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Supercritical Angle Localization Microscopy
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Supercritical angle localization microscopy (SALM) is a conceptually new approach to 3D single-molecule localization microscopy based on the principle of surface generated fluorescence. This near-field fluorescence occurs when a fluorophore is in the vicinity of a water-glass interface. It is emitted into large angles above the critical angle and its intensity strongly depends on the distance of the fluorophore from the interface. By splitting high and low emission angles and imaging them simultaneously we can determine the precise axial position of single molecules from their relative intensities in the two channels. This technique has the prospect of achieving nanometer isotropic resolution in the TIRF range above the coverslip with a setup as simple and robust as in PSF engineering based 3D localization microscopy. However, current approaches to SALM do not achieve the full potential of the method as they are limited by diffraction in the supercritical angle channel. Here we present a new SALM setup intended to optimize the method by using an objective with ultra-high numerical aperture (NA 1.7) and adaptive optics. This microscope will be the perfect tool to image processes close to the plasma membrane with highest 3D superresolution. Examples include membrane receptors, the adhesion machinery, endocytosis or the actin cortex.

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3D Multicolor STED Nanoscope a Super-Resolution Approach to Mammalian Photoreceptor
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Photoreceptors in mammalian retina present two distinct subcellular regions: the Inner Segment (IS) and the Outer Segment (OS). The OS shows an unique architecture and is responsible for the photo-transduction, despite the metabolic activity of photo-transduction remains controversial and still unclear(1), recent studies suggest that the presence of OXidative PHOSphorylation proteins (OXPHOS) in rods OS support the energetic requirements of photo-transduction(2).
We propose a STimulated Emission Depletion (STED) superresolution approach to investigate the structural proteins and the OXPHOS proteins distribution in photoreceptor cells. STED nanoscopy allows overcoming the diffraction limit, enabling to distinguish details of cellular and molecular structures at the nanoscale(3). Our 3D multicolor STED nanoscope is based on a supercontinuum pulsed laser with 20MHz repetition frequency and three synchronized outputs: a supercontinuum laser beam in the 450-2000nm spectral range, used for excitation, and two laser beams at 710 and 745nm respectively, which have a bandwidth of about 10nm, used for depletion(4). In order to obtain 3D super-resolution we divide the STED beam and shape the two beam in donut and bottle profiles. Such beam profiles are obtained by two phase plates, a vortex (0-2π) and a homemade 0-π one respectively(5). Since we can adjust the power ratio in the two depletion pathways we are flexible in the choice of the final three-dimensional resolution.
Nevertheless we have optimized the system and the fluorophores choice in order to separate three channels spectrally. Thus we show three colors image with two colors super-resolved.
References
1. Hsu, S. C. et al. J.-Biol. Chem. 269, 17954-17959 (1994).
2. Calzia, D. et al.Biol. Cell 105, 345-58 (2013).
3. Hell, S. W. et al. Opt Lett. 19, 780-2 (1994).
4. Galiani, S. et al. Opt. Express 20, 7362 (2012).
5. Harke, B. et al. Nano Lett. 8, 1309-1313 (2008).

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Boost Your Microscope by Exploring New Dimensions
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Due to diffraction of light, the conventional fluorescent microscope is a system with a band-limited information spatial channel. Every super-resolved fluorescent microscope circumvents this limitation by avoiding the simultaneous signaling of adjacent (< diffraction limit distance) fluorophores recording them sequentially in time. Notably, the confocal microscope also agrees to this perspective. Thus, the current super-resolved systems encode extra spatial information in the temporal channel.
Here we discuss if other channels (dimensions) can be used to further improve the resolution of a fluorescence microscope. In particular, we describe time-gated STED microscopy and image-scanning microscopy (ISM) as examples of exploring extra dimensions, respectively the photon arrival time and the spatial image plane dimensions.
Confocal scanning microscopy (CSM) can theoretically surpass the diffraction limit by a factor of $\sqrt{2}$. Practically, this improvement is sacrificed to obtain a good signal-to-noise ratio (SNR). Image scanning microscopy (ISM) solved this limitation by substituting the single point detector with a 2D array of detector. We showed that ISM can be straightforwardly implemented by using a quadrant detector. This implementation offers resolution close to the CSM theoretical value, improves the SNR by factor of 1.5 with respect to the CSM counterpart, and may be implemented without losing the optical sectioning capability and the system versatility.
Time-gated detection increases the spatial resolution of a gated continuous-wave-(CW-) STED microscope, with the drawback of reducing the SNR. Thus, in sub-optimal conditions, such as a low-photons budget regime, the SNR reduction can cancel-out the expected gain in resolution. We developed a method which does not discard photons, but instead sorts all the photons in different time-gates according to their arrival time and recombines them through a multi-image deconvolution. Our results show that the SNR of the restored image improves, thereby improving the effective resolution.

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A Novel Fast Volumetric Light Sheet Microscopy
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Fast noninvasive three-dimensional (3D) imaging is crucial for the quantitative understanding of highly dynamic biological processes. Over the last decades, several fluorescence microscopy techniques have been developed in order to provide a faster and deeper imaging of thick biological samples [1]. Within this framework, Light Sheet Fluorescence Microscopy (LSFM) has emerged as a powerful imaging tool for 3D imaging of thick samples ranging from single cells to entire animals [2,3].
However, to obtain a 3D reconstruction either sample or microscope parts usually need to be moved limiting the acquisition speed and inducing possible interferences in volume recording. To solve this problem, herein we propose a new light-sheet-based optical scheme that enables fast volumetric recording at unprecedented temporal resolution. 3D imaging speeds up to 100 Volumes per Second (faster than three times the volumetric video rate) have been achieved without sample rotation or translation. Volumetric acquisition speed is limited only by the acquisition frame rate of the CCD camera and by a reasonable signal to noise ratio. Our optical approach allows invariant acquisition along the detection axis avoiding extensive processing and deconvolution methods to restore image quality. We demonstrate imaging performance of the microscope by fast volumetric acquisition of flowing beads and live Paramecium movements.
1. Diaspro A. Nanoscopy and Multidimensional Optical Fluorescence Microscopy, Chapman and Hall/CRC, (2010).
2. E.H. Stelzer. Light-sheet fluorescence microscopy for quantitative biology. Nat Methods. 12(1):23-6. (2014).
3. Huisken J and Stainier DYR. Selective plane illumination microscopy techniques in developmental biology. Development, 136(12):1963-1975, (2009).

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The Effects of Glucagon-Inhibiting Factors on Second Messengers in a Pancreatic α-Cell Line
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Abnormalities in glucagon secretion from the pancreatic α-cells worsen the hyperglycemia in diabetic patients, and a treatment to correct the glucagon output from their α-cells would be beneficial towards improving euglycemia in these patients. Glucose and various paracrine factors strongly inhibit glucagon secretion from islet α-cells, but the mechanisms of action remain elusive. Here, we present our approach to quantitatively measure the effects of these glucagon modulators on intracellular second messengers, such as Ca^{2+} and cAMP, and correlate those results with secretion. We use αTC1clone6 cells as a model of glucagon-secreting cell, and we transfect them with the fourth generation