Emerging Techniques in Modern Microscopies

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STED nanoscopy of the living mouse brain

KI Willig, H Steffens, SW Hell – Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

Confocal or two-photon microscopy are powerful techniques for imaging of structures inside living cells, tissue or living animals. However, they cannot show fine details or substructures of the cell because of their diffraction limited resolution of about half of the wavelength of light (~200-350nm). Recently, however, this limit has been overcome by a whole family of superresolution microscopy or nanoscopy concepts, including STED, RESOLFT, PALM, STORM and others[1]. These concepts are based on modulating the fluorescence emission such that adjacent labeled features fluoresce sequentially in time. The first technique to attain such diffraction-unlimited resolution was STED microscopy, which stands out for its recording speed and the ability to record 3D images from deep inside transparent specimens. Further, STED microscopy is live-cell compatible, especially when using fluorescent proteins.
It was shown that STED microscopy is capable to image dendritic spines up to 120 µm deep inside living organotypic brain slices and to resolve distinct distributions of actin inside dendrites and spines[2]. The basic function of spines is to interconnect with the neighboring cells by forming a synapse. It is therefore important to study them in a natural environment, which is ideally the living animal. We therefore developed an upright STED microscope to image the cerebral cortex of a living mouse through a glass window, so that we could observe the dynamics of dendritic spines in the molecular layer of the visual cortex[3]. The results show that STED nanoscopy is a highly suitable tool for neuroscience which can play a substantial role in the study of the living brain.

1. Hell, S.W., *Microscopy and its focal switch.* Nature Methods, 2009. 6(1): p. 24-32.
2. Urban, N.T., et al., *STED Nanoscopy of Actin Dynamics in Synapses Deep Inside Living Brain Slices.* Biophysical Journal, 2011. 101(5): p. 1277-84.
3. Berning, S., et al., *Nanoscopy in a living mouse brain.* Science 2012. 335(6068): p. 551.