85-Plat
STED Nanoscopy in Living Cells using Live Cell Compatible Markers
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We demonstrate far-field optical imaging with subdiffraction resolution in the interior of living mammalian cells and tissue by applying stimulated emission depletion (STED) microscopy.
Utilizing a yellow fluorescent protein (YFP) to image individual structural elements of the endoplasmatic reticulum (ER) and the tubular network (Fig.1) revealed a focal plane (x,y) resolution < 50 nm inside the living cell, corresponding to a 4-fold improvement over that of a confocal microscope and a 16-fold reduction in the focal spot cross-sectional area. Time lapse STED imaging of dendritic spines of YFP-positive hippocampal neurons in organotypic slices outperforms confocal microscopy in revealing important structural details. As an alternative to the fluorescent protein we employed a genetically encoded protein tag which can be stained in vivo with modified organic dyes. Using a rhodamine dye of high photostability enabled us to image structures in the living cell with a resolution of ~ 40 nm.
Thus nanoscale imaging of structures in the interior of living cells greatly expands the scope of light microscopy in cell biology.

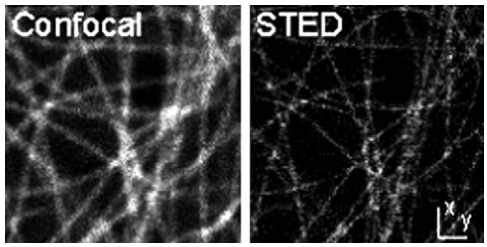

Fig. 1. Citrine labeled microtubules.

86-Plat
Multilayer Three-dimensional Super-resolution Imaging of Thick Biological Samples
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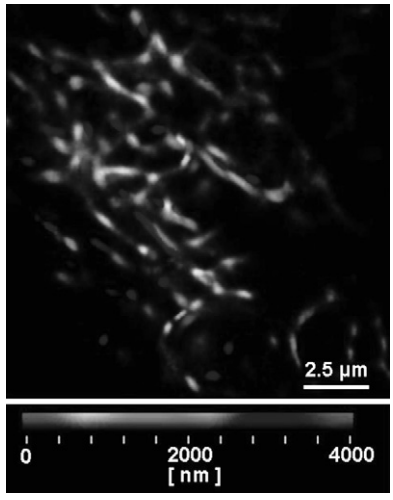
Recent advances in optical microscopy have created the capability of creating images in biological samples beyond the diffraction limit at nanometre resolution. A general feature of most of the techniques based on photoactivated localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM) has been the use of thin biological samples and a sample geometry using total internal reflection that limits the imaging depth to a fraction of an optical wavelength. However, in order to study whole cells or organelles which are typically up to ~15μm deep into the cell, the extension of these methods to a 3D super-resolution technique is required.
Here we report an advance in optical microscopy that enables imaging of protein distributions in cells with a lateral localization precision better than 50 nm at multiple imaging planes deep in biological samples. The approach is based on combining the lateral super-resolution provided by PALM with two-photon temporal focusing that provides optical sectioning. We have generated super-resolution images over an axial range of ~10μm in both mitochondrially-labeled fixed cells, and in the membranes of living S2 Drosophila cells.



87-Plat
Single Molecule Tracking With Light Sheet-Based Microscopy In Vivo
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Autofluorescence, rapid photobleaching and high particle concentrations present restrictions to single molecule observation in biological systems by epi-illumination. Light sheet-based microscopy overcomes these limitations [1]. By combining the speed of parallel image acquisition and the optical sectioning produced by light sheet illumination, we created a powerful tool to study single molecule dynamics on a millisecond timescale [2].
We illuminated the sample perpendicular to the detection axis with a thin light sheet (FWHM 2-3μm). In this manner a simple optical sectioning microscope is created, because only the focal plane of the detection optics is illuminated and no out-of-focus fluorescence is generated. The background fluorescence is strongly reduced and the signal-to-noise-ratio (SNR) greatly improved.
We constructed a miniaturized glass specimen chamber, which can be illuminated from the side in a very flexible manner and directly be mounted on a commercial inverse microscope. The specimen is easily accessible for micromanipulation and can be observed via the 0.17mm thin glass bottom of the chamber using high NA objective lenses.
With this setup it was straightforward to observe trajectories of single protein molecules in aqueous solution with a $D = 90\mu m^2/s$, and also in the cellular interior. By analysis of the diffusion behavior of single fluorescent dextran molecules we determined the viscosity of living *C. tentans* salivary gland cell nuclei. Similarly, molecular dynamics in adherent cells can be observed with greatly improved contrast.
With this new experimental setup we use the ideal imaging scheme for single molecule visualization and push the limit of sensitivity far beyond the potential of conventional epi-illumination.
[1] Huisken, J. et al. (2004). *Science* **305**(5686): 1007-9.
[2] Ritter, J.G. et al. (2008). *Opt Express* **16**(10): 7142-52.

88-Plat
Ultra-high resolved Multi-Beam-Two-Photon-Striped-Illumination-Microscopy (MBTPSIM): Studying the Molecular Nature of Cell-Cell Interactions
Raluca Niesner, Jan Leo Rinnenthal.
Charite, University of Medicine, Berlin, Berlin, Germany.
Two-photon microscopy (TPM) has become a very popular tool in life sciences because of its essential advantages (extended penetration depth, intrinsic z-resolution, reduced photobleaching outside the focal plane etc.). However, resolution in TPM is usually limited due to the diffraction limit of focused laser light (xy: ca 200 nm, z: ca 800 nm for a 100x lens, NA 1.4). If structured-illumination methods are used, the two-photon microscope is in principle capable to overcome this limitation. We show that by means of Multi-Beam-Two-Photon-Striped-Illumination-Microscopy (MBTPSIM) resolution can dramatically be improved in the xy-plane as well as in z-direction. The experimental setup is based on a tunable Ti:Sa laser system and a multi-beam scan-head allowing simultaneous interference-free scanning of up to 64 beamlets. The excitation beamlets are focused into the sample by a high NA objective lens with a sufficient working distance (>200 μm). Fluorescence is detected synchronously by a CCD camera. Image reconstruction is performed via a customized mathematical algorithm. In order to quantify the improvement in resolution we determined the point-spread-function of the system in agarose media as a bench-marking model as well as in brain tissue by using fluorescent nano-beads. We propose this technique to be a suitable tool for studying the molecular nature of cell-cell interactions at the nanometer scale for instance in autoimmune reactions.

89-Plat
A Programmable Light Engine For Quantitative TIRF And HILO Imaging
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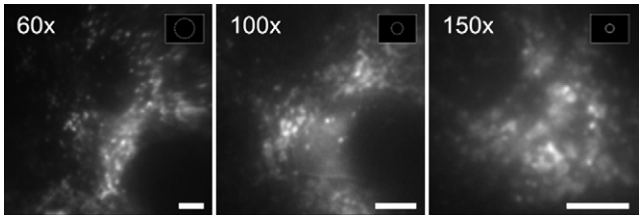
We report on a simple yet powerful implementation of objective type total internal reflection fluorescence (TIRF) and highly inclined laminated optical sheet (HILO) illumination microscopy. Both rely on an asymmetric oblique illumination to confine spatially fluorescence excitation to a dielectric boundary or a thin sheet of light, respectively. Undesired side effects are intensity variations across the field of view, due to scattering and interference. Utilizing an acousto-optical deflector (AOD) based scanner, we restore the symmetry of illumination by generating a hollow cone of light, impinging at the sample at an angle adjustable within μs. Parameters relevant for quantitative image analysis are measured on-line, during fluorescence image acquisition by capturing an objective back-focal plane image on a second small CCD detector. Our device affords background-free variable-angle TIRF/HILO even for the shortest practical exposure times.
We expect it to be useful for evanescent-wave imaging involving multiple excitation wavelengths, multiple penetration depths (Variable-angle TIRF)

and experiments using objectives with different magnification (and hence different back pupils). We illustrate the system performance by demonstrating ultra-low background TIRF imaging of 200 Hz Qdot blinking, vinculin-EGFP labeled cellular adhesion sites and lysosomal dynamics in cortical astrocytes.



Platform H: Membrane Physical Chemistry I

90-Plat
Charges in phospholipid layers
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The interfacial properties of a membrane are determinant for interaction among bio-membranes / lipid bilayers, or for establishing contact among layer surfaces and substrates approaching from the bulk. The access to the bilayer and its local structural modifications upon interaction with an adsorbing guest molecule are influenced significantly by the presence of charges, and local changes in surface charge density. Results are being presented on model mono- and bilayers prepared from zwitterionic POPC (DPPC) (1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine) and its cationic sibling lipids E-POPC (E-DPPC) (1-palmitoyl-2-oleoyl-sn-glycero-3-ethyl-phosphocholine/ di-1,2-palmitoyl-sn-glycero-3-ethyl-phosphocholine) that served to inoculate charge densities at different mol percentages. Monolayer compression isotherms obtained for the mixtures are compared with isotherms of pure POPC as a reference system. The presence of layer charges is manifested in an earlier onset of interaction, the range of interaction is increased. POPC bilayers with the same charge densities as the monolayers studied were then investigated by single molecule tracking using the fluorophore DiI-C18 for diffusion tracing. Initial results indicate a linear decrease of the lateral diffusion coefficient with increasing charge density. In the course of the study indications for domain formation in pure POPC layers were observed as novel peculiarities; these will be presented and discussed. Preliminary results about the adsorption of partially charged phospholipid layers onto hydrogel polyelectrolyte cushions on solid supports will be presented.

91-Plat
Domain/Raft Exploration in Lipid Mono- & Bilayer by Freeze-fracture Electron Microscopy on Nano-Resolution Scale
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Lateral chemical and physical inhomogeneities of biological membranes such as domains/rafts seem to play an important role in signal transduction, membrane traffic, and diseases.
Freeze-fracture electron microscopy (ff-em) as a cryofixation TEM technique is a powerful to explore such small and highly dynamic domains in a probe-free mode. Since the resolution of this technique is 2 nm we are able to study lipid-, protein-, toxin-, as well as drug domains on a nano-resolution scale. Since replica, resistant to beam damage, can be produced from large, micro-meter size objects, ff-em allows us to study nano-scale events in micro-scale biological as well as artificial assemblies. The fact that the fracture plane follows the area of weakest forces, allows insides into the hydrophobic center of lipid bilayer [1-3] as well as into the lipid/gas interface of lipid monolayer stabilizing gas bubbles [4].
Examples will be given for domains in liposomal bilayer made of drugs [5], proteins, and toxin. Lipid-induced modulation of 2-D crystals of bacteriorhodopsin in liposomal bilayer will be shown as an extreme example for domain formation of intrinsic proteins [6-8]. Additionally, liquid ordered (Lo) domains will be shown recently detected in lipid monolayer, stabilizing hydrophobic gas bubbles.
[1] B. Sternberg, Liposome Technology, CRC Press I (1992) 363.
[2] B. Sternberg, Handbook Nonmedical Applications of Liposomes CRC Press (1996) 271.
[3] B. Sternberg, Medical Applications of Liposomes, Elsevier (1998) 395.
[4] C. Brancewicz et al. J. Disp. Sci. & Techn. 27:5 (2006) 761.
[5] K. Merz and B. Sternberg J. Drug Targ. 2 (1994) 411.
[6] B. Sternberg et al. Biochim. Biophys. Acta 980 (1989) 117.
[7] B. Sternberg et al. Biochim. Biophys. Acta 1108 (1992) 21.
[8] B. Sternberg et al. J. Struc. Biol. 110 (1993) 196.

92-Plat
Sterol Uptake From Liposomes By MβCD Is Influenced By The Extent Of Sterol Superlattice In The Membrane
Berenice Venegas, **Parkson L.-G. Chong** .
Temple University School of Medicine, Philadelphia, PA, USA.
Cholesterol transfer regulates the intracellular distribution and the metabolism of cholesterol, thus having a direct impact on cholesterol homeostasis in cells. The present work investigates the effect of lipid lateral organization (i.e., the extent of sterol superlattice) on sterol transfer from liposomes to methyl-Beta-cyclodextrin (MβCD), a water-soluble macrocyclic compound able to pick up sterols from the membranes. Several sample sets of large unilamellar vesicles (LUVs) composed of POPC, dehydroergosterol (DHE) and Dansyl-PE were examined. Each sample set contained ~15 samples centered at one of critical sterol mole fractions (C_r) theoretically predicted for maximal sterol superlattice formation (e.g., 20.0, 22.2, 25.0, 33.3, 40.0 and 50.0 mol%). Within the same sample set, the DHE content in the sample was varied using 0.4 mol% increments. The molar ratio of DHE to Dansyl-PE was kept constant (15:1) in all samples. The rate of sterol transfer was monitored in real time based on the resonance energy transfer between DHE (donor) and Dansyl-PE (acceptor). The fluorescence intensity of Dansyl-PE versus time was monitored at 500 nm upon addition of MβCD. When DHE is transferred from LUVs to MβCD, the energy transfer efficiency is decreased and, consequently, the fluorescence intensity of Dansyl-PE is decreased over time. The initial rate of DHE transfer was determined by a linear fit of the data collected in the first few seconds of the transfer process. The initial rate of the DHE transfer was found to vary with DHE content in a biphasic manner at C_r. This result demonstrates that the rate of DHE transfer from LUVs to MβCD is governed by the extent of sterol superlattice in the liposomal membrane. (Supported by AHA, NSF and PDOH)

93-Plat
Lipid Diffusion In Domain-forming Bilayers Studied By Pfg-nmr
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Chemistry, Umea, Sweden.
The pulsed field gradient (pfg)-NMR method for measurements of translational diffusion of molecules in aligned lipid bilayers is presented. Lateral phase separation of lipids has been successfully studied as well as their dynamics within the bilayer organization. Support was obtained for that the lateral diffusion depends on lipid packing and acyl chain ordering. Therefore, investigations of order parameters of perdeuterated acyl chains, using 2H NMR quadrupole splittings, were useful complements. Here, some of our recent achievements on lipid membranes will be summarized. In particular, bilayers exhibiting two-phase coexistence of liquid disordered (ld) and liquid ordered (lo) phases are considered in detail. Among our major results are that the lateral diffusion is the same for all components, independent of the molecular structure (including cholesterol (CHOL)), if they reside in the same domain in the membrane. Furthermore, quite unexpectedly CHOL seems to partition into the ld and lo phases to roughly the same extent, indicating that CHOL has no strong preference for any of these phases. We propose that the lateral phase separation in bilayers containing one high Tm and one low Tm lipid together with CHOL is driven by the increasing difficulty of incorporating an unsaturated or prenyl lipid into the highly ordered bilayer formed by a saturated lipid and CHOL, i.e. the phase transition is entropy driven to keep the disorder of the hydrocarbon chains of the unsaturated lipid.

94-Plat
Lipid Sorting In Membranes Nanotubes
Benoit Sorre[1], Andrew Callan-Jones[1], Jean-Baptiste Manneville[1], Pierre Nassoy[1], Jean-Francois Joanny[1], Jacques Prost[1,2], Bruno Goud[1], Patricia Bassereau[1].
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Several studies have shown that lipids are sorted at every step of intracellular trafficking [1], for example in [2] it has been shown that COPI-coated vesicles have a different lipid composition than the Golgi apparatus they originate from. But general principles governing lipid sorting are not fully understood yet. In particular, general physical principles in sorting must be investigated closely. As transport intermediates are highly curved, the role of membrane curvature must be considered. As a driving force for sorting we propose, that composition