ISOSTED NANOSCOPY FOR THICK SPECIMENS

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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.

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\textsubscript{xy} and STED\textsubscript{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