STED MICROSCOPY OF THE LIVING MOUSE VISUAL CORTEX USING RED FLUORESCENT PROTEINS

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Far-field fluorescence microscopy and the use of genetically encoded fluorescent proteins is a powerful technique to image structures inside living cells, tissue and living animals. However, small structures such as synapses which form the junctions of neurons are difficult to study with conventional light microscopy because of their minute size. From all the novel light microscopy or nanoscopy techniques available presently, STED microscopy stands out for its imaging capabilities in tissue: It is live-cell compatible, able to record 3D images from inside transparent tissue and the imaging speed is fast. Recently, we have developed an upright scanning STED microscope to image the dynamics of dendritic spines and implemented virus infection methods (adeno-associated virus) to label filamentous actin in the living mouse with Lifeact, an actin binding protein, and the yellow fluorescent protein [1]. However, the tissue penetration and therefore the imaging capability could be increased by using more red-shifted light which is ideally within the optical window (600-1000 nm). In the optical window the absorbance of hemoglobin and myoglobin is relatively low and less autofluorescence is generated. The drawback, however, is the low quantum yield of all red-emitting proteins which are available so far.

Here, we report STED microscopy of neuronal structures in the visual cortex of the living, anaesthetized mouse using red-fluorescent proteins. We fused mNeptune2 (excitation / emission of 599 / 651 nm) and mNeptune2.5 [2], monomeric, red-emitting proteins with Lifeact to highlight actin filaments in dendritic spines. We adapted the STED microscope to the red emission by excitation of 560 nm and STED of 730 nm. We recorded actin dynamics at sub-diffraction resolution without any sign of photo destruction.

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