LOWER-BACKGROUND CONFOCAL MICROSCOPY USING A COHERENT-HYBRID DEPLETION BEAM

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Sub-diffraction resolution fluorescence imaging explores the fact that the fluorescence microscope is not governed by optics laws alone, but involves a (generally non-linear) sample response. Stimulated emission depletion (STED) microscopy, which pioneered the approach [1], works by illuminating an excited spot with a doughnut-shaped, red-shifted, depletion beam. The depletion beam is generated by using i) a helical phase ramp ('vortex'), for maximal lateral resolution [2], ii) a Pi-shifted top-hat, for maximal axial resolution [3] or iii) their incoherent combination. The vortex (or 2D-STED) mode is, by far, the most widely used in xy scans due to its notable lateral resolution and resilience to radial aberrations, such as an axial misalignment in the optics train or spherical aberration. However, depth sectioning in 2D-STED is still limited to that provided by the confocal pinhole.

Building on the aberration resilience of the 2D-STED doughnut dip, we proposed a 2D-STED-perturbed beam featuring a tunable ampoule-shaped (concave) dip [4], defining a 'coherent-hybrid' (CH-) STED mode. This depletion beam, generated by a bi-vortex plate (Fig. 1), allows a balance to be found between lateral resolution and background suppression.

Here, we focus on the high-sectioning end of the CH-STED regime ($\rho<0.85$) which, for a given energy, displays only a mild (super) resolution level. This regime has a high sectioning strength, which can be used at relatively low STED power for a 'low-noise confocal' mode, as we show by imaging a complex biological object: the mitotic spindle.

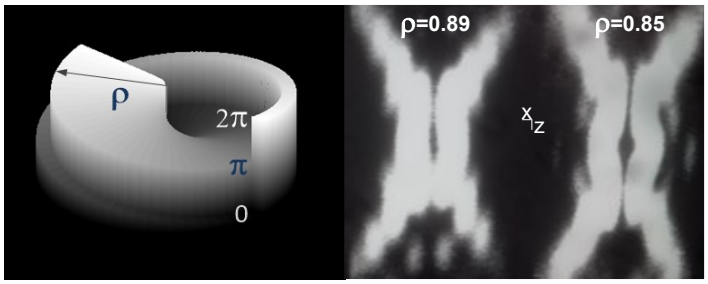

Figure 1: Bi-vortex phase plate and depletion beam axial section at different ρ.
Lower ρ values lose lateral resolution for improved sectioning.

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