ISOSTED NANOSCOPY FOR THICK SPECIMENS

Xiang Hao[1, 2], Edward S. Allgeyer[2, 3], Joerg Bewersdorf[2,4], Xu Liu[1]

[1] State Key Laboratory of Modern Optical Instrumentation, College of Optical Science and Engineering, Zhejiang University, Hangzhou 310027, China

[2] Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, USA

[3] The Gurdon Institute, University of Cambridge, Cambridge CB2 1QN, UK

[4] Department of Biomedical Engineering, Yale University, New Haven, Connecticut, USA

Email: haox@zju.edu.cn

KEY WORDS: isoSTED, 3D imaging, adaptive optics, thick specimen

Far-field light nanoscopy techniques are exceptional in their ability to noninvasively visualize cellular structures below the diffraction limit in three dimensions (3D). To further improve axial resolution, current nanoscopy methods can be combined with a 4Pi geometry. However, although the compatibility of 4Pi nanoscopy with cell samples has been shown, its application in tissues has been unexplored, due to the ghost images and the quickly accumulated aberrations in thick specimens.

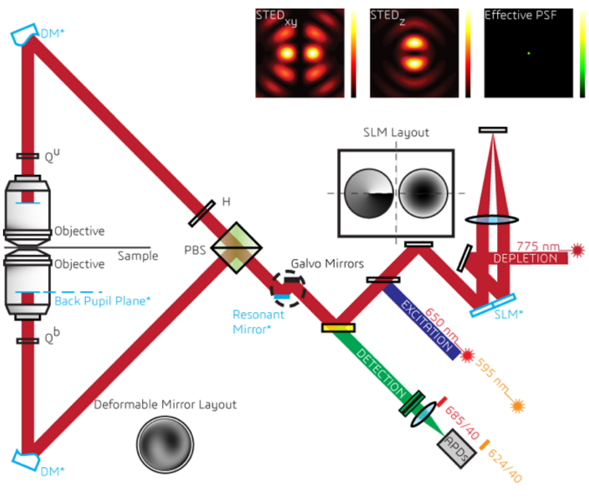

Fig. 1 Simplified schematic of adaptive optical isoSTED nanoscopy.

To extend the imaging capabilities to thick specimens without compromising resolution, we present a new implementation of 4Pi-STED (isoSTED) nanoscopy. A simplified schematic is shown in **Fig. 1**. This new design provided three major improvements with respect to the original isoSTED nanoscope [1]. First, using orthogonal polarization components from the same laser to generate the $STED_{xy}$ and $STED_z$ depletion patterns leads to intrinsic co-alignment of the two depletion beams. Second, we converted the linear polarization of the laser sources to circular polarization to minimize excitation and depletion selectivity with respect to fluorophore dipole orientation and also reduces laser-induced background in the recorded images. Third, two deformable mirrors (DMs) were respectively placed into the upper and lower beam paths. Aberrations were corrected using a sensorless adaptive optics (AO) architecture. These endeavors enable us to recover the aberration-free point-spread function (PSF) dynamically and to enable the discrimination of the ghost images induced by the side-lobes above and below the focal plane.

Furthermore, to demonstrate the wide applicability of our newly developed isoSTED nanoscopy, we imaged complex biological architectures in 3D ranging from organelles inside the cells to intercellular structures in the tissue specimens.

Reference

[1] R. Schmidt, C. A. Wurm, S. Jakobs, J. Engelhardt, A. Egner, and S. W. Hell, "Spherical nanosized focal spot unravels the interior of cells," *Nat. Methods* **5**, 539-544 (2008).