BP 22: Bioimaging

Time: Wednesday 15:00–17:45 Location: H46

Invited Talk BP 22.1 Wed 15:00 H46

From DNA Nanotechnology to biomedical insight: Towards single-molecule spatial omics — •Ralf Jungmann — LMU and MPI of Biochemistry, Munich, Germany

Super-resolution fluorescence microscopy is a powerful tool for biophysical and biological research. The transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT) can be leveraged for easy-to-implement multiplexed super-resolution imaging that achieves molecular-scale resolution across large fields of view. This seminar will introduce recent technical advancements in DNA-PAINT including approaches that achieve sub-10-nm spatial resolution and spectrally unlimited multiplexing in whole cells followed by recent developments in novel protein labeling probes that have the potential to facilitate DNA-barcoded labeling of much of the proteome within intact cellular environments. Applications of these new approaches will be discussed in cell surface receptor imaging and neuroscience. Visualization and quantification of cell surface receptors at thus far elusive spatial resolutions and levels of multiplexing yield fundamental insights into the molecular architecture of surface receptor interactions thus enabling the future development of more refined *pattern*-based therapeutics. A key approach in implementing these methods has been to leverage standard off-the-shelf fluorescence microscopy hardware as a tool for spatial omics, thus democratizing the ability to visualize most biomolecules and probe their network-wide interactions in single cells, tissues, and beyond with single-molecule-based "Localizomics".

BP 22.2 Wed 15:30 H46

3D Single-Nanoparticle Tracking with Fluorescence Lifetime Imaging for Investigating Lipid Nanoparticles Endosomal Pathways — •Thomas Kellerer[1,2], Judith A. Müller[2], Tanja Grawert[1], Lukas Moser[1], Joachim O. Rädler[2], and Thomas Hellerer[1] — [1]Multiphoton Imaging Lab, Munich University of Applied Sciences, 80335 Munich, Germany — [2]Faculty of Physics and Center for NanoScience, Ludwig Maximilians-University, 80539 Munich, Germany

Lipid nanoparticles (LNPs) are vital for delivering mRNA in drug delivery systems, but the kinetics and intracellular pathways of their cargo release remain often unclear. To address this, we developed a microscopy technique to track single nanoparticles in 3D over extended periods. This approach integrates a lock-in amplifier for simultaneous image based 3D tracking and fluorescence lifetime measurement, enabling analysis of the microenvironment, such as pH changes. Achieving a frame rate of 7.6 fps at 1024x1024 resolution and lifetimes measured within 102 ns, our method provides novel insights into LNP dynamics and endosomal acidification in live cells. This technique was applied to study the acidification kinetics of endosomes during transfection. By measuring pH changes in real time, we provided insights into the intracellular behavior of LNPs and their role in mRNA delivery. This approach establishes a new standard for tracking nanoparticles and analyzing their microenvironments with high spatial and temporal resolution.

BP 22.3 Wed 15:45 H46

The dynamics of binding and uptake of SARS-CoV-2 virus-like particles investigated by ROCS and fluorescence microscopy — •Alexander Rohrbach and Dominik Huber — Lab for Bio- and Nano-Photonics, University of Freiburg, Germany

Viruses such as coronavirus SARS-CoV-2 are challenging to observe during interactions with sales in life-cell imaging to their small size and remarkable speed. Techniques like fluorescence microscopy often struggle to visualize these interactions, especially due to their susceptibility to bleaching and the difficulty to label different structures without altering their function.

In our research we use 200 Hz Rotating Coherent Scattering (ROCS) microscopy in order to visualize the diffusion of 100 nm sized virus-mimicking particles (VLPs) and their interactions with macro*phages or epithelial cells. ROCS is a label-free imaging technique at 160 nm resolution, using coherent backscattering of a rotating laser. By tracking VLPs with ROCS and with fluorescence, we are able to analyze their fluctuations and thereby the dynamics of diffusing VLPs close to A549 lung epithelial cells. Using spatiotemporal and spectral analysis methods, we can investigate for the first time diffusion, binding and uptake events of single VLPs at the cell periphery.

BP 22.4 Wed 16:00 H46

Visualising immune cell interactions in lymph nodes — •Anna Schepers[1], Joannah Fergusson[1], Helena Coker[1], Robert Kochl[2], and Marco Fritzsche[1] — [1]Kennedy Institute of Rheumatology, Oxford, UK — [2]King's College London, UK

The inherently multiscale immune response is regulated by diverse cell interactions, relying on cues from tissues down to single cells and subcellular structures. The intricate dynamics of the immune system present challenges for the observation of the immune response. A technological advance has been achieved with the introduction of lattice light sheet microscopy (LLSM), allowing fast and gentle imaging of live samples while achieving subcellular resolution. By complementing LLSM-based volumetric imaging with advanced sample handling of *ex vivo* tissue samples and perfusion imaging chambers, we provide a system that preserves critical physiological complexity. The perfusion system ensures oxygen and nutrient supply to maintain and sample viability while, at the same time, enabling imaging of the perfused samples. We show that in our setup, we can follow single cells and their interactions in volumes several cell layers deep in living samples within their environment, providing nuanced insights into the immune response.

15 min. break

BP 22.5 Wed 16:30 H46

Platelet biomechanics in biochemical confinement — •Vincent Gidlund, Aylin Balmes, and Tilman Schäffer — Institute of Applied Physics, University of Tübingen, Tübingen, Germany

Platelets, as part of the human blood, play a critical role in wound healing, hemostasis, and thrombotic diseases. When platelets accumulate and form plugs during wound healing, they can experience confining microenvironments. For a deeper understanding of platelet biomechanics, it is important to investigate the effects of a confining microenvironment on platelets. It is known that platelets can adapt to line-shaped microenvironments, but the mechanical properties of platelets subjected to two-dimensional confining microenvironments remain unexplored. We use microcontact printing of fibrinogen patterns of different shapes (circles, triangles) and areas to create a biochemically confining microenvironment for platelets. We then apply epifluorescence microscopy and scanning ion conductance microscopy (SICM) to measure F-actin distribution, topography, and stiffness of platelets confined to these shapes. We found that platelets adapt their morphology to match the shape of the underlying fibrinogen pattern. They show a redistribution of F-actin towards the periphery, as has been observed in other cell types. Additionally, a reduced shape area leads to decreased platelet stiffness.

BP 22.6 Wed 16:45 H46

Near infrared fluorescent silicate nanosheets for Bioimaging — •Bjoern F. Hill and Sebastian Kruss — Physical Chemistry II, Ruhr-University Bochum, Bochu, Germany

Fluorophores emitting in the near-infrared (NIR) are highly advantageous in photonics and biosensing due to reduced light scattering, low phototoxicity, and minimal autofluorescence in this spectral region.

Egyptian Blue (CaCuSi4O10) combines properties that make it a promising material for bioimaging and -photonics: It exhibits bright and stable NIR fluorescence (λ_em=935 nm), its layered structure enables exfoliation into 2D nanosheets (EB-NS), additionally it features a high quantum yield, proven biocompatibility and low production costs.

We present a surfactant-assisted exfoliation route to produce monodisperse EB-NS, tailored to nm-scale diameters, with thicknesses as low as single monolayers, while retaining their NIR fluorescence [1].

Addidionally, we demonstrate the integration of EB-NS with single-walled carbon nanotubes (SWCNTs) to create a ratiometric fluorescence sensor for dopamine. This sensor achieves robust, non-invasive imaging of neurotransmitter release from live cells, while the remarkable stability of the EB-NS fluorescence compensates for environmental fluctuations and enhances measurement reliability [2].

In summary, EB-NS represent a novel, accessible, and highly stable NIR fluorescent nanomaterial with broad applications in bioimaging

and -photonics.

[1] B. Hill, et. al., RSC Adv., 2023,13, 20916-20925
[2] B. Hill, J. Mohr, et.al., Nanoscale, 2024,16, 18534-18544

BP 22.7 Wed 17:00 H46

Lysosomal activity in response to incubation of pristine and functionalized carbon nanodots — •Carla Sprengel[1], Céline David[2], Lena Berning[2], Cathrin Nollmann[1], Björn Stork[2], and Thomas Heinzel[1] — [1]Condensed Matter Physics Laboratory, Heinrich Heine University, Düsseldorf, Germany — [2]Institute of Molecular Medicine I, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University, Düsseldorf, Germany

Fluorescent carbon nanodots (CNDs) have emerged as promising carriers for drug delivery systems due to their high biocompatibility and functionalizability. We could not find an influence of CNDs on cellular lysosomal functions, as characterized via the cathepsin B and L activity and autophagic markers p62 and LC3B-II, even under high CND concentrations. Functionalization of CNDs with branched polyethylenimin (bPEI) as a model drug conjugate leads to a greater accumulation of bPEI-CND compounds within lysosomes compared to native CNDs. Here, changes in the lysosomal size and function can be explained exclusively by bPEI. This leads us to conclude that CNDs are highly efficient and inert carriers for delivering functional molecules into lysosomes as target while minimizing lysosomal escape and therefore preventing unintended side effects on other cell organelles.

BP 22.8 Wed 17:15 H46

Grating Based X-ray Phase Contrast CT with Laboratory Setups — •Luka Gaetani[1,2], Josef Scholz[1,2], Lorenz Birnbacher[2,3], and Julia Herzen[1,2] — [1]Research Group Biomedical Imaging Physics, Department of Physics, TUM School of Natural Sciences, Technical University of Munich, 85748 Garching, Germany — [2]Chair of Biomedical Physics — [3]Institute for Diagnostic and Interventional Radiology, TUM Klinikum rechts der Isar, 81675 München, Germany

Grating-based X-ray Phase Contrast Computed Tomography (CT) represents a significant advancement in imaging, offering enhanced sensitivity to soft tissues and low-density materials by capturing phase information complementary to absorption contrast. This technique utilizes a series of optical gratings to measure phase shifts introduced by the sample, enabling the reconstruction of high-resolution phase contrast images. Laboratory-based implementations of this method, facilitated by compact X-ray sources and precise grating alignment, have extended its accessibility and applicability to diverse fields. However, optimizing these setups necessitates addressing challenges such as coherence management, efficient data acquisition, and advanced reconstruction algorithms to maximize their performance in non-synchrotron environments. This presentation will demonstrate how a grating-based interferometer enables the quantitative determination of electron density, a physical property of the sample, by accurately correlating the sample's influence on the gray values in the recorded X-ray images.

BP 22.9 Wed 17:30 H46

Nanomaterials: A Versatile Sensitizer for Enhanced Singlet Molecular Oxygen Generation — •Zahid Ullah Khan[1], Latif Ullah Khan[1,2], Hermi Felinto Brito[1], and Paolo Di Mascio[1] — [1]Institute of Chemistry, University of São Paulo (USP), 05508-000, São Paulo-SP, Brazil — [2]Synchrotron-light for Experimental Science and Applications in the Middle East (SESAME) P.O. Box 7, Allan 19252, Jordan

Singlet molecular oxygen (1O2) plays a crucial role in various fields, including optoelectronics, photooxygenation reactions, and biomedical therapies, particularly as a major contributor to the success of photodynamic therapy (PDT). Since direct excitation of oxygen from the triplet ground state (3O2) to the singlet-excited state is spin-forbidden, thus, making the design of heterogeneous sensitizers crucial for efficient 1O2 production. For this purpose, nanomaterials, such as quantum dots (QDs) and rare earth fluoride nanoparticles (NPs), have emerged as versatile sensitizers for 1O2 generation, either individually or in combination with other inorganic or organic materials. Hence, conjoining the photophysical properties of QDs and rare earth NPs with other materials, e.g., coupling/combining with other inorganic materials, doping with the transition metal ions or lanthanide ions, and conjugation with a molecular sensitizer provide the opportunity to achieve high-efficiency quantum yields of 1O2 which is not possible with either component separately. Hence, the current work focuses the development of semiconductor QDs and rare earth-based nanosensitizer for efficient production of 1O2.