Dual-color superresolution imaging of synapses and glia cells in living brain slices using STED microscopy

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Neuronal synapses are complex structures composed of pre- and postsynaptic membrane specializations ensheathed by glia processes, forming elementary functional compartments for rapid and flexible signaling in the central nervous system. Understanding how synapses are built during development and modified by experience is a central theme and challenge for neuroscience.

As synapses and glial processes are typically very small (< < 1 µm), dynamic and reside inside three-dimensional, light-scattering tissue, it is difficult to study them by conventional, diffraction-limited light microscopy.

However, major advances in superresolution imaging and fluorescence labeling are greatly improving our ability to investigate the inner life and dynamics of synapses using life-cell imaging approaches. We have shown that superresolution STED microscopy is a powerful technique for live-cell imaging of synapse morphology using YFP as a genetically encoded volume-label.

We will review our recent progress in developing STED microscopy for live-cell nanoscale imaging of synaptic structures deep inside brain slices and in two colors simultaneously. Specifically, we will demonstrate the powerful potential of these methodological advances for several applications concerning superresolution imaging of synapses: 1) spine plasticity and actin dynamics using lifeact 2) nanoscale imaging up to 100 µm deep below tissue surface and 3) dual-color live-cell imaging of pre- and postsynaptic structures as well as astrocytic processes with nanoscale spatial resolution under realistic experimental conditions.

References