BP 9: Bioimaging

Time: Tuesday 9:30–13:00 Location: H16

BP 9.1 Tue 9:30 H16

Nano-infrared spectroscopic imaging (NanIRim): Promises and Challenges for Application in Biophotonics — •Daniela Täuber — Leibniz Institute of Photonic Technology, Jena, Germany — Friedrich-Schiller University Jena, Germany

A number of nano IR spectroscopic methods have been developed, which provide chemical information at subcellular and single molecule level. High spatial resolution leads to a reduction of the number of chemical bonds contributing to the signal. Thus, variations have to be identified above a heterogeneous background. Since 2019, I have investigated advantages and limitations of mid IR photo-induced force microscopy (PIF-IR) together with my team and collaborators. We applied PIF-IR to materials ranging from organic monolayers on various substrates, and biopolymer compositions to single bacteria and human retina. Recently, we studied interference effects in layered systems comparing experimental and calculated FTIR spectra of polymer films on different substrates to PIF-IR spectra. PIF-IR enables hyperspectral imaging at a fascinating spatial resolution of ~5 nm. A drawback are the small data sets. We applied PIF-IR to well-known interactions of antibiotics with Bacillus subtilis. To meet the challenge of finding the local interactions in hyperspectral images of single bacteria, we developed an advanced cluster analysis together with colleagues in the Heintzmann Lab. Our findings are very promising for successful applications of PIF-IR to the investigation of local variations in the surface areas of cells and tissues. Such visualization at the single cell level will boost our understanding of interactions in the Life Sciences

BP 9.2 Tue 9:45 H16

Phase reconstruction of low-energy electron holograms of individual proteins — •Hannah Ochner[1], Sven Szilagyi[1], Moritz Edte[1], Stephan Rauschenbach[1,2], Luigi Malavolti[1], and Klaus Kern[1,3] — [1]Max Planck Institute for Solid State Research, Stuttgart — [2]Department of Chemistry, University of Oxford — [3]Institut de Physique, École Polytechnique Fédérale de Lausanne

Low-energy electron holography (LEEH) can image proteins and their conformational variability on the single-molecule level [1,2]. However, the technique does not yield a real-space image, but rather a hologram from which the information about the molecule needs to be recovered via a reconstruction process. While a one-step reconstruction process can reproduce molecular size and shape via amplitude imaging, it cannot directly recover the phase information encoded in the hologram. Here, we apply an iterative phase retrieval algorithm to experimentally acquired low-energy electron holograms of proteins. This allows us to reconstruct the phase shift induced by the protein along with its amplitude distribution. We provide evidence that phase imaging is sensitive to changes in local potential, as indicated by the strong correlation between reconstructed phase shift and the number of scatterers in the electron path, and the strong phase signatures induced by localised charges. LEEH phase imaging thus yields insights into structural features beyond size and shape and could, at high spatial resolution, open up the possibility of chemically sensitive single-molecule imaging.

[1] PNAS,2017;114(7) [2] PNAS,2021;118(51) e2112651118

BP 9.3 Tue 10:00 H16

Investigation of human platelet volume changes with scanning ion conductance microscopy (SICM) — •Konstantin Krutzke, Jan Seifert, Johannes Rheinlaender, and Tilman E. Schäffer — Institute of Applied Physics, Eberhard-Karls-Universität Tübingen, Germany

Human blood platelets (thrombocytes) are anucleate cells that play an important role in wound closure in the case of vessel injury. Changes in morphology and activation of platelets are linked to blood vessel diseases such as atherosclerosis or can cause thrombosis. Water-induced swelling promotes procoagulant activity and possibly initiates thrombosis. Volume changes of platelets can be measured by light transmittance or light scattering techniques. However, these studies have only qualitatively shown that platelets regulate their volume as a response to different osmotic conditions and usually have not been performed on a single-cell level. To elucidate the volume regulatory mechanisms of platelets, we used scanning ion conductance microscopy (SICM) to quantitatively measure dynamic volume changes of single adhered platelets under different osmotic conditions with down to sub-minute time-resolution. SICM is a nanopipette-based, contact-free imaging technique ideally suited for sensitive live cells such as platelets. Our data show that rapid volume regulation of non-activated adherent platelets occurs in direct response to different osmotic conditions. Activated platelets, however, seem not to be able to regulate their volume when the osmolarity changes. We thereby highlight the usability of SICM for high-speed volume measurements.

BP 9.4 Tue 10:15 H16

Pool formation of synaptic vesicles by synapsin investigated by X-ray diffraction and cryo-EM — •Jette Alfken[1], Charlotte Neuhaus[1], Moritz Stammer[1], Marcelo Ganzella[3], Arsen Petrovic[4], Rubén Fernández-Busnadiego[4], Reinhard Jahn[3], Dragomir Milovanovic[2], and Tim Salditt[1] — [1]Georg-August-Universität, Institute for X-ray Physics, 37077 Göttingen — [2]Laboratory of Molecular Neuroscience, German Center for Neurodegenerative Diseases (DZNE), 10117 Berlin — [3]Laboratory of Neurobiology, Max Planck Institute for Multidisciplinary Sciences, 37077 Göttingen, Germany — [4]Institute of Neuropathology, University Medical Center Göttingen, 37099 Göttingen

Synaptic vesicles (SVs) are organized in dense pools close to the synaptic membrane. A key protein for this structural arrangement within the synapse is synapsin, which forms droplets containing SVs, due to liquid-liquid phase separation. To study the structure and interactions underlying pool formation in a controlled in vitro model, we have investigated phases made of SVs purified from rat brain and varied synapsin concentration. We have studied the pools by two complementary techniques: cryo-EM yielding the 3D structural arrangement of the adhering vesicles in pools at resolution of a few nanometers in the vitrified state, and solution SAXS under varied buffer conditions and concentrations. In addition, the pools were studied for comparison in a controlled system consisting of artificially prepared lipid vesicles and synapsin. We report these experiments and preliminary results (data analysis still ongoing).

15 min. break

BP 9.5 Tue 10:45 H16

Understanding calcareous biomineralization on the nanoscale through in-vivo growth imaging by x-ray nanodiffraction — •Tilman Grünewald[1], Jeremie Vidal-Dupiol[2], Julien Duboisset[1], Bruno Petton[3], Jacqueline Legrand[3], Michael Sztucki[4], Manfred Burghammer[4], and Virginie Chamard[1] — [1]Institut Fresnel, Marseille, France — [2]Ifremer, Montpellier, France — [3]Ifremer, Plouzané, France — [4]ESRF, Grenoble, France

Biomineralized tissues combine properties such as low weight with high-strength and are formed from abundant atoms via low-energy processes. However, the nanostructural formation process of biominerals is not well understood, relying on post-mortem investigations of the bivalve growth edge [1]. Insights by in-vivo experiments requires studying a live organism in its environment at the crystalline level with sub-um spatial resolution. The associated problems have been overcome by 4th generation synchrotrons, enabling faster measurements.

Here, an experimental approach we developed and validated is outlined, enabling us to observe the first nanoscale-resolved, temporal follow-up of the shell growth in a living, mineralizing Crassostrea gigas oyster shell by nanofocus x-ray Bragg diffraction.

We show that crystallization occurs without the presence of the animal mantle, over several hours and follows a layer-by-layer deposition scheme with slightly misaligned grains. These results imply a cyclic crystallization, driven by a physico-chemical mechanism. This provides the animal with an efficient way of building its shell.

[1] Duboisset et al. 10.1016/j.actbio.2022.01.024

BP 9.6 Tue 11:00 H16

An open-top scanned oblique lightsheet microscope for neuronal network imaging — •Achim Theo Brinkop[1], Stefan Stöberl[1], Florian Schorre[1], and Friedhelm Serwane[1,2,3] — [1]Faculty of Physics, LMU Munich, Germany — [2]Munich Cluster for Systems Neurology (SyNergy), Germany — [3]Graduate School of Systemic Neuroscience (GSN), Munich, Germany

Understanding signal processing in neuronal networks such as brain

organoids on a single-neuron level has remained a challenge. Imaging network activity requires a millisecond temporal resolution with single-neuron spatial resolution, all in an observation volume containing the 3D network. Advances in lightsheet microscopy have brought this goal closer to experimental reach, but at the cost of complex optical set-ups which (i) impose geometrical constraints to sample mounting or (ii) require multiple imaging objectives with custom optical components.

We report on the development of an open-top single-objective oblique lightsheet microscope which reduces the complexity compared to existing set-ups. We implement the open-top geometry by using only two primary objectives. The lightsheet is digital scanned by a fast galvo mirror to maintain high image quality. Our first prototype with excitation wavelengths of 561 (488, 638) nm is expected to allow for a $1/e^2$-resolution of 1.84 (1.47, 1.95) μm axially and 0.27 (0.23, 0.30) μm laterally. It offers a volumetric temporal resolution of 8 (2) Hz for a volume of $400 \times 90\,(360) \times 100\,\mu m^3$.

With this set-up, we aim to gain insights into large neuronal networks of retina organoids, both in wildtype and disease condition.

BP 9.7 Tue 11:15 H16

Extraction of Calcium Traces from Volumetric Lightsheet Images of 3D Neuron Ensembles — •Filippo Kiessler[1], Paulina Wysmolek[4], Katja Salbaum[1,2], Elijah Shelton[1], Selina Sonntag[1], and Friedhelm Serwane[1,2,3] — [1]Faculty of Physics and Center for NanoScience, Ludwig-Maximilians-Universität München, Munich — [2]Graduate School of Systemic Neuroscience (GSN), Munich, Germany — [3]Munich Cluster for Systems Neurology (SyNergy), Germany — [4]Max Planck Institute for Medical Research, Heidelberg, Germany

In vitro systems resembling brain regions, such as brain organoids, are slowly changing the field of neuroscience. However, characterization of their electrical activity has remained a challenge as this requires electrophysiological readout in 3D at single-neuron resolution. We use a custom-built single-photon light-sheet microscope to record calcium activity in 3D neuron ensembles which we grow from mouse embryonic stem cells. To extract calcium intensities from the volumetric lightsheet data, we developed a custom software pipeline that augments the CaImAn software. Our pipeline includes a median filter to remove sample bleaching effects. In addition, typical artifacts arising from the illumination of of our light-sheet microscope are removed with a custom Fourier filter. With this setup we obtained connectivity graphs based on correlation of the extracted calcium traces. We envision this platform as a non-invasive toy-model to understand neuronal information generation and processing.

BP 9.8 Tue 11:30 H16

Thermal fluctuations of the trapped bead as the complementary tool to the microscopy for investigation of a phagocytosis. — •Tetiana Udod and Alexander Rohrbach — Lab for Bio- and Nano-Photonics, Department of Microsystems Engineering (IMTEK), University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany

Phagocytosis, the uptake of particle by cells, is typically investigated in vivo by different microscopy technics, such as Brightfield, DIC, or Fluorescence microscopy. But even with highest possible resolution we can*t observe receptor binding or derive binding strengths to the cell membrane during binding and uptake. In addition to continuously recording 3D stacks of J774 macrophages cells by DIC microscopy, we record the thermal fluctuations of beads during the engulfment process. We measure the bead*s position in 3D with nanometer precision at MHz rates with back focal plane interferometry. Running both methods in parallel we can correlate the bead position relative to the cell to record changes in binding parameters like stiffnesses or, viscous drags derived from position fluctuations. Furthermore, remaining measurement ambiguities are resolved by Brownian Dynamic simulations.

To better understand such processes we use a combination of experiments with Photonic Force Microscopy, Brownian Dynamic simulation and analytical theory.

BP 9.9 Tue 11:45 H16

Assessing the cochlear morphology from the whole organ down to cellular resolution with multi-scale phase-contrast x-ray tomography — •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 phase-contrast x-ray tomography (PC-CT) 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

15 min. break

BP 9.10 Tue 12:15 H16

Studying biomolecular dynamics and structure with high-speed atomic force microscopy — •Dimitar Stamov, Andreas Kraus, André Körnig, and Heiko Haschke — JPK BioAFM, Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany

Studying the molecular dynamics and structural conformations is important for understanding the function and biological significance of samples ranging from single membrane proteins to complex macromolecular systems. Recent atomic force microscopy (AFM) developments have led to unprecedented imaging rates in fluid, enabling temporal resolution on the sub-20-milisecond scale.

Annexin V (A5) serves as an important regulator of membrane repair in eukaryotic cells, where it shows a strong Ca2+ binding affinity to phosphatidylserine. We have used high-speed AFM to study the 2D crystal formation in a model system containing supported lipid bilayers and A5 molecules. We demonstrate the lateral dynamics and preferred structural orientations of the mobile A5 trimers.

We previously demonstrated that pUC19 plasmids bind to poly-L-ornithine substrate in supercoiled states that are very high in torsional energy, thereby driving dehybridization of the double-helical DNA strands. Here we have quantified the process kinetics with a temporal resolution of 25 ms per frame and identified stages that include formation of metastable dehybridization bubbles, thermodynamic single strand fluctuations, and ultimately rehybridization to an intact double-stranded state.

Prize Talk BP 9.11 Tue 12:30 H16

Super-resolution STED and MINFLUX Nanoscopes by Abberior Instruments — •Gerald Donnert — Abberior Instruments GmbH, Göttingen, Germany — Laureate of the Technology-Transfer-Prize 2022

Abberior Instruments GmbH was founded 10 years ago from the laboratory of Nobel Laureate Stefan Hell at the Max Planck Institute in Göttingen. In 2022, Abberior Instruments was awarded the Technology Transfer Prize of the German Physical Society (DPG).

Abberior Instruments develops and markets super-resolution light microscopes, namely confocal plus STED microscopes and MINFLUX microscopes. The latter are the latest generation of super-resolution instruments with resolutions down to the molecular level, i.e. 1 nm resolution; unrivaled in resolution today. Understanding life at the molecular level - both in terms of structure and dynamics - is a human dream and is becoming feasible with the latest generation of super-resolution instruments with multicolor capabilities. We expect to soon gain new insights into the dynamic structural changes of e.g. protein machines in living cells.

In this talk, I will present the latest imaging and tracking results with our super-resolution STED and MINFLUX nanoscopes, such as single-particle tracking of lipids in lipid membranes, the structure of nuclear pore complex (NPC) subunits, and the nanoscale assembly of proteins in neuronal synapses.