DEEP 3D LOCALIZATION MICROSCOPY USING A DEFORMABLE MIRROR.

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Point spread function (PSF)-shaping is a popular technique for 3D localization microscopy (PALM/STORM), wherein the axial position of individual molecules can be computed from individual 2D image sequences. The most accessible method – astigmatism generated by a cylindrical lens – is limited to ~1 um depth. Several alternative methods have been proposed to improve the axial range, including double-helix or saddle-point PSFs. Previously described systems used either spatial light modulators (SLMs), which have low photon collection efficiency (below 30%), or expensive transmission masks that cannot be adjusted.

We describe a PSF shaping system based on a deformable mirror, an affordable device that provides 100% photon efficiency and is flexible enough to generate a wide range of PSFs including Gaussian, astigmatism or saddle-point PSFs [1]. This approach is beneficial for 3D single molecule localization as we can easily adapt the desired axial range by changing the mirror's shape via software to the particular needs of the experiment. Our localization and super-resolution image reconstruction algorithm uses phase retrieval with a PSF model based on a single pupil function composed of Zernike modes and an image formation model that accounts for Poisson noise. We implemented a maximum likelihood estimator (MLE) algorithm to retrieve the phase from a stack of calibration images, a detection algorithm based on cross-correlation with the PSF dictionary, and another MLE algorithm for single molecule localization. Our algorithms were implemented using highly-parallelized Python code, enabling the localization of millions of molecules in reasonable time on a multicore machine or computing cluster.

We test our system by imaging beads and biological structures such as immuno-stained microtubules in U373 cells. We show that an astigmatic PSF created by a deformable mirror can outperform a cylindrical lens in 3D localization precision. We also show that a saddle-point PSF allows to improve the axial range from 1 μm to 3 μm with minor loss in localization precision compared to the astigmatic PSF. On microtubules, our astigmatic imaging mode achieves a 3D resolution of 30x30x50 nm (XYZ) with a detectable range of 1 μm. The saddle-point PSF achieves a resolution of 50x50x70 nm with the detectable range of 3 μm. The localization precision is also in good agreement with the fundamental theoretical limit (Cramer-Rao lower bound).

In summary, we describe a flexible optical and computational solution for 3D localization microscopy by PSF shaping.

[1] Shechtman, Y., Sahl, S. J., Backer, A. S., & Moerner, W. E. "Optimal Point Spread Function Design for 3D Imaging", *Physical Review Letters*, **113**(13), 133902 (2014).