COMBINING STED MICROSCOPY WITH SINGLE-MOLECULE IMAGING TO REVEAL THE MORPHOLOGY OF SYNAPSES AND THEIR DYNAMIC MOLECULAR ORGANIZATION

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The advent of super-resolution microscopy has provided exciting opportunities for neuroscientists to study the structure and molecular dynamics of synapses of living neurons. Excitatory synapses are hugely complex signaling machines housed in submicron-sized membranous protrusions in the postsynaptic neuron, called dendritic spines. Understanding their function and regulation requires a detailed view of spine morphology as well as their molecular composition and dynamics, which is not attainable with traditional experimental approaches.

In order to achieve this goal, we have combined two complementary super-resolution microscopy techniques; on the one hand, STED microscopy, which is a bulk imaging technique of ensembles of fluorophores, and lends itself to volumetric imaging of synaptic morphology, whose nanoscale details are invisible to conventional light microscopy, and on the other, single molecule imaging like PALM/STORM/uPAINT, which gives access to a wealth of quantitative information on the organization, dynamics and stochiometry of biomolecules, and are therefore suited for pinpointing and tracking proteins at synapses.

To harness these distinct strengths for synaptic research, we have built a microscope that combines STED with single molecule imaging. The STED part uses pulsed lasers for fluorescence excitation and stimulated emission of GFP, a helical phase mask to generate the focal STED doughnut, a galvo-based beam scanner and confocal fluorescence detection. Single molecule imaging is implemented on the same microscope by full-field excitation under oblique excitation with an EMCCD camera for fluorescence collection and single-molecule localization for image reconstruction. By changing the input port of the microscope, it is possible to switch between STED and single molecule imaging modes within seconds.

We tested the microscope using fluorescent nanospheres and validated its performance by imaging mammalian cells expressing several fluorescent proteins. We then imaged cultured hippocampal neurons obtained from fetal rat brains that expressed cytosolic GFP and various constructs to label synaptic proteins (PSD95-mEos3.2, GluA1-mEos3.2 and GluA2-Atto647N). Neuronal morphology was imaged by STED and the organization and mobility of synaptic proteins using (spt)PALM and uPAINT. STED and single molecule data were acquired sequentially within seconds, making it possible to overlay and quantitatively correlate, with 50 nm accuracy, the locations and motility of the labeled synaptic proteins with the morphological parameters of the dendritic spines.