# BP 1: Imaging

Time: Monday 9:30–13:00 Location: H 1028

**Invited Talk** BP 1.1 Mon 9:30 H 1028

**Light sheet-based fluorescence microscopy for quantitative biology** — •Ernst H.K. Stelzer — Physical Biology, BMLS, Goethe Universität, D-60438 Frankfurt am Main

As long as we rely on epifluorescence microscopes, we are faced with serious challenges. Fluorophores and specimens are essentially wasted during the observation process, since all fluorophores and many endogenous organic compounds in the specimen are excited whenever we record a single plane. Obviously, the situation becomes even more challenging when we perform complex biological experiments and observe the behavior of multiple targets in three dimensions as a function of time. In light sheet-based fluorescence microscopy (LSFM), planar optical sectioning in the excitation process minimizes fluorophore bleaching and phototoxic effects. Since biological specimens survive long-term three-dimensional imaging at high spatio-temporal resolution, LSFM has become the tool of choice in developmental biology. LSFM makes a sincere and honest effort to reduce bleaching and phototoxicity. LSFM allows one to record millions of pixels in parallel. Laser light sheet-based devices, including a macroscope, had been built several times, but their capability to perform at a microscopic level was unknown until we described a diffraction-limited microscope in 2002, observed living biological samples and evaluated multiple-view imaging. This developed from theta microscopy (1993) and a systematic evaluation of diffraction-limited microscopes with two to four lenses, in theory and practice.

BP 1.2 Mon 10:00 H 1028

**Reflected light-sheet microscopy reveals single molecules in Drosophila melanogaster embryo** — •Ferdinand Greiss[1], Myrto Deligiannaki[2], Christophe Jung[2], Ulrike Gaul[2], and Dieter Braun[1] — [1]System Biophysics, Department of Physics, Ludwig Maximilians University, Amalienstr. 54, 80799 Munich, Germany — [2]Gene Center, Department of Biochemistry, Ludwig Maximilans University, Feodor-Lynen-Str. 25, 81377 Munich, Germany

Searching the growing field of light microscopy for dynamic high resolution imaging in vivo suggests total internal reflection microscopy (TIRF) as one of the most popular candidates. However, TIRF has the major limitation that it is constrained to the imaging depth of only several hundred nanometers close to the coverslip surface. The need for alternative imaging techniques is therefore evident.

Here we report a method to reveal single molecules at various imaging depths and thicker tissues. We adapted and optimized reflected light-sheet microscopy (RLSM) as described by Gebhardt et al. (2013) and used it to study the epidermal barrier of Drosophila embryos.

Epidermal cells seal their intermembrane space by septate junctions, which impede free diffusion through the paracellular route. We were able to detect single 10 kDa Alexa647-conjugated Dextran diffusing in and around epidermal tissue. As discovered with ensemble measurements, mutants with disrupted septate junctions exhibit leaky epithelial barrier. We were able to confirm these findings with our optical setup on the single molecule level.

BP 1.3 Mon 10:15 H 1028

**Single laser beam photothermal microscopy** — •Andre Heber, Markus Selmke, Marco Braun, and Frank Cichos — Molecular Nanophotonics Group, Institute of Experimental Physics I, Leipzig University, 04103 Leipzig, GERMANY

Photothermal microscopy enables the selective detection of single absorbing nanoparticles and molecules in the presence of scatterers. It is based on refractive index changes upon optical excitation. Typically, it utilises two different laser beams at distinct wavelengths to induce and detect changes of the optical properties in the vicinity of an absorbing nanoobject. Here, we show that a single modulated heating laser with an intensity offset suffices to selectively image absorbers. The modulated optical heating creates a thermal wave around the absorber and results in a retardation of the refractive index field. The out-of-phase component of the scattering on the heated nanoparticle and the refractive index profile provides a selective contrast for absorbers. The use of a single laser beam simplifies the application of photothermal microscopy in existing microscopy schemes.

BP 1.4 Mon 10:30 H 1028

**3D-Refractive Index Measurements of Single Cells by Optical Diffraction Tomography** — •Paul Müller[1], Mirjam Schürmann[1], Christoph Faigle[1], Moritz Kreysing[2], and Jochen Guck[1] — [1]Biotechnology Center of the TU Dresden — [2]Max Planck Institute of Molecular Cell Biology and Genetics

The refractive index is an inherent property of biological cells. The 3D distribution of the refractive index within a cell determines its light scattering properties. It is possible to reconstruct the refractive index distribution, and thus the internal structure of a cell, from scattering data using optical diffraction tomography (ODT). ODT combines computerized tomography (CT) with the Born approximation to address the wavelike propagation of light through a specimen. In ODT, the phase of the scattered field behind a rotating specimen is measured. From these 2D phase images one can reconstruct the 3D refractive index map of the specimen. Here, we demonstrate quantitative 3D ODT imaging of single biological cells with sub-cellular resolution. The technique does not require a marker and the acquisition of images is contact-free. In this particular study, suspended cells are held in an optical trap and rotated using microfluidic flow or, all-optically, by means of an optical cell rotator (OCR). The phase of the scattered field is measured using digital holographic microscopy (DHM), a quantitative phase microscopy technique. The resulting 3D refractive index map allows us to determine properties such as cell volume, dry mass, or density and refractive index of sub-cellular compartments. Many future applications in biology and medicine are envisioned.

BP 1.5 Mon 10:45 H 1028

**Wide-field fast frame-rate FLIM and simultaneous FCS for applications in molecular biology** — •Csongor Keuer[1], Danilo Bronzi[2], Marco Vitali[1], Franco Zappa[2], Cornelia Junghans[1], Thomas Friedrich[1], and Franz-Josef Schmitt[1] — [1]Institute of Chemistry, Bioenergetics, TU Berlin, Straße des 17. Juni 135, D-10623 Berlin, Germany — [2]Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy

The application of a 64x32 SPAD-based CMOS image sensor for single molecule spectroscopy is presented. Fluorescence amplitudes and lifetimes are measured simultaneously with correlation of fluorescence singal for each pixel. The system is suitable to spatially resolve diffusion constants and concentrations of single molecules in total internal reflection (TIR) excitation of the sample. An image splitter has been added to simultaneously perform both FLIM and FCS of diffusing fluorophores in aqueous solution in two wavelength or polarization channels. The system is suitable for particle size measurement, fast concentration determination and imaging of molecular dynamic processes.

BP 1.6 Mon 11:00 H 1028

**Label-free optical detection of single proteins** — •Katharina König, Marek Piliarik, and Vahid Sandoghdar — Max Planck Institute for the Science of Light, Erlangen, Germany

Detection of small amounts of biomolecules down to the single molecule level is highly desirable in a variety of fundamental and technological investigations. Conventional modern methods rely on strong fluorescence or absorption properties of marker molecules. However, labeling strategies have many disadvantages since they are nontrivial to implement and can often alter the behavior of analyte molecules. In this work we report on the direct, optical detection of single proteins without the need for any labels. This sensitivity is achieved via interferometric detection of the scattering (iSCAT) from a single protein. By combining this approach with microfluidics and functionalized surfaces, we are able to count single protein binding events and localize their positions with a precision of 5 nm. A wide-field detection arrangement and direct protein imaging capability make the method readily suitable for biomolecular analytics, outperforming previously reported label-free sensing approaches. We will show first emerging applications in ultra sensitive single-cell analysis.

**15 min break**

BP 1.7 Mon 11:30 H 1028

**Helium Ion Microscopy of Biological Cells** — •Natalie Frese[1], André Beyer[1], Matthias Schürmann[2], Barbara Kaltschmidt[2],

Christian Kaltschmidt[2], and Armin Gölzhäuser[1] — [1]Physics of Supramolecular Systems, University of Bielefeld, 33615 Bielefeld, Germany — [2]Cell Biology, University of Bielefeld, 33615 Bielefeld, Germany

In this presentation HIM images of biological cells are presented. The presented study focuses on neuronal differentiated human inferior turbinate stem cells, mouse neurons and mouse fibroblasts. The cells were prepared by critical point drying and a flood gun was used to compensate charging, so no conductive coating was necessary.

Therewith, extremely small features at native cell surfaces were imaged with an estimated edge resolution of 1.5 nm. Due to the size of the structures and the preparation methods of the cells the observed features could be an indicator for lipid rafts. This hypothesis will be discussed.

BP 1.8 Mon 11:45 H 1028

**PEEM and SEM of Magnetospirillum magnetotacticum's Magnetosome Chains** — •Christoph Keutner[1], Alex von Bohlen[2], Ulf Berges[1], Philipp Espeter[1], Claus M. Schneider[3], and Carsten Westphal[1] — [1]DELTA/Experimentelle Physik I, TU Dortmund, Maria-Goeppert-Mayer-Straße 2, 44221 Dortmund, Germany — [2]ISAS Dortmund, Bunsen-Kirchhoff-Straße 11, 44139 Dortmund, Germany — [3]PGI-6, FZ Jülich, 52425 Jülich, Germany

Magnetotactic bacteria are of great interdisciplinary interest, since a vast field of applications from magnetic recording media to medical nanorobots is conceivable. A key feature for a further understanding is the detailed knowledge about the magnetosome chain within the bacteria.

Here, we present two preparation procedures of Magnetospirillum magnetotacticum suitable for UHV experiments. These allow us to preform photoemission electron microscopy (PEEM) on magnetotactic bacteria, for the first time. We show that PEEM combined with x-ray absorption spectra (XAS) can access the magnetic particles (magentosomes) within intact magnetotactic bacteria.

By combining scanning electron microscopy (SEM) with energy dispersive x-ray spectrometry (EDX), magnetosome chains of intact bacteria become directly visible through the cell envelope. Even single magnetosomes as individual parts of the chains can be imaged.

BP 1.9 Mon 12:00 H 1028

**Visualization of subcellular temperature changes in living cells utilizing the nitrogen vacancy center** — •Torsten Rendler, Zhiqin Chu, Andrea Zappe, and Joerg Wrachtrup — 3. Physikalisches Institut, Universität Stuttgart

For mammals which can maintain thermal homeostasis, temperature is vital to their life. Visualization of temperature change on subcellular organelles in living cells thus give a basic understanding of life science such as cell metabolism, cell division and gene expression. Despite the high impact to life science brought by such measurement, developing a precise and long term reliable thermometer in living cells has not been achieved. Although intracellular temperature gradients in the range of ~ kelveins in living cells have been found, it is still under debate if the existence of such temperature gradients on cellular level is reasonable. In our study, we use the nitrogen vacancy (NV), a color center in diamond, to monitor the temporal and spatial temperature change inside living cells. Embedded in nanometer sized diamond crystals, the NV centers becomes a localized sensor, capable of measuring the local temperature independently of their environment, i.e., pH, ionic strength and surrounding biomacromolecules. Our findings indicated that nanodiamonds with NV centers can serve as a nano-scaled thermometer in cellular thermal biology.

BP 1.10 Mon 12:15 H 1028

**Three-dimensional orientation imaging of single fluorescent emitter transition dipoles** — •Narain Karedla, Anna Chizhik, Ingo Gregor, Alexey Chizhik, and Jörg Enderlein — 3. Physikalisches Institut, Georg-August-Universität Göttingen

Fluorescent molecules behave as electric dipole emitters with fixed but typically distinct excitation and emission dipole orientations. In the vicinity of dielectric or conducting surfaces, the emission properties of a dye molecule such as the emission rate (Purcell effect) and angular distribution of radiation are dramatically changed. Utilizing these effects, we develop a first-ever method, by combining radially-polarized laser scanning together with imaging on a defocused EMCCD camera in a way that provides excitation and emission patterns of each individual dye molecule, which gives us the three-dimensional orientations of both the excitation and emission dipole. Furthermore, using the orientation information of the emission dipole with respect to the interface, we localize single emitters axially from the interface with nanometer accuracy, a technique which we term as single-molecule metal-induced energy transfer (smMIET). This method is ideal for investigating the dimensionality and orientations of the TDMs of quantum dots, which are substantially modified in the vicinity of metal nanostructures.

BP 1.11 Mon 12:30 H 1028

**High resolution surface charge mapping with a scanning ion-conductance microscope** — •Lasse Hyldgaard Klausen, Thomas Fuhs, Flemming Besenbacher, and Mingdong Dong — Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark

Characterising the surface charge density (SCD) is important in interface and colloid science, and especially local variations in SCD of biological samples are of keen interest. The surface charge of lipid bilayers governs the uptake of charged particles and guides cell-cell interactions. As the electrostatic potential is screened by high physiological salt concentrations, direct probing of the potential can only be performed at a sub nanometre distance; therefore it is challenging to measure the local SCD under physiological conditions.

In this study we measure SCD using a scanning ion-conductance microscope (SICM) setup, where the electrolyte current through a nanopipette is monitored as the pipette is positioned in the vicinity of the sample. We investigate the current dependency of SCD and pipette potential using numerical solutions to Poisson and Nernst-Planck equations and characterise a complex system governed by a multitude of factors such as pipette size, geometry and charge. We then propose an imaging method and prove its feasibility by mapping the surface charge density of phase separated lipid bilayers with sub micrometre spatial resolution.

BP 1.12 Mon 12:45 H 1028

**Propagation-based phase contrast tomography of neuronal tissues** — •Mareike Töpperwien[1], Martin Krenkel[1], Jürgen Goldschmidt[2], and Tim Salditt[1] — [1]Institute for X-Ray Physics, Göttingen, Germany — [2]Leibniz Institute for Neurobiology, Magdeburg, Germany

In order to visualize the 3D native structure of neuronal tissues with cellular and sub-cellular resolution in macroscopically large volumes, hard x-ray tomography offers a unique potential beyond the current capabilities of histology. However, classical x-ray tomography based on absorption gives nearly no contrast for soft biological tissues. In order to visualize also non-absorbing or weakly absorbing structures, the much stronger phase shifts which the sample induces in a (partially) coherent wavefront can be exploited for contrast formation. During free space propagation behind the object, these phase shifts are converted to a measurable intensity image by interference of the disturbed wave fronts. Thus, the original phase distribution has to be reconstructed from the intensity images using suitable phase retrieval algorithms.

As proof-of-concept, in this work we present propagation-based x-ray phase contrast tomography of neuronal tissues. In specially dried samples, sub-cellular resolution in mm sized tissue volumes has been achieved yielding three dimensional renderings which are consistent with classical histology results. We also show that complementing the synchrotron radiation results, a compact laboratory setup with a high brilliance liquid-metal microfocus source can be used.