quantitative fluorescence microscopy and cryo-electron microscopy, have become a powerful approach to extract the underlying rules of how the microtubule cytoskeleton acts as a dynamic system. For example, through this approach the hierarchical functioning of a regulatory protein interaction network at growing microtubule ends, formed around so-called end binding (EB) proteins, can now be understood from the atomic to the micrometer scale. In the future, the challenge will be to reconstitute even more complex systems to be able to test directly our understanding of higher-order cytoskeletal functions.

1125-Symp
The Forces that Center the Mitotic Spindle

Jonathon Howard[1], Horatiu Fantana[1], Jacques Pecreaux[2], Carlos Garzon-Coral[1], Stefanie Redemann[1], Anthony A. Hyman[1].
[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [2]Institute of Genetics and Developmental Biology, Rennes, France.

Precise positioning of the mitotic spindle is important for specifying the plane of cell division and the subsequent partitioning of the cell's contents to the daughter cells. Studies on different organisms and cell types have suggested diverse centering mechanisms: astral microtubules grow out from the spindle and push against the cortex, cortical dynein motors pull on astral microtubules, and dynein-dependent organelle transport on astral microtubules leads to a reactive force on the spindle. The different mechanisms lead to different predictions for the precision of centering, how mutations effect the precision, and the magnitude of the forces associated with spindle centering. We used image processing to accurately track the position and orientation of the mitotic spindle during the first cell division in the C. elegans embryo. The high precision of centering, < 1% of cell diameter transverse to the anterior-posterior axis, increased after RNAi against gpr-1/2, genes encoding activators of the cortical force generators; this suggests that centering is not mediated by gpr-1/2-dependent cortical pulling forces. To measure the forces associated with spindle positioning, we built a magnetic tweezers apparatus so that forces could be exerted on the spindle via beads incorporated into the embryo: forces of approximately 20 pN were required to displace the spindle through 1 μm. These mechanical experiments constrain molecular models of the centering process.

Platform: Imaging & Optical Microscopy: Superresolution Imaging & Single Molecules

1126-Plat
Sub-100 nm 3D Detection Volumes by Total Internal Reflection STED Microscopy

Travis J. Gould, **Joerg Bewersdorf**.
Yale School of Medicine, New Haven, CT, USA.

Stimulated Emission Depletion (STED) Microscopy (1–2) has revolutionized far-field fluorescence microscopy by breaking the classical diffraction limit: 25 nm resolution and better are routinely achieved in the focal plane.

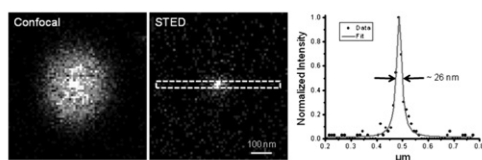

Fig. 1. 20 nm fluorescent bead imaged in confocal and STED mode. The profile across the white box demonstrates <26 nm resolution.

While comparable axial resolution values have been obtained using two opposing objectives , single-objective STED microscopy can usually not realize values on this size scale.
Here we present results obtained with a recently realized STED variant that combines total internal reflection excitation for ~70 nm axial sectioning capabilities with stimulated depletion for ~50 nm lateral super-resolution.
TIRF STED microscopy represents an attractive super-resolution alternative for live cell microscopy featuring fast scanning with sub-100 nm 3D detection volumes and reduced photo-damage through TIRF excitation.
References:

1. Hell, S. W., and J. Wichmann. 1994. Opt. Lett. 19:780–782.
2. Toomre, D., and J. Bewersdorf. 2010. Annu. Rev. Cell. Dev. Biol. 26:285–314.
3. Schmidt, R., C. A. Wurm, S. Jakobs, J. Engelhardt, A. Egner, and S. W. Hell. 2008. Nat. Methods 5:539–544.
4. Gould, T. J., J. R. Myers, and J. Bewersdorf. 2011. Opt. Express. 19:13351–13357.

1127-Plat
Super-Resolution dSTORM Imaging of Human Galectin-1 Interacting with Neuroblastoma Cells

Antonia Goehler, Nadja Bertleff, Malte Timm, Sören Doose, Juergen Seibel, Markus Sauer.
University Wuerzburg, Wuerzburg, Germany.

Galectins are a family of carbohydrate-binding proteins with an affinity for beta-galactosides. They share a core sequence consisting of 130 amino acids, and the beta-sandwich fold. Human Galectin-1 (hGal-1) is a well studied representative of prototype galectins, non-covalently linked homodimers with two specific carbohydrate recognition domains (CRD). It is differentially expressed by various normal and pathological tissues and is involved in intra- and extracellular processes like cell adhesion, formation of galectin-glycoprotein lattices, signal transduction and regulating immune responses, inflammation, allergies, and host-pathogen interactions. Furthermore oxidized galectins are associated with the regeneration of the central nervous system after injury.
We use direct stochastic optical reconstruction microscopy (dSTORM) to study the spatial organization of hGal-1 interacting with glycans like ganglioside GM1 presented on the membrane of human SK-N-MC neuroblastoma cells. Using the photoswitchable fluorophore ALEXA 647 as specific galectin marker, we employ fluorescence on/off switching with standard widefield microscopy and spot analysis of single molecules in order to resolve clustering, localization, and cross-linking of galectins on the cell surface with a spatial resolution of less than 50 nm.
We study spatial organization and its dependence on galectin concentration and oxidation state, as well as inhibition of the specific recognition.

1128-Plat
Direct Live-Cell Super-Resolution Imaging of Cellular DNA

Alexander Benke, Xavier Meylan, Suliana Manley.
EPFL, Lausanne, Switzerland.

Direct stochastic optical reconstruction microscopy (dSTORM) is an imaging method that relies on the stochastic photoswitching of single fluorophores and enables resolution of structures down to tens of nanometers in biological samples. Recently, several proteins have been visualized with dSTORM in live cells by using genetically encoded tags labeled with chemical dyes, including DNA-associating proteins. However, despite its importance in cellular processes, live-cell super-resolution imaging of DNA structure itself has never been demonstrated. We present the imaging of DNA with dSTORM based on direct DNA labeling. We optimized buffer conditions to achieve the reversible photoswitching required for dSTORM in living cells and used it to resolve nuclear and mitochondrial DNA structures. Furthermore, due to the excellent preservation of the dyes, we were able to perform time-lapse super-resolution imaging. This illustrates that is possible to monitor the sub-diffraction limited organization of DNA in individual cells over time. This protocol in combination with protein super-resolution imaging provides an advantageous tool to study processes related to DNA dynamic structural rearrangements such as those occurring during cell division or in response to cell stress.

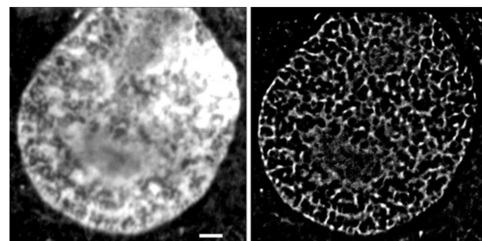

Figure: Wide-field (left) and dSTORM (left) images of cellular nucleus. Scale bar 2.5μm.

1129-Plat
Live Cell Super-Resolution Imaging of Transverse Membrane Tubules in Heart Failure

Eva Wagner[1], Marcel Lauterbach[2], Tobias Kohl[1], Volker Westphal[1], George S.B. Williams[3], Julia H. Steinbrecher[1], Jan-Hendrik Streich[1], Hoang-Trong M. Tuan[4], Brian Hagen[3], Stefan Luther[5], Ulrich Parlitz[5], M. Saleet Jafri[4], Stefan W. Hell[2], W.J. Lederer[3], **Stephan E. Lehnart**[1].
[1]Georg August University, Heart Research Center Goettingen, Goettingen, Germany, [2]Max Planck Institute for Biophysical Chemistry, Goettingen, Germany, [3]BioMET, Center for Biomedical Engineering & Technology, University of Maryland, Baltimore, MD, USA, [4]School of Systems Biology, College of Science, George Mason University, Manassas, VA, USA, [5]Max Planck Institute for Dynamics and Self-Organization, Goettingen, Germany.

Transverse tubules (TTs) are hollow intracellular membrane structures, coupling action potential propagation at the cell surface to intracellular