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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.

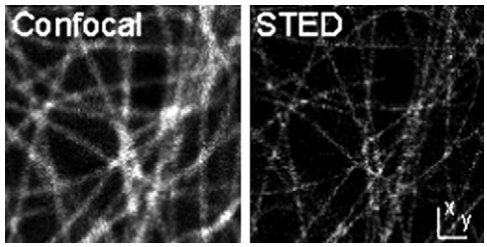

Fig. 1. Citrine labeled microtubules.

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Multilayer Three-dimensional Super-resolution Imaging of Thick Biological Samples
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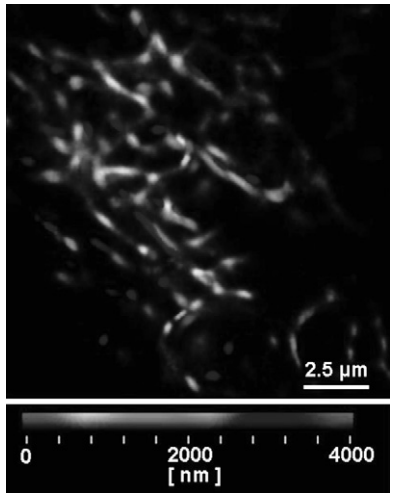
Recent advances in optical microscopy have created the capability of creating images in biological samples beyond the diffraction limit at nanometre resolution. A general feature of most of the techniques based on photoactivated localization microscopy (PALM) or stochastic optical reconstruction microscopy (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
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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.

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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)