BEAM SHAPING FOR CONFOCAL AND STED MICROSCOPY

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KEY WORDS: super-resolution, ISM, adaptive optics, wavefront engineering.

Since 1952, when Toraldo di Francia proposed to subdivide the pupil in a proper number of concentric annular filters [1], the engineer of the three-dimensional (3D) volume surrounds the focus of an optical microscope has been deeply studied and exploited. Although the advent of super-resolution techniques like stimulated emission depletion (STED) overcome the diffraction limit in the three dimensions, there still an open challenge in how the axial resolution can be improved and optimized. In this work we investigate the possibility of enhancing z-resolution in image scanning microscopy and STED microscopy by designing proper pupil filters. While for confocal microscopy we take inspiration from some pioneering Sheppard' [2] and Martinez-Corral' [3] articles, for STED the unlimited improvement in resolution is achieved by confining the excitation volume via stimulated emission both in lateral and axial directions. This is traditionally achieved by superimposing two beams with different shapes, i.e. vortex beam and bottle beam, on top of the excitation beam at the focus of an objective lens [4]. However, the vortex beam and the bottle beam behave differently under certain aberrations, and preserving the intensity-zero under the compound aberration effects caused by the optical inhomogeneity is a challenge [5]. One approach proposed lately utilizes a hybrid phase masks, a combination of the helical phase and the pi shifts [6]. Our recent results show that the tilting of two vortex beams relative to the optical axis of the microscope can enable 3D super-resolution [7]. In this study, we explore the potential of this new approach toward deep tissue 3D STED microscopy.

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