BP 7: Superresolution Optical Microscopy (focus session)

Time: Monday 14:30–17:15 Location: H 1028

Invited Talk BP 7.1 Mon 14:30 H 1028

Super-resolution imaging of small, fast moving cellular structures — •Alexander Rohrbach — Lab for Bio- and Nano-Photonics, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany

Many new, exciting imaging techniques have emerged during the last decade, providing significantly improved spatial resolution and contrast. However, this extra information comes at the cost of more photons required to illuminate the cell, which requires more time and energy and often damages biological structures. The smaller the structures to be investigated, the faster they usually move inside living cells, because of both Brownian motion and coordinated work of molecular motors. Therefore, alternative imaging approaches have to be developed. In this talk I will demonstrate how fluorescence-based super resolution microscopy uncovers the work of polymerization motors driving the cytoskeleton in bacteria. I will make the switch to ultra- fast, label-free, coherent imaging through scattering of a rotating laser beam, which reveals unexpected biophysical transport processes at the periphery of macrophages. And last, I will show how fast shape changes of a tiny helical bacterium held in a scanning optical trap can be monitored in 3D at 1000 Hz giving insights of molecular processes inside the 200 nm thin cell body.

BP 7.2 Mon 15:00 H 1028

Molecular architecture of native fibronectin fibrils — •Ingmar Schoen[1], Susanna Früh[1], Jonas Ries[2], and Viola Vogel[1] — [1]ETH Zurich, Zurich, Switzerland — [2]European Molecular Biology Laboratory, Heidelberg, Germany

Fibronectin fibrils within the extracellular matrix play central roles in regulating cell anchorage and behavior, particularly in early development, wound healing, but also in cancer and other pathologies. However, their hierarchical structure at the molecular level remained elusive. Using single-molecule localization microscopy combined with site-specific labeling techniques, we found that the most elemental fibronectin protofibrils consist of overlapping dimeric fibronectin molecules that show a quasi-periodic order. The spatial autocorrelation of regular, punctate label patterns along these fibrils yielded an average spacing of ca. 95 nm which was consistent for different antibody epitopes along the fibronectin molecule. Dual-color cross-correlation revealed alternating N- and C-terminal regions. Single end-labeled fibronectin molecules incorporated into protofibrils displayed an average end-to-end distance of ca. 133 nm. Together, these results suggest a staggered arrangement with an antiparallel 30-40 nm broad overlap of the N-termini of adjacent molecules involving the first five type I and type III repeats of each molecule. While demonstrated here using fibronectin fibers, this powerful super-resolution approach can be extended to elucidate the build-up of other filamentous protein structures in their physiological environment.

BP 7.3 Mon 15:15 H 1028

Quantitative Analysis of Nuclear Genome Nanostructure using Super-resolution Fluorescence Microscopy — •Christoph Cremer[1,2,3], Aleksander Szczurek[1], Hyun Keun Lee[1,3], Kirti Prakash[1,2], and Udo Birk[1,3] — [1]Institute of Molecular Biology (IMB), D-55128 Mainz/Germany — [2]Institute for Pharmacy and Molecular Biotechnology (IPMB), University Heidelberg & Kirchhoff-Institute for Physics (KIP), D-69120 Heidelberg/Germany — [3]Department of Physics, University Mainz (JGU), D-55128 Germany

Numerical models of nuclear genome structure have provided quantitative predictions on various length scales, from the micrometer to the nanometer range. Until recently, experimental tests of such models using far field light microscopy were limited by the conventional resolution limit of about 200 nm in the object plane and 600 nm along the optical axis (*Abbe/Rayleigh-limit*). These limits have been overcome by various super-resolution fluorescence microscopy (SRM) methods, such as Stimulated Emission Depletion (STED) and Photoactivated Localization Microscopy (PALM). Here, we report on quantitative nuclear nanostructure analysis based on complementary SRM approaches. Presently, these approaches allow us to analyze nuclear nanostructures down to few tens of nanometer in 3D using a special variant of localization microscopy, Spectral Precision Distance/Position Determination Microscopy (SPDM).

BP 7.4 Mon 15:30 H 1028

Recent Advances in Pattern Matching based Multi-Species FLIM Analysis — •Felix Koberling[1], Benedikt Krämer[1], Thomas Niehörster[2], Anna Löschberger[2], Marcelle König[1], Paja Reisch[1], Matthias Patting[1], Ingo Gregor[3], Markus Sauer[2], and Rainer Erdmann[1] — [1]PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany, info@picoquant.com — [2]Julius-Maximilians-University Wuerzburg, Germany, Department of Biotechnology & Biophysics, Am Hubland, 97074 Wuerzburg, Germany — [3]Georg-August-University Göttingen, 3rd Institute of Physics, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

A pattern matching based fluorescence decay analysis is a successful alternative to multi-exponential decay fitting and to the phasor analysis approach. We describe the complex single pixel decay with a linear combination of reference decays (patterns) and calculate analytically the individual amplitudes for the reference patterns followed by a re-iteration with MLE fitting. This procedure is now further accelerated by a purely analytical vector projection based method. We will compare the different approaches for multi-species FLIM analysis and present latest application examples also combining spectral and lifetime information for a multidimensional fluorescence pattern analysis.

15 min break

BP 7.5 Mon 16:00 H 1028

Superresolution with Transient Binding: Getting all the Photons — •Philip Tinnefeld — Institut für Physikalische & Theoretische Chemie, TU Braunschweig, Germany

The resolution of localization based superresolution microscopy is intricately connected to the number of photons detected from a single molecule per localization event. We developed DNA PAINT, a superresolution technique with single-molecule switching induced by transient binding of a short labeled oligonucleotide to structures labeled with a complementary nucleic acid strand. Because no photophysical dark-states are involved, the maximum number of photons can be extracted from each fluorescent dye. With DNA PAINT, we resolved two distinct locations (docking strands) at a distance of 6 nm on DNA origami nanostructures. Besides resolution, DNA PAINT offers exquisite multiplexing capabilities without inducing chromatic aberrations. Just by using different sequences, different structures can be imaged. As the number of detectable photons is ultimately connected to the photostability of fluorescent dyes we will also present a new single-protectant mechanism that represents an advancement over the established ROXS-concept for photostabilization and blinking.

BP 7.6 Mon 16:15 H 1028

Optical nanoscopy with self-healing fluorophores — Jasper H. M. van der Velde, Jingyi Huang, Andreas Herrmann, and •Thorben Cordes — Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands

Customized buffer cocktails are so far the method of choice to facilitate photoswitching and to enhance signal stability for various implementations of optical super-resolution microscopy - a strategy that is not applicable for live-cell imaging.

In this contribution, we tested organic fluorophores with intramolecular photostabilization in STED-type microscopy to improve spatial and temporal resolution without using buffer additives. To obtain fluorophore-photostabilizer conjugates we use a recently published synthesis strategy based on unnatural amino acids. We explored the photostability and achievable spatial resolution of single ATTO647N-labelled oligonucleotides in STED imaging. We further tested KK114-conjugates for antibody labelling and STED imaging of the nuclear pore complex with the aim to increase the number of possible subsequent STED images. Finally, KK114-, Bodipy-Fl, and RhodamineB-derivatives were tested in STED-FCS. Our results show that intramolecular photostabilization is a simple and effective method to increase the photostability of dyes used for STED to achieve high spatial and temporal resolution. Strikingly, ATTO647N-photostabilizer conjugates allowed to generate single-molecule fluorescent time traces with excitation and STED-laser turned on, something that could so

far not be achieved with standard fluorophores.

BP 7.7 Mon 16:30 H 1028

Cryo-Fluorescence Microscopy of Single Molecules — •Weixing Li, Simon Stein, Ingo Gregor, and Joerg Enderlein — DPI, Georg-August-University Goettingen, Germany

The super-resolution fluorescence localization microscopy methods such as Stochastic Optical Reconstruction Microscopy (STORM), Photoactivation Localization Microscopy (PALM), or Ground State Depletion Imaging (GSDIM), routinely achieve a lateral image resolution of ~ 30 nm. This resolution is directly related to the number of photons emitted from a single fluorescent molecule and roughly scales with the invers square root of the detected photon number. Therefore, photo-bleaching is the fundamental bottleneck that limits the achievable resolution. One method to suppress photo-bleaching is to cool a sample down to cryogenic temperatures. For that purpose, we designed and built a dedicated cryostat suitable for single molecule fluorescence microscopy. The system is not only capable of cooling the sample to cryogenic temperatures, but gives also optical access to the sample for high-quality imaging with a conventional microscope employing an objective with high numerical aperture. Another important property of our system is its excellent mechanical stability, enabling long-time observations of samples over several hours with negligible drift. Using our system, we successfully performed photo-bleaching studies on single molecules showing a more than two order of magnitude enhancement in photo-stability, which results in an exceptional molecular localization accuracy in angstrom scale.

BP 7.8 Mon 16:45 H 1028

Optimizing STED Performance — •Marcelle Koenig, Rhys Dowler, Benedikt Kraemer, Felix Koberling, Sebastian Tannert, Matthias Patting, and Rainer Erdmann — PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany, info@picoquant.com

Stimulated Emisson Depletion (STED) microscopy is becoming a standard technique in biological imaging, reaching an optical resolution far below 100*nm. The improvement in optical resolution can be achieved with different optical tools and data acquisition, as well as data processing workflows. These have a significant influence not only on the optical resolution itself but also on the general applicability of the technique for specific labels, specimen and phenomena to be studied. We present results based on a confocal microscope which was upgraded with an EASYDOnut phaseplate to convert the STED laser beam into the required donut-shaped focal spot while leaving the co-aligned excitation beam unaffected [1]. On the way towards suitable imaging conditions, various experimental modalities to minimize irreversible photobleaching and to improve photon statistics will be discussed. Different analysis methods based upon the arrival times of photons have also been compared in order to sharpen the images and to suppress unwanted contributions in addition to spectral filtering. Multilabel STED with only one depletion wavelength can be achieved by employing spectral as well as temporal information which act as a fingerprint for individual dyes. Pattern Matching analysis allows for a fast and simple separation of the different fluorescent labels.

BP 7.9 Mon 17:00 H 1028

A Scanning Cavity Microscope — •Matthias Mader[1,2], Jakob Reichel[3], Theodor W. Hänsch[1,2], and David Hunger[1,2] — [1]Ludiwgs-Maximilans-Universität München, Fakultät für Physik, Schellingstraße 4, 80799 München — [2]Max-Planck-Institut für Quantenoptik, Hans-Kopfermann-Straße 1, 85748 Garching — [3]Laboratoire Kastler Brossel, ENS/UPMC-Paris 6/CNRS, 24 rue Lhomond, 75005 Paris

We present a versatile tool for ultra-sensitive and spatially resolved optical characterization of single nanoparticles.

Using signal enhancement in a scanning optical microcavity made of a micromachined optical fiber and a plane mirror [1] we measure the polarization dependent extinction of a single nanoparticle as well as its birefringence. Harnessing multiple interactions of probe light with a sample within the optical resonator, we achieve a 1700-fold signal enhancement compared to diffraction-limited microscopy. We demonstrate quantitative imaging of the extinction cross section of gold nanoparticles with a sensitivity below 1 nm^2, we show a method to improve spatial resolution potentially below the diffraction limit by using higher order cavity modes, and we present measurements of the birefringence and extinction contrast of gold nanorods [2].

[1] D. Hunger, T. Steinmetz, Y. Colombe, C. Deutsch, T. W. Hänsch and J. Reichel, New J. Phys. 12, pp. 065038 (2010)

[2] M. Mader, J. Reichel, T. W. Hänsch and D. Hunger, arXiv preprint arXiv:1411.7180 (2014)