STED microscopy with optimised doughnut shaped beams

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In a spot-scanning STED microscope[1-2], the excited state of a fluorophore is quenched by stimulated emission in the outer areas of the focal excitation spot, in a saturated manner. Ideally the quenching light should therefore have zero intensity close to the excitation maximum but a uniformly high intensity in a volume surrounding it. Such an intensity distribution is usually created by systematically manipulating the wavefront of the beam used for STED. The focal distribution resulting from the wavefront manipulation can be predicted by diffraction theory. Since the quenching process is polarization dependent, vectorial focusing theory has to be applied.

Based on a careful analysis of the focusing process we developed an algorithm to optimize the wavefront in order to achieve maximal resolution in a STED-microscope under common experimental conditions. Optimal combinations of polarization and wavefront are proposed depending on the aperture angle, the available laser power and the rotational mobility of the dye molecules.

New experiments will be presented, showing a fundamental increase of resolution beyond the diffraction barrier.

[1] S. W. Hell, and J. Wichmann, "Breaking the diffraction resolution limit by stimulated emission.", Opt. Lett. , **19**, 780-782 (1994)

[2] Thomas A. Klar, Stefan Jakobs, Marcus Dyba, Alexander Egner, Stefan W. Hell, “Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission”, *PNAS* , **97**, 8206-8210 (2000)