the receptor organ of the inner ear which transduces sound into neuronal activity. Both fundamental aspects of signal transduction and neuro-physiology as well as biomedical research (implant technology, hearing loss and disorders) require 3D imaging techniques capable to quantify the micro-anatomy (1).

We present multi-scale 3D imaging of small-animal cochleae by X-ray phase-contrast tomography (XPCT) using both synchrotron radiation (SR) and lab μ-CT to assess the morphology of the cochlea, orientation of cochlear implants (CIs), and the number and density of spiral ganglion neurons (SGNs). Due to optimization in sample preparation, image acquisition and phase retrieval we achieve high contrast for unstained soft tissue. Without extensive sample preparation, shape and volume of every SGN in the entire organ can be identified. In the high-resolution PC-CT, and in the parallel beam, we reach cellular resolution in the organ of Corti. Lab μ-CT is suitable to analyze cochlear morphology and to assess the correct positioning of CIs and resulting (non-)optimal signal transduction.

(1) Keppeler et al. (2021), PNAS 118(18), e2014472118 (2) Schaeper et al. (2022), Proc. SPIE 12242