STED MICROSCOPY WITH SUPERCONTINUUM SOURCE, HOLOGRAPHIC PSF CONTROL AND FLIM

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Stimulated emission depletion (STED) microscopy provides far-field resolution beyond the diffraction limit [1]. Fluorescence emanating from the periphery of the focused excitation beam is suppressed by a second beam that depletes the excited state population through stimulated emission thus the PSF of the microscope is effectively narrowed. The ultimate aim of the work presented here is to develop a versatile STED microscope for FLIM-FRET studies of protein interactions localized on scales below the classical diffraction limit, such as microclusters of cell receptor activation. To this end we present a system using a tunable supercontinuum excitation source (to provide relatively straightforward and low-cost spectral versatility) and incorporating a programmable spatial light modulator (SLM) (to facilitate convenient switching between different STED imaging modes and compensate for aberrations in the depletion path) [2]. STED is implemented on an otherwise standard confocal scanning microscope for compatibility with conventional microscopy techniques and instrumentation. Fluorescence lifetime imaging (FLIM) is implemented using time correlated single photon counting, which also provides a convenient means to monitor the STED process. In our current set-up, a femtosecond Ti:Sapphire laser is used to both generate a supercontinuum in a microstructured optical fiber and to provide the depletion beam. Images with resolution improvement beyond the far field diffraction limit in both the lateral and axial directions can be acquired by scanning overlapped excitation and depletion beams in two dimensions using the flying spot scanner of a commercially available laser scanning confocal microscope. The spatial properties of the depletion beam are controlled holographically using a programmable SLM, which can facilitate rapid switching between different STED imaging modes (improving resolution laterally or axially) and can also compensate for aberrations in the optical path. A typical STED FLIM image illustrating resolution below the diffraction limit is shown in Figure 1.

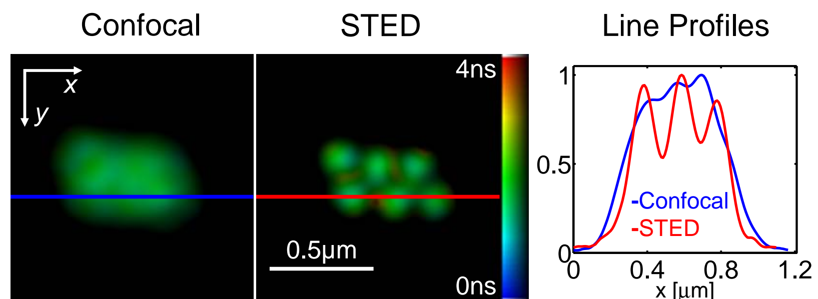

Figure 1: Intensity merged FLIM images (color-coded) of 200 nm beads in confocal and STED modes (adapted from [2]).

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