Abstract Submitted
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Two-color super-resolution imaging of dendritic spines of hippocampal neurons using a custom STED microscope[1] STEPHANIE MEYER, KEVIN WOOLFREY, BARIS OZBAY, DIEGO RESTREPO, MARK DELL'ACQUA, EMILY GIBSON, University of Colorado Denver — We built a 2-color STED microscope and imaged dendritic spines in mouse hippocampal neurons at sub-diffraction limit resolution. The microscope is designed similar to one developed by Johanna Bückers, et. al. (Opt. Exp. 2011) in the lab of Dr. Stefan Hell. The STED microscope images at Atto590/Atto647N wavelengths and is capable of doing so simultaneously. We characterized the resolution of the system by imaging 40nm fluorescent beads as ∼58nm (Atto590) and ∼44 nm (Atto647N). The microscope is part of the UC Denver Advanced Light Microscopy Core, primarily for use by neuroscientists. We then performed 2-color STED imaging on hippocampal neurons immuno-labeled at PSD-95 (a postsynaptic density marker) along with either the GluA1-subunit of the AMPA-type glutamate receptor or the signaling scaffold protein AKAP150 in order to visualize nm-scale compartmentalization of these proteins within single postsynaptic dendritic spines. Importantly, for both GluA1 and AKAP150, STED imaging visualized sub-diffraction dimension clusters in spines located at both synaptic (overlapping or proximal to PSD-95) and extrasynaptic locations. In the future 2-color STED imaging should be useful for studying changes in the localization of these proteins during synaptic plasticity.

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