BP 13: Posters: Imaging and Superresolution Optical Microscopy

Time: Monday 17:30–19:30 Location: Poster A

BP 13.1 Mon 17:30 Poster A

Detecting rare Events for sure: Stitched Field-of-view Imaging on the Intelligent Programmable Array Microscope (iPAM) — •Stephan Kramer, Anthony de Vries, Nathan Cook, Donna Arndt-Jovin, and Thomas Jovin — Labor f. Zelluläre Dynamik, Max-Planck-Institut f. biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen

Imaging rare events by confocal microscopy in populations of live cells requires a macroscopic field of view (FOV) of several millimeters in diameter. Standard laser scanning confocal microscopes or spinning disk systems are too slow to capture FOVs that large. Using the scripting capabilities of the driving software of our iPAM [1,2] we are able to seamlessly image regions of the size of 1 mm^2. The total FOV is subdivided into regular array of tiles corresponding to the size of the FOV of the microscope which depends on the number of pixels in the camera. Usually, the array is of the size of 30×30 where at each position a z stack of 10 to 30 images is recorded within a couple of seconds. The scripting facility of the iPAM allows us to analyze the recorded images concurrent to the acquisition so that after a first scan of the total area only those tiles are retained where cells of interest have been detected. As sample application we discuss the recording of UV-triggered protein transport from the endoplasmatic reticulum to the Golgi apparatus.

[1] W. Caarls et al., Minimizing light exposure with the programmable array microscope, J. Microscopy **241**, 101 – 110 (2010)

[2] P. De Beule et al. Generation-3 programmable array microscope with digital micro-mirror device. SPIE proceedings (2011)

BP 13.2 Mon 17:30 Poster A

Wide Field Detection and Imaging of Atomic Spins using Nitrogen-Vacancy Centers — •Florestan Ziem, Philipp Scheiger, Helmut Fedder, and Jörg Wrachtrup — 3. Physikalisches Institut und SCoPE, Universität Stuttgart

Electron and nuclear magnetic resonance provide information ranging from composition over structure to function of diverse samples in material and life sciences, as well as medical diagnostics. Applied at micro- and nanoscale dimensions, these technique provide label-free imaging and eventually single molecule analysis, e.g. revealing the structure of proteins or membrane channel mechanisms. Traditional induction based magnetic resonance sensing schemes are blind to such vanishing sample volumes, directing the focus at novel sensors. Here, we show how nitrogen-vacancy centers in diamond hosts allow the detection of small ensembles of electronic [1,2] and nuclear [3,4] spins at ambient conditions by optically detected magnetic resonance. In a wide field microscope, parallel magnetic detection and imaging on the microscale are demonstrated.

[1] Steinert, S. *et al.* Magnetic spin imaging under ambient conditions with sub-cellular resolution. Nat. Commun. **4**, 1607 (2013).
[2] Ziem, F. C., *et al.* Highly sensitive detection of physiological spins in a microfluidic device. Nano Lett. **13**, 4093 (2013).
[3] Mamin, H. J. *et al.* Nanoscale Nuclear Magnetic Resonance with a Nitrogen-Vacancy Spin Sensor. Science **339**, 557 (2013).
[4] Staudacher, T. *et al.* Nuclear Magnetic Resonance Spectroscopy on a (5-Nanometer)^3 Sample Volume. Science **339**, 561 (2013).

BP 13.3 Mon 17:30 Poster A

X-ray waveguide optics for nanoscale phase contrast tomography — •Martin Krenkel[1], Andrea Markus[2], Matthias Bartels[1], Christian Dullin[2], Frauke Alves[2], and Tim Salditt[1] — [1]Institute for x-ray physics, University Göttingen, Germany — [2]University Medical Center, Göttingen, Germany

We use propagation based hard x-ray phase contrast tomography to explore the three dimensional structure of soft biological tissues from the organ down to sub-cellular level. As in classical absorption tomography nano-scale structures only barely absorb the radiation, the much stronger phase shift can be used to enhance the contrast.

To reduce beam inhomogeneities and to fulfill the theoretical assumptions needed for proper reconstruction, we use a divergent x-ray waveguide beam geometry at the synchrotron. Thus, the magnification can be easily tuned by placing the sample at different defocus distances. Due to the small Fresnel numbers in this geometry the measured images are of holographic nature which poses a challenge in phase retrieval. With nearly mono modal x-ray beams resolutions down to 23 nm are achieved. We demonstrate this technique on soft hydrated mouse lung tissue yielding 3D reconstructions for a large field of view and region of interest zoom tomography. To further improve the imaging scheme, we develop new reconstruction algorithms that impose minimal restrictions to the objects, based on an optimized measurements or on using an intrinsic consistency constraint of different tomographic projections.

BP 13.4 Mon 17:30 Poster A

Calibration of the diffusion coefficients of the FCS standard Rhodamine 6G in aqueous solutions — •Günter Majer — MPI for Intelligent Systems, Stuttgart, Germany

Precise diffusion measurements of rhodamine 6G (Rh6G) dissolved in D_2O at various concentrations were carried out in the temperature range from 280 to 320 K using pulsed field gradient nuclear magnetic resonance (PFG-NMR). For the diffusion studies at low Rh6G concentrations, a water suppression PFG-NMR sequence was applied. The temperature and concentration dependent diffusion coefficients of Rh6G can be used as calibration references in fluorescence correlation spectroscopy (FCS). Besides measuring the diffusivity of Rh6G, the diffusion coefficient of the solvent in the same system could be determined in parallel by PFG-NMR as the resonances of water and Rh6G are well separated in the ^{1}H NMR spectrum. The isotope effect of the solvent on the diffusion coefficient is determined by FCS measurements on Rh6G dissolved in both D_2O and H_2O.

BP 13.5 Mon 17:30 Poster A

Probing the intracellular pH in magnetotactic bacteria — •Erika Günther, Mathieu Bennet, and Damien Faivre — Max-Planck Institute of Colloids and Interfaces, Biomaterials, Potsdam, Germany

Magnetotactic bacteria are microorganisms that possess encapsulated biomineralised magnetite nanoparticles (magnetosomes) to navigate along the geomagnetic field lines. The formation of magnetite nanoparticles typically requires iron concentrations (200 μM) and pHs ($pH > 8$) that are toxic to cells.

In order to understand how magnetotactic bacteria biomineralise iron nanoparticles in physiological conditions, we aim at measuring their intracellular chemical properties using ratiometric fluorescence microscopy and fluorescence lifetime imaging microscopy.

We have measured the intracellular pH in living *Magnetospirillum gryphiswaldense* MSR-1 cells using confocal laser scanning microscopy. From these measurements, we conclude that the biomineralisation of iron nanoparticles in MTB does not result in the presence of a non-physiological cytoplasmic pH. Magnetotactic bacteria buffer their intracellular pH to pH 7.0 ± 0.25 when exposed to a range of extracellular from pH 6.5 to pH 7.5. We will present the strategy that we are adopting to measure the chemical potential in the cytoplasm and discuss the possibility of better spatially resolving our measurements in order to gain insight into the chemical conditions in the magnetosomes.

BP 13.6 Mon 17:30 Poster A

Embedding of flagellate Trypanosoma brucei for single-molecule microscopy — •Marius Glogger[1], Simone Stichler[2], Jörg Tessmar[2], Jürgen Groll[2], Markus Engstler[1], and Susanne Fenz[1] — [1]Biocenter: Cell and Developmental Biology, University of Würzburg, Würzburg, Germany — [2]Department of Functional Materials in Medicine and Dentistry, University of Würzburg, Würzburg, Germany

The uniflagellate protozoa Trypanosoma brucei are the causal agents of African sleeping sickness. High-resolution microscopy of trypanosoma in vivo is challenging due to the high motility of the parasite. Here, we present an approach for complete cell immobilization suitable for single-molecule fluorescence microscopy (SMM) techniques. Immobilization of trypanosomes requires both efficient trapping to prevent cell motility and mild embedding conditions to ensure cell viability. Biopolymers like agarose or gelatine were used before to immobilize cells sufficiently for fluorescence recovery after photobleaching studies in vivo. However, complete inhibition of the flagellar beat was not achieved. Hence, novel gels are needed for sophisticated SMM methods, which require a high spatial accuracy. We use modified hyaluronic acid and a crosslinking reagent to generate gels that solidify quickly

upon UV-illumination. The rigidity of these hydrogel can be adjusted easily to guarantee complete immobilization of cellular movement. At the same time we verify cell viability using a fluorescent marker. We aim to transfer our results on trypanosomes to flagellates in general.

BP 13.7 Mon 17:30 Poster A

In situ nanoscale imaging of chromosome oscillations in living yeast cells — •Natalja Strelnikova[1], Vasily Zaburdaev[2], Nora Sauter[1], Manuel Guizar Sicairos[3], Ana Diaz[3], Petrina Delivani[4], Mariola Chacon[4], Iva Tolic-Norrelykke[4,5], and Thomas Pfohl[1] — [1]Department of Chemistry, University of Basel, Switzerland — [2]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany — [3]Paul Scherrer Institut, Villigen, Switzerland — [4]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [5]Division of Molecular Biology, Institute Rudjer Boskovic, Zagreb, Croatia

Meiosis is a fundamental process in all eukaryotes leading to genetic diversity, but its details and the mechanism of homologous chromosome recombination is still poorly understood. Currently, there are no data on the change in total length and shape that chromatin undergoes during the oscillations before condensation into chromosomes. We employ a combined coherent scanning transmission X-ray microscopy (STXM) and ptychography setup, which allows us to investigate the dynamics of nuclear oscillations during meiosis of fission yeast Schizosaccharomyces pombe. We show that the combination of STXM and ptychography is an extraordinary tool to image living yeast cells during nuclear oscillation without additional labeling down to the submicron scale. Therefore we can analyze the chromatin compaction, mutual alignment of chromosomes and its impact on the recombination in eukaryotes on the relevant length scales of 30 to 100 nm.

BP 13.8 Mon 17:30 Poster A

Photothermal Detection and Correlation Spectroscopy of Single Gold Nanoparticles in Living Cells — •Romy Schachoff, Alice Abend, and Frank Cichos — Molecular Nanophotonics Group, Universität Leipzig, Linsende. 5, 04103 Leipzig

For better insights into complex cellular processes fluorescence microscopy on the single molecule level has gained large importance. As this technique relies on emission processes, it is restricted to fluorescent probes and hampered by their photophysical processes such as bleaching and blinking. Recently, photothermal microscopy, which is based on the absorption of light, has been pushed to a new level of sensitivity allowing even the detection of single molecules. The technique employs the conversion of optical energy into heat by an absorbing non-fluorescent species. The released heat has been shown to create a nano-lens deflecting a focused probe laser in a microscopy setup. Gold nano particles down to 5 nm in size exhibit large absorption cross sections and high photo stability and, thus, deliver intense and stable optical signals in photothermal microscopy with large signal to noise ratios even in heterogeneous environments. Since this method is highly sensitive to the absorbing species and non invasive, we recently startet to implement photothermal detection and correlation spectroscopy in living cells to study local dynamics in biological samples. Further, we aim to use gold nano particles as single nano heat sources in cells to locally change the physical properties of special cell sites and to manipulate the behavior of the cells.

BP 13.9 Mon 17:30 Poster A

All-optical realization of optical diffraction tomography — •Mirjam Schürmann[1], Paul Müller[1], Moritz Kreysing[2], and Jochen Guck[1] — [1]Biotechnology Center, TU-Dresden, Tatzberg 47/49, 01307 Dresden, Germany — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany

Quantitative phase microscopy is an established marker-free imaging technique for biological cells. Several studies have demonstrated the refractive index (RI) to be a conclusive measure for cellular alterations e.g. due to infection or during differentiation. A 2D mean RI map of a cell can be determined from a phase map if the cell shape is known. However, imaging cells from only one direction leads to a mixed contribution of all cellular components to the measured phase. This prevents an assignment of an RI to individual cellular organelles. Rotating the sample for tomographic imaging can overcome this problem. Here, we present the experimental realization of a contact-free rotation of individual suspended cells in combination with digital holographic microscopy (DHM) for quantitative phase imaging at each of the rotational positions. The rotation is implemented in a dual-beam laser trap. A spatial light modulator (SLM) is used to control the orientation of the LP_{11} output fiber mode of one of the trapping fibers which leads to a subsequent controlled orientation of the cell. The gathered data can be combined to a 3D RI map of the cell using optical diffraction tomography (ODT). This all-optical demonstration of ODT opens the door to many applications in basic biology and biotechnology.

BP 13.10 Mon 17:30 Poster A

GPU-based statistical multi-resolution estimators for image reconstruction — •Jan Lebert[1], Johannes Hagemann[2], and Stephan Kramer[3] — [1]G. A. U. Göttingen, Fakultät f. Physik, Friedrich-Hund-Platz 1, 37077 Göttingen — [2]Institut f. Röntgenphysik, G. A. U. Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen — [3]Max-Planck-Institut f. biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen

We present two implementations of Dykstra's projection algorithm on NVIDIA's compute unified device architecture (CUDA). Dykstra's algorithm is the central step in statistical multi-resolution (SMR) methods (Frick, Marnitz, and Munk, 2012 and 2013) which are a recent development for the deconvolution of noisy images. Unlike other methods its primary parameter is the confidence level with which the reconstruction is considered as valid. Compared with a CPU our CUDA implementation of the standard Dykstra algorithm (SDA) is one order of magnitude faster. For a further speedup we have developed a new variant, which we call *incomplete Dykstra's algorithm* (ICD). Implemented in CUDA it yields an additional speedup of one order of magnitude over the CUDA version of SDA. As sample application we discuss preprocessing super-resolution optical fluctuation imaging (SOFI) methods (Dertinger et al., 2009) by ICD. Our results show that a careful parallelization of Dykstra's algorithm enables its use in large-scale statistical multi-resolution analysis.

BP 13.11 Mon 17:30 Poster A

Localization Precision in Stepwise Photobleaching Experiments — •Ingmar Schoen — ETH Zurich, Zurich, Switzerland

The precise determination of the position of fluorescent labels is essential for the quantitative study of biomolecular structures by various localization microscopy techniques. Localization by stepwise photobleaching is especially suited for measuring nanometer-scale distances between two labels; however, the precision of this method has remained elusive. Here, we show that shot noise from other emitters and error propagation compromise the localization precision in stepwise photobleaching. Incorporation of point spread function-shaped shot noise into the variance term in the Fisher matrix yielded fundamental Cramer-Rao lower bounds (CRLBs) that were in general anisotropic and depended on emitter intensity and position. We performed simulations to benchmark the extent to which different analysis procedures reached these ideal CRLBs. The accumulation of noise from several images accounted for the worse localization precision in image subtraction. Propagation of fitting errors compromised the CRLBs in sequential fitting using fixed parameters. Global fitting of all images was also governed by error propagation, but made optimal use of the available information. The precision of individual distance measurements depended critically on the exact bleaching kinetics and was correctly quantified by the CRLBs. The methods presented here provide a consistent framework for quantitatively analyzing stepwise photobleaching experiments and shed light on the localization precision in some other bleaching- or blinking-assisted techniques.

BP 13.12 Mon 17:30 Poster A

Parallelizing super-resolution optical fluctuation imaging (SOFI) — •Bartosz Kohnke[1], Stephan Kramer[1], Johannes Hagemann[2], and Suzunosuke Nagaoka[3] — [1]Max-Planck-Institut f. biophysikalische Chemie, Am Faßberg 11, 37077 Göttingen — [2]Institut F. Röntgenphysik, Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen — [3]Institut f. Informatik, Universität Göttingen, Goldschmidtstraße 7, 37077 Göttingen

SOFI algorithms [1] are based on the observation that a higher order statistical analysis of a time series may be utilized for generating super-resolved images by computing per-pixel auto-cumulants, e.g. of a fluorescence signal. As the effectiveness of SOFI has been accepted in the microscopy community, our focus is on the speedup due to a careful design of a parallel implementation on current multi- and many-core compute architectures. In addition, we present robust, single-pass algorithms suitable for an adaptive computation of the final SOFI image in case of large-scale data analysis where the usual two-pass algorithms are unfeasible. We compare the performance on different paralleliza-

tion frameworks, in particular Intel's threading building blocks, Qt's QThreads, CUDA and OpenCL. For the CUDA implementation we use our SciPAL library [2,3].

[1] Dertinger et al., PNAS Vol. 106, No. 52, pp. 22287 (2009)
[2] SciPAL: Expression Templates and Composition Closure Objects for High Performance Computational Physics with CUDA and OpenMP, S. C. Kramer and J. Hagemann, ACM TOPC (to appear).
[3] https://code.google.com/p/scipal/

BP 13.13 Mon 17:30 Poster A

Improvements for Stochastic Optical Fluctuation Imaging (SOFI): Sub-pixel super-resolution images with a conventional wide-field microscope — •Simon Christoph Stein, Anja Huss, and Jörg Enderlein — Drittes Physikalisches Institut, Georg-August Universität Göttingen, Deutschland

The last decade has seen a rapid evolution of a wide array of new super-resolution microscopy techniques which are by now widely available and applied in the life sciences. Among these different techniques, super-resolution optical fluctuation imaging (SOFI) stands out due to its algorithmic and experimental simplicity, requiring only the rapid recording, with a conventional wide-field setup, of the intensity fluctuations from a sample which is labeled with blinking emitters.

The visual fidelity of SOFI, however, is limited by the finite size of the camera's pixel grid. We present a new approach for creating sub-pixel resolution images which is completely artifact-free and straightforward to implement, keeping the simplicity of the original algorithm.

Furthermore we show how an estimate for the PSF of an optical system can be extracted from the raw movie of fluctuating emitters. The knowledge about the PSF is used for post-processing deconvolution, enhancing the quality of SOFI images.

BP 13.14 Mon 17:30 Poster A

SIM microscopy to investigate lipofuscine granules in retinal pigment epithelial cells — •Florian Schock[1,2,3,4], Gerrit Best[1,2,4], Nil Celik[2], Alena Bakulina[5,9], Saadettin Sel[2], Udo Birk[1,3], Rainer Heintzmann[6,7,10], Jürgen Hesser[5,9], Stefan Dithmar[2,8], and Christoph Cremer[1,3,4] — [1]Kirchhoff Institute for Physics, University of Heidelberg — [2]Department of Ophthalmology, University-Hospital Heidelberg — [3]Institute of Molecular Biology, University of Mainz — [4]Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg — [5]Experimenatal Radiation Oncology, University Medical Center Mannheim, University of Heidelberg — [6]Institute for Physical Chemistry and Abbe Center of Photonics, University of Jena — [7]Leibniz Institute of Photonic Technology — [8]Department of Ophthalmology, Hospital Wiesbaden — [9]Institute for Scientific Computation, University of Heidelberg — [10]Randall Division of Cell & Molecular Biophysics, King's College London

Age related macular degeneration, the main cause for legal blindness in industrial countries, is accompanied by accumulation of lipofuscine granules inside the retinal pigment epithelial cells (RPE cells). Here we demonstrate that the super-resolution technique "Structured Illumination Microscopy" (SIM) is able to resolve up to over 100 granules inside single cells and compare these results with other microscopy techniques. In addition, we introduce an algorithm to automatically identify, separate and characterise the granules, and present first super-resolution images on spectral discrimination of lipofuscine granules and intra-granule regions.

BP 13.15 Mon 17:30 Poster A

Image Scanning Microscopy — •Jörg Enderlein — 3. Physikalisches Institut, Georg-August-Universität Göttingen

Recent years have seen an explosion in new advanced and super-resolution methods in fluorescence microscopy, which have culminated in the Nobel Prize in Chemistry for 2014. One of the early methods of these techniques was structured illumination microscopy (SIM), which combines wide-field imaging with a structured illumination for doubling the resolution of a wide-field microscope. An alternative approach is image scanning microscopy (ISM), which combines a conventional confocal laser scanning microscope with a wide-field imaging detector. Although the idea was theoretically proposed by Colin Sheppard already in 1988, its first successful realization was only achieved in 2010. Since then, a whole flood of modifications and implementations of this idea have been published, and by now, even the first commercial implementation of ISM (Airy Scan Microscope by Carl Zeiss Jena) is available. I will present the working principle of ISM and its several implementations and applications.