

3D STED nanoscopy by tilting 2D doughnut depletion beams

Takahiro Deguchi¹, Sami Koho², Giorgio Tortarolo², Behjat Kamaran^{1,3}, Eleonora Uriati¹, Giuseppe Vicidomini², Alberto Diaspro^{1,3}, and Paolo Bianchini¹

¹Nanoscopy and NIC@IIT, CHT Erzelli, Istituto Italiano di Tecnologia,

²Molecular Microscopy and Spectroscopy, CHT Erzelli, Istituto Italiano di Tecnologia

³Department of Physics, University of Genoa, Genoa, Italy

E-mail : paolo.bianchini@iit.it, or takahiro.deguchi@iit.it

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Stimulated emission depletion (STED) microscopy allows spatial resolution beyond the diffraction-limit. The improvement in resolution is achieved by confining the effective excitation volume via the stimulated emission process under a doughnut-shaped depletion laser beam. For efficient confinement both in lateral and axial directions, traditionally two beams with different shapes, i.e., vortex and bottle beams, were co-aligned with the conventional Gaussian excitation beam [1]. These beam shapes were formed by wavefront engineering, applying helical phase shift and pi phase shift at the center, respectively.

When 3D STED microscopy is applied for thick biological specimens, adaptive optics is often coupled to preserve the spatial resolution as the loss in resolution and signal becomes significant due to the specimen-induced aberrations [2]. However, the vortex beam and the bottle beam behave differently under specific aberrations. In particular, the two beams preserve differently the “zero-“ intensity points under compound aberration effects, and thus having optimal intensity distribution is a challenge [3]. One approach proposed lately utilizes a hybrid phase mask, a combination of the helical phase and the pi shifts. This approach exhibits a beam shape that confines the excitation volume laterally and axially, demonstrating 3D super-resolution at significantly higher contrast than the conventional approach [4]. However, this method has not been examined at aberrating conditions.

Recently, we have found that, by tilting the vortex beam relative to the optical axis of the microscope, the depletion beam exhibits the confinement of the excitation volume both in lateral and axial directions, enabling 3D super-resolution [5]. In this study, we characterize the performance of the tilted depletion beam in the context of 3D STED microscopy at the presence of optical aberrations. For an experimental demonstration, we form the depletion beam using multiple phase masks, introduced by a spatial light modulator. We explore the potential of this new approach toward deep tissue 3D STED microscopy.

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