Optimal 3D SMLM for any PSF using ZOLA-3D

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PSF engineering is often used for achieving 3D SMLM from 2D images obtained without scanning. The most common engineered PSF is astigmatism [1], but this method is limited to an axial range of ~1 µm. Larger axial ranges can be achieved using more sophisticated PSF such as corkscrew [2], double helix [3], phase ramp [4], saddle-point [5] or tetrapod [6] PSFs. In most cases, a dedicated localization method is employed for each PSF to reconstruct super-resolution images. A recent method based on cubic splines [7] allows to model PSFs of arbitrary shapes, but does not account for depth-dependent variations in the PSF induced by refractive index mismatch. Because phase shaping devices enable an infinite variety of PSFs, a generic approach is needed to localize single molecules and reconstruct super-resolution images.

We recently developed ZOLA-3D, a software that enables 3D SMLM image reconstruction using a realistic modelling of the PSF, and which accounts for depth-dependent spherical aberrations [8]. The Zernike-based phase retrieval modelling was limited to continuous phase masks such as those used for astigmatism, saddle-point or tetrapod PSFs. Here, we propose an extension of ZOLA-3D that uses a pixel-based phase retrieval method using maximum likelihood estimation. This algorithm can now model arbitrary PSFs, including corkscrew, double helix or phase ramp methods whose phase is not continuous. We will show evaluations of our extended ZOLA-3D on simulated data, indicating that the localization algorithm reaches the fundamental (Cramer-Rao) limits, as well as applications to experimental data of nuclear pore complexes imaged using a double helix PSF.

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