Improved resolution in STED microscopy using the Fluorescence transfer pairs as fluorescent probes

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Abstract: In STED microscopy, using higher laser power is often necessary since its spatial resolution increases proportionally with the square root of the intensity of the depletion light. Further improvement of its resolution is hampered mainly by the maximum intensity of the STED beam that can be sustained by the biological samples. Meanwhile, the higher laser power results in accelerated photobleaching produced by absorption of the depletion light by the excited singlet or the triplet state. Here, we theoretically demonstrate that using the FRET pair with a ultrahigh energy transfer efficiency as the probes has the potential of increasing the resolution of the STED microscopy without increasing the power of the depletion light. When excited with a Gaussian-like focal spot, the acceptor molecules have a higher probability of being excited in the center than those at the periphery. The higher the excitation intensity is used, the more likely it becomes that central acceptor molecules can be excited directly into saturation and the donor in this area will be left to be fluorescent for the exited acceptors can't accept the energy. When a depletion beam with the doughnut-shape is added upon the acceptor molecules, the acceptor molecules in the outer region of the focus are forced to transit to the ground state by stimulated emission. This helps to eliminate FRET frustration at periphery and limits the FRET saturation in the center. Consequently, the fluorescent spot of donor is further decreased.

Figure 1 Normalized PSFs in STED microscope for Cy3 fluorophore (a) and Cy3-Cy5 pair (b). (c) Intensity profiles through the centers of the image in (a) (black curve) and (b) (red curve). The resolution is enhanced from 70nm to 45nm when FRET pair is used as the probes.

References: