Revealing the functional anatomy of the neuropil using super-resolution STED microscopy

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Stimulated emission depletion (STED) microscopy is unique among the commonly applied super-resolution techniques in being a laser beam scanning fluorescence microscopy modality. This makes it particularly suited for imaging inside live brain tissue, where it can resolve the morphology and dynamics of the smallest cellular structures, including dendritic spines, boutons and glia processes. More recently, we have conceived a new STED based approach to visualize also the extracellular space in live brain slices, termed super-resolution shadow imaging (SUSHI). Because the SUSHI approach is based on fluorescently labeling of the interstitial fluid, it inherently reveals all cellular structures in the field of view as shadows, and these can be analyzed in the context of the extracellular space. When SUSHI is combined with conventional STED imaging of fluorescently labelled cells, it allows analysis of these in the context of the neuropil.
In this talk, I will first introduce STED/SUSHI and the proof-of-principle findings it has brought about in revealing structural extracellular space dynamics on multiple spatiotemporal scales. I will move on to our current work that aims to understand diffusion in the extracellular space by developing a computational model based on live tissue SUSHI imaging, and finally I will outline our future plans for investigating the interplay between microglia cells and the extracellular space using our newly custom-built STED microscope.