BP 5: Bioimaging and Biospectroscopy I

Time: Monday 15:00–17:15 Location: HÜL 386

Invited Talk BP 5.1 Mon 15:00 HÜL 386

Mirror-enhanced fluorescence for superresolution imaging and spectroscopy — •Katrin G. Heinze[1], Hannah S. Heil[1], Benjamin Schreiber[1], and Markus Sauer[2] — [1]Rudolf Virchow Center, University of Würzburg, Würzburg, Germany — [2]Biocenter of the University of Würzburg, Würzburg, Germany

The "Resolution Revolution" in fluorescence microscopy over the last decade has given rise to a variety of techniques that allow imaging with resolution up to the nanometer range. One remarkable technique is direct stochastic optical reconstruction microscopy (dSTORM), a widely-used type of single molecule localization microscopy (SMLM). The key point here is the achievable localization precision, which mainly depends on the image contrast generated by the individual fluorophore*s emission. We found that reflective metal-dielectric nanocoatings represent a tunable nano-mirror that can do both quenching and boosting fluorescence for high-contrast imaging on the nanoscale. The enhanced resolution is a near-field effect and thus restricted to surface imaging; however, most membrane fluorescence applications benefit, even if classic resolution is not the main concern: Spectroscopic methods in live-cells such as Fluorescence Correlation Spectroscopy and Fluorescence Resonance Energy Transfer also belong to the scope of application. Mirror-enhanced fluorescence is different from other surface methods based on total internal reflection microscopy or optoplasmonics. While surface-plasmon supported methods provide much higher enhancement factors, mirror-enhanced approaches are more versatile and thus highly suitable for modern bio-imaging.

BP 5.2 Mon 15:30 HÜL 386

Trans-membrane Fluorescence Enhancement by Carbon Dots: Energy Transfer, Ionic Effects and pH Dependence — •Florian H. Huber[1], Stefanie D. Pritzl[1], Santanu Bhattacharyya[1], Fernando Pschunder[2], Maria Ana Huergo[2], Theobald Lohmüller[1], and Jochen Feldmann[1] — [1]Chair for Photonics and Optoelectronics, Nano-Institute Munich and Department of Physics, Ludwig-Maximilians-Universität (LMU), Königinstr. 10, 80539 Munich, Germany — [2]INIFTA, Universidad Nacional de La Plata - CONICET, Sucursal 4 Casilla de Correo 16, 1900 La Plata, Argentina

Improved optical biosensors that are sensitive to the membrane potential of cells are highly desirable for imaging applications and microscopy studies of membrane systems. Here, we analyse how transmembrane energy transfer between fluorescent carbon dots (CDs, size ~1.0 - 1.5 nm) and fluorescein labelled phospholipid molecules across the bilayer membrane of giant phospholipid vesicles is influence by ionic interactions and the pH. A system has been designed, where positively charged CDs and negatively charged fluorescein lipids co-localize across a bilayer membrane due to electrostatic attraction [1]. By performing absorbance, photoluminescence (PL), and fluorescence life time decay measurements, we find that ionic interactions do not only facilitate energy transfer, but also result in a PL enhancement of the dye, which is further adjustable by the pH of the vesicle suspension. [1] S.D. Pritzl, F. Pschunder, F. Ehrat, S. Bhattacharyya, T. Lohmueller, M. Huergo, J. Feldmann, Nano Letters, 19 (6), 3886-3891, 2019

BP 5.3 Mon 15:45 HÜL 386

Near Infrared Imaging and Sensing with Carbon Nanotubes — •Sebastian Kruss — Universität Göttingen, Göttingen, Deutschland

Carbon nanomaterials such as semiconducting single-walled carbon nanotubes (SWCNTs) are versatile building blocks for optical biosensors and labels. SWCNTs fluoresce in the near infrared (nIR, 900-1700 nm) and their optoelectronic properties are very sensitive to changes in the chemical environment but a) achieving high selectivity and sensitivity and b) targeting or delivering those sensors to specific locations in cells or organisms is still a great challenge. Therefore, we use novel chemical and physical approaches to tailor SWCNTs. In the past years we have made substantial progress in the chemical design and used it to image complex processes in biological systems. 1. We tailored the corona phase around SWCNTs to enhance selectivity and photophysics of SWCNT-based sensors for the neurotransmitters dopamine and serotonin. Such sensors were used to image release of these signaling molecules from cells (neurons, blood platelets) with unprecedented spatiotemporal resolution. 2.SWCNTs were conjugated to nanobodies that can be targeted to any Green Fluorescent Protein (GFP) moiety. These SWCNTs were used for in vivo tracking of single kinesin motors and microrheology measurements in living drosophila embryos. 3. Peptides were incorporated into the organic corona phase around SWCNTs to target cell surface receptors. This approach enabled us to label for the first time integrins on human blood platelets in the nIR.

BP 5.4 Mon 16:00 HÜL 386

Stimulated Raman scattering microscopy of biomedical systems — •Moritz Floess, Florian Werner, Tobias Steinle, and Harald Giessen — 4th Physics Institute and Research Center SCoPE, University of Stuttgart, Germany

We employ stimulated Raman scattering (SRS) microscopy as a label-free and chemically selective imaging technique to investigate a system of pectin and porcine pleura. This model system is of high interest to improve surgical treatment of lung injuries. SRS is based on addressing molecular vibrational states using two pulsed laser beams with a variable frequency detuning. Hereby, an 8-W, 1032-nm, 450-fs Yb:KGW oscillator with 41 MHz repetition rate serves as the Stokes beam and simultaneously as the pump source for an optical parametric oscillator (OPO). The frequency-doubled OPO output provides the tunable Raman pump beam. Thanks to the favorable noise properties of the solid-state laser system we can address distinctive Raman bands in the fingerprint region with a high signal-to-noise ratio.

15 min. coffee break

BP 5.5 Mon 16:30 HÜL 386

Motion-based segmentation for particle tracking: A fully-convolutional neuronal network that analyses movement — •Till Korten[1], Walter de Back[2], Christoph Robert Meinecke[3], Danny Reuter[3,4], and Stefan Diez[1] — [1]B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden, Dresden, Germany — [2]Institute for Medical Informatics and Biometry (IMB), Carl Gustav Carus Faculty of Medicine, Technische Universität Dresden, Dresden, Germany — [3]Center for Microtechnologies, TU-Chemnitz, Chemnitz, Germany — [4]Fraunhofer Institute for Electronic Nanosystems (ENAS), Chemnitz, Germany

For single-particle tracking it is often necessary to separate particles of interest from background particles based on their movement pattern. Here we introduce a deep neuronal network that employed convolutional long-short-term-memory layers in order to be able to perform image segmentation based on the motion pattern of particles. Training was performed with ≈ 500 manually annotated 128x128 pixel frames. The segmentation result was used as input for a conventional single particle tracking algorithm. With this workflow 100% of all tracks belonged to microtubules that were propelled by kinesin-1 motor proteins along guiding channels and no tracks belonged to microtubules diffusing in the background. Furthermore, microtubules moving in a different orientation than the guiding channels during training, did not show up during inference. In conclusion, the deep-learning-based tracking resulted in almost twice as many (2800 vs. 1500) usable tracks that were 35 % longer compared to filtering after tracking.

BP 5.6 Mon 16:45 HÜL 386

Self-organization of endoplasmic reticulum exit sites — •Konstantin Speckner, Lorenz Stadler, and Matthias Weiss — Experimentalphysik 1, Universität Bayreuth

The endoplasmic reticulum (ER) is a highly dynamic organelle that pervades the entire cell and hosts a variety of vital processes. For example, the exchange of proteins with the secretory pathway occurs at specialized and long-lived membrane domains, called ER exit sites (ERES). In mammalian cells, ERES form droplet-like protein assemblies that arrange as hundreds of dispersed punctae with a quasi-crystalline ordering. Although ERES were seen to diffuse on short timescales, they appear stationary on longer periods. Notably, their dynamics is different from the cytoskeleton-dependent, shivering motion of ER tubules. To gain insights into the underlying physical mechanisms of ERES self-organization, we have studied the emerging pattern of ERES when perturbing the ER morphology in different ways. As a result, we found a significantly changed spatial arrangement of

ERES components when affecting the cytoskeletons integrity or reducing the amount of curvature-inducing membrane proteins. Even more pronounced changes were observed when the ER was transformed into vesicular structures by osmotic swelling. Our findings strongly indicate that the self-organization of ERES on the ER membrane system is caused by a diffusion-driven condensation phenomenon, similar to a liquid-liquid phase separation.

BP 5.7 Mon 17:00 HÜL 386

Particle tracking via an electrically focus tunable lens (ETL) — •Oliver Köhn and Christian Wagner — Universität des Saarlandes

We describe a method for the 3D tracking of particles using an electrically focus tunable lens (ETL). Applying a current to the ETL adapts the focal plane of the lens, allowing us to track particles in three dimensions without any mechanical interactions with the sample. Based on the sharpness of the tracked particles we are able to recalculate the current z-position of the particle. This calculation can be performed during the tracking-process, enabling us to perform live-tracking of particles. We show how this tracking-method can be applied to track 1.) bacteria (Bacillus subtilis) and 2.) um-sized beads, both for several minutes.