Least-Square PSF fitting for localization microscopy

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Localization-based forms of fluorescence microscopy predominantly rely on efficient computation of Gaussian approximations of the point spread function (PSF) for the calculation of fluorophore positions. Theoretical predictions show that under specific experimental conditions, localization accuracy is significantly improved when the localization is performed using a more realistic model. Here, we show how this can be achieved in practice by considering 3-D point spread function (PSF) models for the widefield microscope.

To this aim, we formulate the Gibson and Lanni PSF model in terms of readily accessible parameters of the experimental setup and provide an algorithm for an efficient implementation. We then focus on the least-square criterion and demonstrate its performance under various acquisition conditions. In particular, we estimate the 3-D location of particles from z-stack data and the defocus measure of the detector plane.

Our work provides a complementary approach to currently available algorithms that rely on simplified PSF models. The latter can be used for obtaining preliminary results and for having an immediate feedback about the quality of the experiment, while the more realistic models can be used for a more accurate analysis that can be performed at a later stage.