

Tilted depletion beam enables 3D super-resolution in STED microscopy

Takahiro Deguchi¹, Giorgio Tortarolo², Sami Koho², Giulia Zanini¹, Luca Pesce¹, Alberto Diaspro¹, Giuseppe Vicidomini², and Paolo Bianchini¹

1. Nanoscopy and NIC@IIT, Istituto Italiano di Tecnologia,
via Morego 30, 16155, Genova Italy

2. Molecular Microscopy and Spectroscopy, Istituto Italiano di Tecnologia
via Morego 30, 16155, Genova Italy

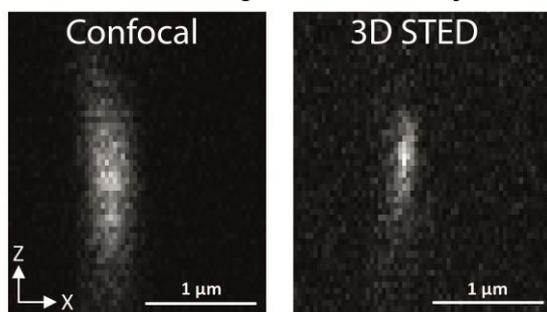
E-mail : paolo.bianchini@iit.it, or takahiro.deguchi@iit.it

KEY WORDS: super-resolution, PSF engineering, SLM, STED microscopy.

Stimulated emission depletion (STED) microscopy allows surpassing the diffraction-limited resolution 3D microscopy and opened up the studies of nanostructures in biological applications¹. This improvement in spatial resolution is realized by employing two laser beams, excitation and depletion, and superimposing the two at the focus of an objective lens. The depletion beam engineered with a helical phase steps (vortex beam) exhibits an intensity distribution of a doughnut-like pattern in lateral plane, thus zero at the center and peaks in a ring shape. Under the doughnut, excited molecules are de-excited through stimulated emission processes, and the fluorescence signal from the spontaneous emission is detected only from the central region of the excitation beam. This effectively reduces the size of the point spread function of confocal microscopy, leading to an improved resolution.

To achieve 3D super-resolution, in addition to the vortex beam, one employs an additional depletion beam, which is formed with π phase shift at the center (bottle beam)². The bottle beam exhibits intensity peaks above and below the objective focus, and this generates de-excitation at the top and bottom of the excitation volume, resulting in an axial resolution improvement. However, the bottle beam is known to be sensitive to refractive index mismatch and/or optical inhomogeneity, and when the zero-center is lost the resolution and the signal-to-background ratio declines.

Because the vortex beam is known to be less sensitive to the refractive index inhomogeneity, we exploit the use of an inclined vortex beam for the resolution improvement both in lateral and axial directions³. In order to introduce the tilt to the vortex beam, we examined the following two approaches; (i) shifting the depletion beam relative to the center of an objective lens, or (ii) by using a spatial light modulator, introducing a specific phase pattern at the back aperture of the objective lens. The second approach is typically used for



adaptive optics to correct specimen-induced aberration⁴. We utilize the method to induce a specific aberration mode, coma and lateral shift, to achieve the tilted depletion beam.

The experimental results of imaging a 60 nm fluorescent particle with the above (i) approach showed a resolution improvement of 2-fold in all the directions, compared with a standard confocal microscopy image (Fig.).

References:

1. G. Vicidomini, P. Bianchini, and A. Diaspro, “STED super-resolved microscopy,” *Nature Methods* (2018) [doi:10.1038/nmeth.4593].
2. D. Wildanger et al., “A compact STED microscope providing 3D nanoscale resolution,” *Journal of Microscopy* **236**(1), 35–43 (2009) [doi:10.1111/j.1365-2818.2009.03188.x].
3. “Italian priority application IT 102017000137607; priority filing 11/30, 2017. International application claiming priority PCT/IB2018/059492; Bianchini et al. filing date 11/30, 2018.”
4. T. J. Gould et al., “Adaptive optics enables 3D STED microscopy in aberrating specimens,” *Opt. Express* **20**(19), 20998–21009 (2012) [doi:10.1364/OE.20.020998].