A study of 3D beam shaping for axial resolution enhancement in STED nanoscopy

Takahiro Deguchi[1], Giuseppe Vicidomini[2], Sami Koho[2,3], Pekka Hänninen[3], Alberto Diaspro[1] and Paolo Bianchini[1]
[1]Nanoscopy, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genoa, Italy
[2] Molecular Microscopy and Spectroscopy, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genoa, Italy
[3] Laboratory of Biophysics, University of Turku, Tykistokatu 6A, 20520 Turku Finland
E-mail: takahiro.deguchi@iit.it

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Super-resolution fluorescence microscopy has achieved spatial resolution well beyond the diffraction-limit in all three dimensions (3D) even in biological specimen that are optically heterogeneous [1]. In stimulated emission depletion (STED) microscopy the conventional excitation beam of a scanning fluorescence microscope is overlapped with a doughnut-shaped second beam, so called depletion beam, which silences fluorophores under it *via* stimulated emission; only those fluorophores located at the zero-center of the depletion "doughnut" are allowed to emit light. This process reduces the size of effective fluorescence volume below diffraction limit – and thus, spatial resolution is enhanced.

For the resolution enhancement in all three dimensions, the 3D depletion intensity pattern is commonly formed by two different phase plates; one with helical phase steps (0-2π) forming the lateral doughnut-shaped depletion beam, and the other with a π shift at the center forming the axial depletion beam with maxima above and below the actual focal plane [2]. The axial depletion shape formed with a π phase shift is known to be extremely prone to aberrations e.g. in biological samples, and therefore wavefront correction with adaptive optics has been proposed [3]. Axial resolution may also be enhanced using two opposing objectives [4] or by tomographic approach [5], however, the former leads to a complex system and the latter in limits in sample preparations.

We have demonstrated 3D super-resolution STED microscopy in biological specimen using axial depletion beam [6,7]. In this work, we studied the effectiveness of different 3D patterns of the depletion beam in order to achieve super-resolution in all the three dimensions.

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