AN EXPERIMENTAL PSF MODEL FOR 4PI SINGLE-MOLECULE LOCALIZATION

Yiming Li, Jonas Ries
Cell Biology and Biophysics Unit
European Molecular Biology Laboratory
Meyerhofstr. 1, 69117 Heidelberg, Germany
E-mail: yiming.li@embl.de

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Interferometric single-molecule localization microscopy (iPALM [1], 4Pi-SMS [2]) uses multiphase interferometry to localize single molecules and currently achieves the highest axial resolution of all 3D superresolution approaches. In theory, 3D sub-10 nm resolution can be achieved with only 250 photons collected in each objective for an individual molecule [3]. However, the resolution achievable with the current image analysis workflow is substantially worse than the theoretical limit. Here, we developed an experimental PSF fitting method for the interference 4Pi-PSF. As the interference phase is not fixed with respect to the shape of the PSF, we developed a new 4Pi-PSF model, which decouples the phase term from the shape of the PSF. Using a spline-interpolated experimental PSF model [4] and by fitting all 3 or 4 phase images globally, we showed on simulated data that we can achieve the theoretical limit of 3D resolution, the Cramér-Rao lower bound (CRLB), also for 4Pi microscope.

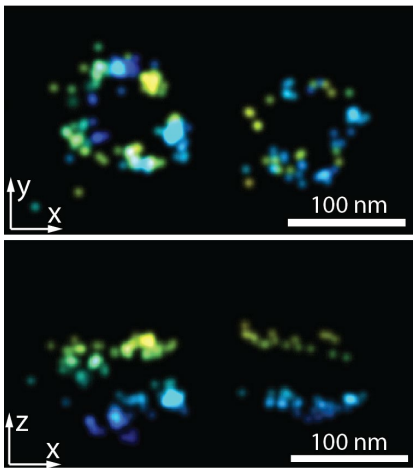

Figure 1: 4Pi-SMS image of Nup96-SNAP-AF647.

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