FAST, ROBUST AND PRECISE 3D LOCALIZATION FOR ARBITRARY POINT SPREAD FUNCTIONS

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We present a fitter for 3D single-molecule localization of arbitrary, experimental point spread functions (PSFs) that reaches minimum uncertainty for both sCMOS and EMCCD cameras. Implemented on the graphics processing unit, it achieves more than 10^5 fits/s. We provide a tool to robustly and accurately model the experimental PSF by averaging several bead stacks and by regularization, which avoids common artifacts often associated with fitting of experimental PSFs.

Using the simple astigmatic 3D method, these tools allowed us to take data with a 3D resolution to date only achieved with much more complex interference-based methods. For instance, we could easily resolve the 3D hollow, cylinder-like structure of immunolabeled microtubules and extract the precise geometry of the clathrin coat on endocytic pits.

Finally, our new fitter enables high-resolution 3D imaging directly on standard microscopes without any 3D optics, by exploiting subtle differences between the upper and lower half of realistic experimental PSFs.

We report on recent progress towards global multi-channel fitting that improves the accuracy in ratiometric dual-color, multi-plane and 4Pi single-molecule localization microscopy.

The fitter is compatible with any PSF engineering approach and it is insensitive to optical aberrations and thus makes accurate 3D superresolution microscopy broadly accessible.

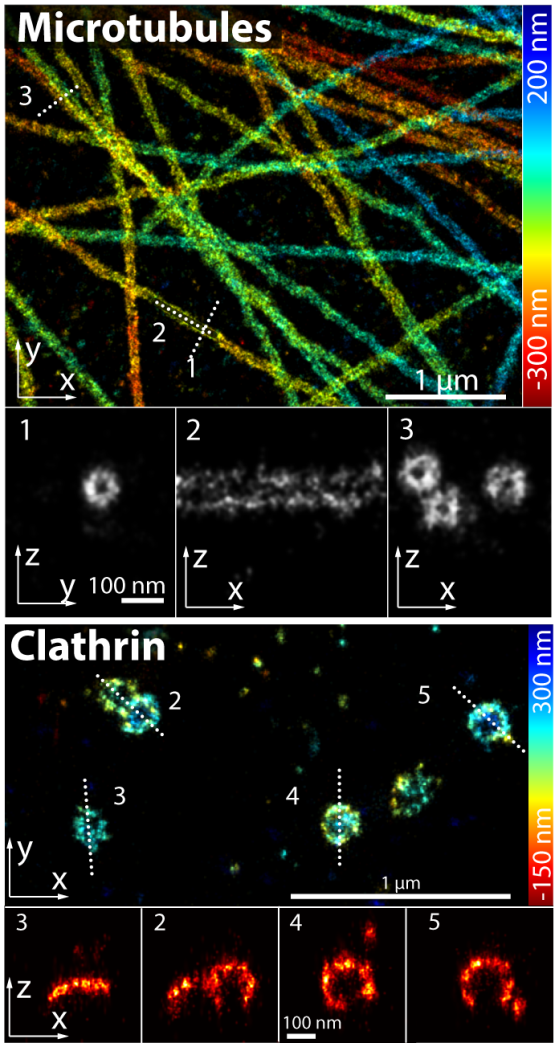

Figure: Validation of the 3D fitter on biological samples. Top-view and side-view reconstructions of immunolabeled microtubules and clathrin-coated pits.

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