BP 31: Imaging

Time: Friday 9:30–11:30 Location: H 1058

Topical Talk BP 31.1 Fri 9:30 H 1058

High-speed imaging of organogenesis in entire zebrafish with SPIM — •Jan Huisken — Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

In the past the recording speed of a time-lapse experiment has ultimately been limited by the amount of light the specimen could tolerate. Lately, it has been shown that light sheet microscopy such as Selective Plane Illumination Microscopy (SPIM) reduces photo-toxic effects to a minimum. Due to the illumination of the sample in a thin volume around the focal plane no tissue outside the plane of interest is exposed and bleached. In addition, the fluorescence is collected with very high sensitivity cameras. SPIM benefits from the latest camera technology and is therefore constantly improving in speed and sensitivity.

Experiments have become possible that run at full speed using the best possible hardware, without being limited by the fragility of the sample. The speed advantage of the SPIM over other fluorescence technique can be utilized to image rapid events in developing tissue or to record a large number of views for multi-view reconstruction. The large amount of data that is accumulated when modern cameras are run at high-speed for hours or days is enormous and innovative data processing solutions are needed. One key application of this emerging technology includes the multi-dimensional imaging of the developing zebrafish larvae over extended periods of time. I will give some examples of the unique capabilities of SPIM, especially for monitoring the development of the cardiovascular system and the early endoderm.

M. Weber and J. Huisken, Curr Opin Genet Dev, 21, 566-72 (2011).

BP 31.2 Fri 10:00 H 1058

Revival of prism-based TIR microscopy - Versatile tracking of fluorescent and scattering probes with nm-precision — •René Schneider and Stefan Diez — B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden

Current single-molecule microscopy experiments mostly rely on the fluorescence signal of fluorescent proteins, organic dyes or quantum dots attached to proteins of interest. However, these probes suffer from limitations, namely limited number of photons before photobleaching, photon blinking, and fluorescence saturation. Consequently, the temporal and spatial resolution by which single molecules can be tracked is limited. Promising candidates for replacing fluorescent probes in-vitro are gold nanoparticles (GNPs). GNPs exhibit a large scattering cross section in the optical spectrum due to plasmon resonance, provide long-term stability and allow for versatile surface chemistry. Furthermore, their emission rate does not saturate.

Here, we present a camera-based wide-field imaging technique for GNP-labeled proteins using a novel parabolic prism-type total-internal reflection (TIR) microscope. We demonstrate the advantages of GNPs over commonly used fluorescent probes and discuss the pros and cons of prism-type versus objective-type TIR microscopy. We demonstrate that prism-based TIR microscopy allows imaging of fluorescent and scattering probes with high signal-to-noise and excellent control over a wide range of incidence angles.

Our method allows for precise localization of biomolecules within short acquisition times over long time scales.

BP 31.3 Fri 10:15 H 1058

Cryogenic Colocalization with Nanometer Resolution — •Siegfried Weisenburger and Vahid Sandoghdar — Max Planck Institute for the Science of Light, 91058 Erlangen, Germany

The advent of super-resolution microscopy methods in the past decade caused a stir in the fluorescence microscopy community [1]. In particular, wide-field localization microscopy has pushed the resolution by more than one order of magnitude. Here, the centers of the point-spread functions of individual fluororescent molecules are determined with very high accuracy only limited by the number of collected photons [2]. At room temperature, the localization accuracy typically reaches a few tens of nanometers restricted by photobleaching. From low-temperature optical studies of single biomolecules, it is known that fluorophores are much more photostable at cryogenic temperatures [3] allowing for the detection of more photons. To overcome the accuracy limitation given by photobleaching, we introduce a new method of colocalization microscopy utilizing liquid helium temperature.

We will demonstrate our technique by colocalization measurements of a double-stranded DNA which is specifically labeled with two fluorescent molecules at a distance of ten nanometers. Furthermore, we will discuss the perspectives of this method for other biological applications.

1) S. Hell, *Nat. Methods* **6**, 24 (2009).
2) R. Thompson et al., *Biophys. J.* **82**, 2775 (2002).
3) R. Zodervan et al., *J. Phys. Chem. A* **108**, 1657 (2004).

BP 31.4 Fri 10:30 H 1058

Multimodal imaging of the human cerebellum: phase contrast tomography, magnetic resonance microscopy and histology — •Georg Schulz[1], Timm Weitkamp[2], Irene Zanette[3,4], Franz Pfeiffer[4], Conny Waschkies[5], Christian David[6], and Bert Müller[1] — [1]BMC, Uni Basel, Switzerland — [2]Synchrotron Soleil, Gif sur Yvette, France — [3]ESRF, Grenoble, France — [4]Biomedical Physics, TUM, Garching, Germany — [5]IBT, ETH / Uni Zurich, Switzerland — [6]LMN, PSI, Villigen, Switzerland

To visualize a part of the human cerebellum we use grating based phase contrast CT, MR microscopy and histology. Phase contrast tomography yields the 3D distribution of the X-ray refractive index and is much more sensitive than conventional absorption imaging. Using a grating interferometer we are able to detect deflection angle differences of around 20 nrad. The CT results were acquired at ESRF Grenoble using a 5 μm pixel size detector. The high sensitivity of the method even provides contrast between structures within the gray matter. The MR microscopy data set was acquired using a T2*-weighted 3D FLASH sequence with an isotropic pixel length of 45 μm. The method allows a clear differentiation between white and gray matter but shows marginal contrast between the two structures within the gray matter. During histology a Nissl staining was applied. While the micrographs have a high spatial resolution of less than a μm and a contrast comparable to the X-ray phase contrast data, they do not provide 3D information. The complementarity of X-ray phase-contrast CT, MR microscopy and histology gives better insights into the morphology of the human brain.

BP 31.5 Fri 10:45 H 1058

Planar structured AlGaN/GaN High Electron Mobility Transistor sensor for recording of Physarum cell activity — •Hartmut Witte[1], Thomas Lippelt[1,2], Christian Warnke[1,2], Marcus J. B. Hauser[2], and Alois Krost[1] — [1]Otto-von-Guericke-Universität Magdeburg, Inst. Exp. Phys., Abt. Halbleiterepitaxie — [2]Otto-von-Guericke-Universität Magdeburg, Inst. Exp. Phys., Abt. Biophysik

Planar multi-electrode AlGaN/GaN High Electron Mobility Transistors (HEMTs) arrangements are very useful for spatial and time resolved stimulation and recording of extended and excitable biological cultures such as neurons or yeast cells. In this contribution, the spatially resolved sensitivity of a multiple AlGaN/GaN HEMT structure is used for detection of migration and growth of Physarum cells in situ. Physarum polycephalum displays remarkably *intelligent* abilities inducing intensive studies for instance on learning and on memory of past events. We have investigated the impact of Physarum cell motion on the source-drain current and impedance of a sensor structure presented by ten circular arranged AlGaN/GaN HEMTs described in detail in Witte et al; J. Phys. D, 44, 355501 (2011). All time tracks were correlated with video pictures of the area around the actual HEMT and the Physarum cell. So, we detected the cell growing across a HEMT sensor. Based on the detailed analysis of the sensor/medium interface we are able to distinguish between the signals from the cell and the medium. Additionally, the cell dispersions were investigated by impedance spectroscopy for more information about the properties of inner structures.

BP 31.6 Fri 11:00 H 1058

4D imaging: a versatile suite for image analysis — •Bhavna Rajasekaran[1], Jean-Yves Tinevez[2], Koichiro Uriu[3], Guillaume Valentin[1], Frank Jülicher[3], and Andrew Oates[1] — [1]Max Planck Institute for Cell Biology and Genetics, Dresden, Germany — [2]Institut Pasteur, Paris, France — [3]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

Fluorescence microscopy can capture in vivo time-space visualization of cellular dynamics, changes in spatio-temporal pattern of gene ex-

pression within cellular structures, tissue growth and morphogenesis. Computational image analysis techniques serve to translate such image data into meaningful quantitative measurements that can further be analyzed and understood to draw precise description of a biological phenomena or allow hypothesis testing. Here, we demonstrate novel computationally efficient 3D nuclei segmentation algorithm based on image derivatives combined with semi-automated method for post rectification of segmented data to reliably extract individual cell identity and track cells over time based on nearest neighborhood for the developing pre-somitic mesoderm (PSM) tissue in the zebrafish embryo. The PSM undergoes rigorous morphological changes and has juxtaposed cells that exhibit continuous diverse and dynamic cell motions, thus providing a technically challenging platform for image analysis. We use synthetic data and transgenic chimeric embryos to assess and validate the performance of the algorithm. Algorithm development and testing was done in Matlab 7.10.0 (R2010a) and exported to the Fiji library- an open source, user-friendly platform for biological image analysis.

BP 31.7 Fri 11:15 H 1058

In-focus phase contrast electron cryo-microscopy of biological samples with an electrostatic phase plate — •Daniel Rhinow[1], Andreas Walter[1], Manfred Lacher[2], Siegfried Steltenkamp[2], Sam Schmitz[2], Peter Holik[2], and Werner Kühlbrandt[1] — [1]Max-Planck-Institut für Biophysik, 60438 Frankfurt, Deutschland — [2]caesar research center, 53175 Bonn, Deutschland

Although the instrumental resolution limit of the latest generation of transmission electron microscopes reaches 0.5 Å, a variety of physical factors limit the experimental resolution achievable with biological samples. Biological macromolecules are pure phase objects that are visualized by phase contrast, which in conventional cryoEM is generated by defocusing. Disadvantages of defocusing are weak contrast and incomplete transfer of object information, which impairs data collection and 3D reconstruction. A powerful alternative to defocus phase contrast is the use of a physical phase plate in the back focal plane of the electron microscope. The Boersch phase plate (BPP) comprises an electrostatic einzel lens shifting the phase of the unscattered electron beam by 90°, thus maximizing phase contrast for in-focus TEM. The PACEM (Phase Contrast Aberration-Corrected Electron Microscope) is a TEM prototype developed by Carl Zeiss NTS in collaboration with the MPI of Biophysics. BPPs have been tested in the PACEM. First BPP images of stained, unstained, and cryogenic biological specimens have been obtained.