BP 8: Bioimaging and Spectroscopy I

Time: Monday 11:30–12:45 Location: H43

Invited Talk BP 8.1 Mon 11:30 H43

Prospects of super-resolution optical microscopy for studying membrane bioactivity — •Christian Eggeling, Marco Fritzsche, and Erdinc Sezgin — Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Molecular interactions in the plasma membrane of living cells are key in cellular signalling. Protein-protein or protein-lipid complexes, the formation of lipid nanodomains (often denoted "rafts"), or diffusional restrictions by the cortical cytoskeleton are considered to play a functional part in a whole range of membrane-associated processes. The direct and non-invasive observation of such interactions in living cells is often impeded by principle limitations of conventional far-field optical microscopes, specifically with respect to limited spatio-temporal resolution. We present how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution STED microscopy with fluorescence correlation spectroscopy (STED-FCS). We will focus on new insights into the lipid "raft" theory, and on the role of plasma membrane and cytoskeleton organization in the triggering of immune cells, specifically during T-cell activation.

BP 8.2 Mon 12:00 H43

Label-free high contrast superresolution microscopy at 100Hz using coherent dark field illumination in TIR mode (TIR-CODAF) — •Dominic Ruh, Felix Jünger, Philipp von Olshausen, and Alexander Rohrbach — University of Freiburg, Department of Microsystems Engineering - IMTEK, Laboratory for Bio- and Nano-Photonics, Germany

Cells are the smallest units of life. A variety of forces constantly act on these complex systems, pushing them out of equilibrium and causing a manifold of signaling events. The investigation of fast dynamic processes in living cells resulting from these forces require fast measurement techniques - faster and more precise than currently available. The technique presented in this work uses a laser beam that illuminates a living, unlabeled cell under an oblique angle. The monochromatic light is multiply scattered at the cellular structures yielding an image of the cell on the camera that is strongly distorted by speckles. However, by sweeping the laser beam along a circular path in the back focal plane of the objective lens during the integration time of the camera (e.g. only some milliseconds), the speckles cancel out and a high contrast image of the cell is obtained. We call this new technique *total internal reflection coherent dark field (TIR-CODAF) microscopy*. In this talk we present a resolution of 150 nm at a frame rate of 100 Hz. Since TIR-CODAF does not rely on fluorescence many thousands of images without visible loss of image quality can be acquired.

BP 8.3 Mon 12:15 H43

A novel membrane label for STED nanoscopy of living cardiomyocytes — •Elke Hebisch[1], Stephan E. Lehnart[2], and Stefan W. Hell[1] — [1]Max Planck Institute for Biophysical Chemistry, Department NanoBiophotonics, Am Fassberg 11, 37077 Goettingen, Germany — [2]Research Unit for Cellular Biophysics and Translational Cardiology, Heart Research Center Goettingen, Robert-Koch-Str. 40, 37099 Goettingen, Germany

In heart muscle cells the fast and cell-wide propagation of rhythmic action potentials crucially depends on the architecture and composition of the plasma membrane (sarcolemma) and its extensive invaginations that form a transverse-axial tubular system (TATS). Here we report on the first application of the novel fluorescent membrane label cholesterol-PEG-KK114 (Chol-KK114) for STED nanoscopy of living mouse cardiomyocytes. Chol-KK114 enables fast and nontoxic in vivo labeling of cholesterol-rich cardiac membrane nanodomains. We could observe complex sarcolemmal and intracellular cholesterol signal patterns representing nanodomains sized far below the confocal resolution limit. These signal patterns are rich in detail and highly cell type specific since they could not be observed in HeLa or PtK2 cells. On the sarcolemma, we identified individual cholesterol-rich membrane nanodomains and higher order arrangements into ring structures and patches. Conclusively, we established a novel membrane label for superresolution microscopy of nanodomains in living primary cells.

BP 8.4 Mon 12:30 H43

Low-intensity STED microscope with increased image brightness and uncompromised resolution — •Jennifer-Rose Schubert, Claudia Geisler, Britta Vincon, and Alexander Egner — Optical Nanoscopy Dept., Laser-Laboratory, D-37077 Göttingen

Far-field optical microscopy represents a well-established method in the life sciences. Due to diffraction, the resolution is limited to $\sim \frac{\lambda}{2}$ in the focal plane. This constraint can be surpassed by nanoscopic techniques [1]. Amongst others, STED microscopy provides a resolution of up to 20 nm [2]. By definition, resolution enhancement in STED microscopy is achieved by narrowing the effective fluorescent area [3]. This reduction of detection volume depends on the factor of resolution enhancement and is directly linked to a decrease in fluorescence signal which limits the acquisition rate in many cases.

Here, we present a STED technique based on a rotating 1D depletion pattern. This novel STED variant can achieve a higher resolution for a given depletion light intensity as compared to the classical implementation. Furthermore, the overall fluorescence signal detected is higher than for conventional STED microscopy working at the same resolution. Consequently, not only identical super resolution conditions can be realized at lower depletion laser powers but also the acquisition can be sped up. Moreover, both aspects have the potential to drastically reduce photobleaching and sample damage.

[1] Huang, B. et al., Annu. Rev. Biochem., 78, 993-1016 (2009)
[2] Harke, B. et al., Opt. Express, 16, 4154-4162 (2008)
[3] Hell, S. W., Science, 316, 1153-1158 (2007)