BP 33: Bioimaging

Time: Thursday 15:00–18:30 Location: BAR/0205

Invited Talk BP 33.1 Thu 15:00 BAR/0205

Expanding the Bag of Optical Tricks for (Neuro)Biology — •Fabian F. Voigt — Department for Molecular and Cellular Biology, Harvard University, Cambridge, USA — Max Planck Institute for the Science of Light, Erlangen, Germany — Max Planck Center for Physics and Medicine, Erlangen, Germany

Seeing is believing and thus, optical imaging techniques are extremely useful to study brain structure and function. I will present several projects aimed at providing the neuroscience community with better instrumentation: These range from open-source light-sheet microscopes for imaging cleared tissue (https://mesospim.org/) to novel multi-immersion microscope objectives that take inspiration from scallops and astronomical telescopes as well as utilizing metasurfaces for improving light collection efficiency in light-sheet microscopy.

BP 33.2 Thu 15:30 BAR/0205

Using Rotating Coherent Scattering (ROCS) Microscopy for Binding and Uptake Analysis of Virus-Mimicking Particles — •Dominik Huber and Alexander Rohrbach — Lab for Bio- and Nano-Photonics, University of Freiburg, Germany

The investigation of viruses with their host cell is an important topic in medical research, yet direct observation of these nanoscale dynamics remains challenging, since conventional fluorescence microscopy is limited by photobleaching and labelling constraints. To address these limitations, we employ label-free Rotating Coherent Scattering (ROCS) microscopy, an imaging approach that enables high-speed, high-resolution visualization of virus-mimicking particle behaviour at the cell.

ROCS microscopy uses backscattering of oblique illumination of a rotating laser beam to achieve around 160 nm spatial resolution at imaging rates up to around 200 Hz. By combining several illumination wavelengths and illumination angles, ROCS microscopy can be used in brightfield and darkfield as well as in total internal reflection mode. Leveraging this flexibility in imaging, allows us to capture the binding dynamics of particles to cells and to investigate them from their first attachment up to the investigation of single particle uptake events.

The analysis of particle fluctuation widths and mean square displacements enabled us to detect differences in binding characteristics of two different types of virus-mimicking particles, thus demonstrating that ROCS microscopy provides a powerful tool for investigation of particle-cell interactions at the single-particle level.

BP 33.3 Thu 15:45 BAR/0205

Pharmacological modulation of intrinsic tissue transparency for enhanced microscopy — •Adrián Puerta, Susan Wagner, and Moritz Kreysing — Institute of Biological and Chemical Systems, Karlsruhe Institute of Technology, 76344 Eggenstein Leopoldshafen, Germany

Light microscopy remains as one of the primary methods for data acquisition in biological research, yet its performance is frequently limited by the strong scattering properties of experimental tissues. Hence, the use of clearing agents that are incompatible with living samples is often required (1). To overcome these limitations, we aim to modulate the intrinsic transparency of biological samples, enabling more effective live-cell imaging.

Conventional clearing strategies rely on chemicals that modify the refractive index of aqueous or lipid-rich tissue components (2). In contrast, our approach involves screening small molecules with pharmacological properties that may enhance transparency in mammalian cells. To investigate this, we have developed a high-throughput screening system based on flow cytometry that will allow us to identify potential changes at a single-cell scale.

(1) Yu, T. et al. Physical and chemical mechanisms of tissue optical clearing. iScience 24, 102178 (2021).

(2) Ou, Z. et al. Achieving optical transparency in live animals with absorbing molecules. Science 385, eadm6869 (2024).

BP 33.4 Thu 16:00 BAR/0205

Imaging viral infection in live cells with confocal interferometric scattering microscopy (iSCAT) — •Anirudh Khemani[1], David Albrecht[1], Jan Renger[1], Marco Heisig[1], Kiarash Kasaian[1], and Vahid Sandoghdar[1,2] — [1]Max Planck Institute for the Science of Light, 91058 Erlangen, Germany — [2]Max-Planck-Zentrum für Physik und Medizin, 91054 Erlangen, Germany.

Fluorescence-based microscopy can suffer from observational bias, functional perturbation, phototoxicity, or insufficient signal. Confocal interferometric scattering microscopy [1] provides quantitative, label-free imaging of nanoscale dynamics in live-cell processes. iSCAT is a shot-noise-limited homodyne interferometric technique. By rejecting out-of-focus light, confocal iSCAT yields high structural contrast of nano-bioparticles, like vesicles and viruses, and we validate structural assignments with fluorescence. We apply this platform to study rare, dynamic events in the vaccinia virus life cycle. To improve experimental control and statistics, we developed a microfluidic system to deliver individual virions with defined timing and position. Furthermore, we combine confocal SCAT with concomitant wide-field iSCAT [2] to span a large range of spatial and temporal resolutions. We report on complex processes such as virus-induced nucleation of actin tails and high-speed tracking of single virions at cell-cell junctions and on membranes. [1] Küppers, M., Albrecht, D., *et al. Nat. Commun.* **14**, 1962 (2023). [2] Mazaheri, M., Kasaian, K., *et al. Optica* **11**, 1030 (2024).

BP 33.5 Thu 16:15 BAR/0205

Image segmentation of treated and untreated tumor spheroids by fully convolutional networks — Matthias Streller[1], Soňa Michlíková[2], Katharina Lönnecke[1], Leoni A. Kunz-Schughart[2], Anja Voss-Böhme[1], and •Steffen Lange[1,2] — [1]HTW Dresden — [2]OncoRay, TU Dresden, HZDR, Germany

Multicellular 3D tumor spheroids (MCTS) are advanced preclinical cell culture systems for assessing the impact of combinatorial radio(chemo)therapy as they exhibit therapeutically relevant in vivo-like characteristics. State-of-the-art assays quantify long-term curative endpoints based on collected brightfield image time series from large treated spheroid populations, which requires laborious spheroid segmentation of up to 100,000 images per treatment arm. While several image analysis algorithms are available for spheroid segmentation, they all focus on compact MCTS with a clearly distinguishable outer rim throughout growth and often fail for the common case of treated MCTS, which may partly be detached and destroyed and are usually obscured by dead cell debris. To address these issues, we successfully train 2 fully convolutional networks, UNet and HRNet, and optimize their hyperparameters to develop an automatic segmentation for both untreated and treated MCTS[1]. We extensively test the automatic segmentation on larger, independent datasets and observe high accuracy for most images with Jaccard indices around 90%, with deviations consistent to inter-observer variability. We also successfully test against previously published datasets and spheroid segmentations.

[1] Streller et.al, GigaScience 2025, doi.org/10.1093/gigascience/giaf027

BP 33.6 Thu 16:30 BAR/0205

Predicting treatment response of tumor spheroids from radiomics analysis of post-treatment dynamics — Pejman Shojaee[1,2], Tom Bischopink[1], Daria Bolotova[1], Sona Michlikova[2], Leoni A. Kunz-Schughart[2], Steffen Lange[1,2], and •Anja Voss-Böhme[1] — [1]DataMedAssist Group, Faculty of Informatics/Mathematics, HTW Dresden — [2]OncoRay - National Center for Radiation Research in Oncology Dresden

Radiomics has significantly advanced radiation oncology by providing quantitative, objective metrics to predict therapeutic efficacy. However, these methods have not been applied to three-dimensional, multicellular tumor spheroids yet, which are the preferred in vitro model for pre-animal, pre-clinical selection of novel, future-oriented treatment modalities. We present an AI-driven predictive modeling workflow to predict long-term tumor spheroid relapse using radiomics data from early post-treatment imaging of spheroids of two human cancer cell lines subjected to radiation therapy and hyperthermia. Our approach integrates multiple feature selection methods and machine learning algorithms for optimal classification performance. A detailed evaluation of the model performance reveals a time gain by early prediction of 2-14 days, while cases of late relapse remain challenging. The presented radiomics-based approach reduces the resource-intensive demands associated with prolonged experimental monitoring and allows accurate prediction for up to three days beyond the observation horizon.

15 min. break

BP 33.7 Thu 17:00 BAR/0205

Photothermal chemical imaging of nano-structured cells and cell organelles with less than 5 nm resolution — Maryam Ali[1,2], Christin David[1,3], and •Daniela Täuber[1,2] — [1]Friedrich Schiller University Jena — [2]Leibniz Institute of Photonic Technology, Jena — [3]University of Applied Sciences Landshut, Germany

Mid-infrared photoinduced force microscopy (PiF-IR) is a new imaging technique that enables the chemical characterization of surfaces with an unprecedented spatial and high spectral resolution. PiF-IR bridges the gap between high-resolution structure elucidation using electron microscopy, fluorescence microscopy and conventional infrared spectroscopy. It is complementary to tip-enhanced Raman spectroscopy (TERS). PiF-IR was successfully applied to map the local chemistry and structure of the antibiotic interaction on the surface of individual bacterial cells with a resolution of a few nanometers using the model system Bacillus subtilis and vancomycin [Ali et al., Anal. Chem., 2025, 97, 23914] and to map the surface of retina pigment organelles. Frequently observed anisotropic signal distributions on soft nanostructures in tip-enhanced photothermal imaging methods could be attributed to hybrid field coupling in a study combining modeling and experiment [Anindo et al., J. Phys. Chem. C, 2025, 129, 4517].

BP 33.8 Thu 17:15 BAR/0205

Phase Analysis of Photothermal Signal Enables Sub-Cellular Chemical Mapping of Complex Biological Systems — •Felix Hermann Patzschke and Frank Cichos — Molecular Nanophotonics Group, Peter Debye Institute for Soft Matter Physics, Leipzig University, Linnéstraße 5, 04103 Leipzig

Optically probed Photothermal Infrared (O-PTIR) microscopy is a powerful technique for label-free chemical imaging at sub-micron resolution, promising for cell and tissue analysis. However, the mechanism of signal generation is complex, suffering from the dynamic crosstalk between background and target heating. This lack of a rigorous, quantitative understanding of these dynamics currently forces users to sacrifice either chemical specificity or spatial resolution.

We conducted systematic experiments on defined nanoscale structures to motivate and validate a quantitative theoretical model based on the transient heat equation. This model reveals that the phase of the photothermal signal, a dynamic quantity often neglected, contains essential, localized information.

This phase directly encodes the time delay associated with thermal wave propagation, allowing for precise quantification of sub-cellular structural detail through effective thermal distance, even when the amplitude signal is insufficient to resolve closely-spaced features. By leveraging the full phase and amplitude response, we overcome existing spatial resolution limits, achieving enhanced, label-free chemical mapping for next-generation bioimaging, using existing hardware.

BP 33.9 Thu 17:30 BAR/0205

Effects of Optical Stimulation on the Adhesion of Human Osteoblasts — •Franziska Dorn[1], Wiebke Wollenberg[2], Meike Bielfeldt[3], Regina Lange[1], Susanne Stählke[3], Ingo Barke[1], Henrike Rebl[3], Berit Zeller-Pulmhoff[2], and Sylvia Speller[1] — [1]Physics of Surfaces and Interfaces, University Rostock — [2]Data-Driven Analysis and Design of Materials, University of Rostock — [3]Institute of Cell Biology, University Medical Center Rostock

Accelerated growth of autologous bone tissue is a promising strategy in regenerative medicine. In this study, we investigated how optical stimulation influences cell adhesion. We cultured the osteoblast-like (MG-63) cells in fetal bovine serum-free physiologic medium. As substrates, we used plain glass and glass surfaces with structured gold nanotriangular islands fabricated by nanosphere lithography. After cell seeding, the samples were illuminated with green light, inducing a spatially varying light intensity due to reflection and plasmons. Afterwards, we examined the cells using scanning electron microscopy and applied machine-learning-based/random-forest-based segmentation to extract morphological features. We found that opto-stimulated cells form more concave borders than unstimulated controls, suggesting that the cytoskeleton and the focal adhesions develop at non-uniform speed. Additionally, the adhesion area of illuminated cells is substantially larger than that of control cells on glass surfaces. However, when cells are seeded on surfaces with gold nanotriangular islands, this effect is absent. In a next step the peripheral region of the cells, in terms of lamellipodia and filopodia is addressed. (SFB 1270-299150580)

BP 33.10 Thu 17:45 BAR/0205

Imaging biomolecules for improving single-molecule diffraction — •Stefanie Lenzen[1,2], Lukas V. Haas[1,2], Kevin Janson[1], Amit K. Samanta[1,2], and Jochen Küpper[1,2] — [1]Center for Free-Electron Laser Science (CFEL), Deutsches Elektronen-Synchrotron DESY, Hamburg — [2]Department of Physics & Department of Chemistry & Center for Ultrafast Imaging, Universität Hamburg

Determining the structure and dynamics of single native biomolecules is still a challenge. In protein-crystallography and cryo-EM the molecule needs to be fixed, which might lead to structural disentegration, and the temporal resolution of these methods are limited. X-ray free-electron lasers (XFELs) provide ultrashort pulses, enabling diffraction before destruction, and a large number of photons, promising the observation of diffraction patterns *off* single nanoparticles [1]. Aerodynamic-lens stacks were used to deliver focused dense particle beams for such experiments on large nanoparticles [2]. Localization microscopy (LM), based on Mie-scattering is used to study and optimize these beams, an important step in improving single particle imaging. Due to the limitation in particle size [3], small biomolecules do not provide sufficient intensity for being detected with LM. We optimized the optical and analysis system and developed a promising method for the detection of smaller biomolecules, based on fluorescence.

[1] Poudyal, Schmidt, Schwander, *Struct. Dyn.* **7**, 024102 (2020);
[2] Ayyer, et int (39 authors), Chapman, *Optica* **8**, 15-23 (2021);
[3] Worbs, et int (2 authors), Maia, *Commun Phys* **8**, 155 (2025)

BP 33.11 Thu 18:00 BAR/0205

Optically addressable spins in proteins — •Dominik Bucher — Technical University of Munich, TUM School of Natural Sciences, Chemistry Department

Optically addressable spins have attracted significant interest for their potential in quantum sensing technologies. The current workhorses rely on spin defects in solids (such as the nitrogen-vacancy center in diamond), which lack the possibility for precise synthetic control over their properties. In my talk I will highlight our latest research on optically addressable spins in proteins, specifically flavin-based proteins. Upon excitation, these proteins generate radical pairs that can be detected optically via their flourescence and manipulated through radio wave-controlled spin chemistry. We further show that this optical spin interface is tunable by the protein structure. I will explain how these systems differ from the well-established NV center and discuss their potential as genetically encoded quantum sensors for future applications.

K. Meng et al. Optically detected and radio wave-controlled spin chemistry in cryptochrome, BioRxiv https://doi.org/10.1101/2025.04.16.649006 (2025)

BP 33.12 Thu 18:15 BAR/0205

Beyond Molecular Sensitizers: Illuminating the Role of Nanomaterials in Singlet Oxygen Photochemistry — •Zahid Ullah Khan[1], Latif Ullah Khan[2], Hermi Felinto Brito[1], and Paolo 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.