“Volumetric STED/RESOLFT Imaging of Neuronal Cells”

Abstract: Elucidating the volumetric architecture of organelles and molecules inside cells requires microscopy methods with a sufficiently high spatial resolution in all three dimensions. Current methods are limited by insufficient resolving power along the optical axis, long recording times and photobleaching when applied to live cell imaging. Here, we present a 3D, parallelized, reversible, saturable/switchable optical fluorescence transition (3D pRESOLFT) microscope capable of delivering sub-80-nm 3D resolution in whole living cells. We achieved rapid (1–2 Hz) acquisition of large fields of view (~40 × 40 µm²) by highly parallelized image acquisition with an interference pattern that creates an array of 3D-confined and equally spaced intensity minima. This allowed us to reversibly turn switchable fluorescent proteins to dark states, leading to a targeted 3D confinement of fluorescence. We visualized the 3D organization and dynamics of organelles in living cells and volumetric structural alterations of synapses during plasticity in cultured hippocampal neurons.

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