Point Spread Function Engineering enables Light-Efficient Extended Depth 3D Single Molecule and Whole Cell Imaging

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Widefield fluorescence optical microscopy has become a ubiquitous tool for applications in biology, medicine, pharmacology, etc. due to its ability for specific labeling and reduced background. The imaging resolution and imaging volume is typically dictated by the numerical aperture and magnification of the microscope objective. Although, photon starved applications like single molecule (SM) imaging can benefit from high NA objectives, this in turn leads to a reduced depth of field and hence imaging volume. As an example, the depth of field (DOF) of 1.49 NA objective using a Cy5 label is about ~0.5 μm.

To date, researchers have either used axial stitching at the cost of temporal resolution or low NA objectives at the cost of spatial resolution. Here, we propose the use of engineered point spread function (PSF) that enable the extension of the DOF without the need for axial scanning while making use of the full aperture of the high NA objectives. Engineered PSFs work by modifying the wavefront (phase) of light to alter optical system response to a desired shape constrained by the wave equations. More specifically, we categorize these as localization PSFs (e.g., Double Helix (DH) PSF, Tetrapod (TP) PSF) and Extended depth of field (EDOF) PSFs (e.g., Single Helix (SH) PSF, and Deep Focus (DF) PSF)

The DH-PSF presents two lobes with the axial position of an emitter encoded in their orientation, while the lateral position is obtained from the center of the pattern [1]. The DH-PSF can extend the depth range of a 1.49 NA objective from 0.5 um to about 2-12 um. The Tetrapod PSFs [2] on the other hand, can extend the DOF to up to 21 um. The Tetrapod PSF features two main lobes that vary in separation distance as a function of emitter depth. The axis defined by the two lobes rotates 90^{o} when the emitter moves from below to above the focal plane. We will show the applications of these PSFs in localization-based microscopy, specifically in SM imaging [1], single particle tracking [3] and light-sheet imaging [4].

The SH-PSF [5] is similar to the DH-PSF but exhibits a single lobe rotating about its's axis. The DF-PSF on the other hand maintains the circular symmetry of the PSF while extending the DOF. Thus, these PSFs result in photons being distributed over a smaller area and the resulting PSF shape is similar to a Gaussian albeit across a much larger DOF. We will show results from reconstructions of single-shot images captured with these EDOF-PSFs exhibiting an extended depth of field image. Even without any post-processing the images have better overall quality over the volume than a traditional lens. With post-processing, we achieve near diffraction limited resolution depending on the SNR available.

References

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