

ALGORITHM FOR 3D LOCALIZATION MICROSCOPY USING REALISTIC MODELING OF THE POINT SPREAD FUNCTION BY PHASE RETRIEVAL

Benoit Lelandais, Andrey Aristov, Mickaël Lelek, Christophe Zimmer
Unité Imagerie et Modélisation
Institut Pasteur
25-28 rue du Docteur Roux, 75015, Paris, France
E-mail: benoit.lelandais@pasteur.fr

KEY WORDS: PALM/STORM, single molecule, phase retrieval, PSF model, CRLB

1. INTRODUCTION: Super-resolution localization microscopy relies on the computational localization of single molecules with sub-diffraction precision. Precise localization is usually achieved by fitting single molecule images to a point spread function (PSF) model. For optimal precision, this model should be as close as possible to the real PSF. The Gaussian PSF model function used in most algorithms, and most alternative models that do not take account for optical aberrations are therefore suboptimal. Realistic models of the PSF are even more important in 3D localization microscopy approaches that use PSF engineering to encode the axial position of molecules in 2D images. Determining a theoretical model that accurately models arbitrary 3D PSFs in presence of optical aberrations is challenging. Instead, we here propose a method that determines a realistic PSF model directly from experimental data.

2. METHOD: We implemented an algorithm based on phase retrieval to model the PSF from noisy images of fluorescent beads. Our method assumes that the phase is a combination of Zernike polynomials and fully accounts for Poisson noise. We compute a maximum likelihood estimation (MLE) of the phase using Newton-Raphson optimization. This provides a numerical model of the PSF for arbitrary subpixelic positions. First, we used this model to compute the fundamental limit to localization precision given by the Cramér-Rao lower bound (CRLB). Second, we implemented a full reconstruction algorithm for 3D localization microscopy that uses cross-correlation for detection and MLE for localization using the PSF obtained by phase retrieval.

3. RESULTS: Our method was evaluated on simulated and experimental data from an optical system that uses a deformable mirror for PSF shaping. Our results show that the 3D localization precision reached the CRLB for an axial range of 3 μm , indicating that the MLE has optimal precision. We will also present results on 2D data and on 3D data obtained with a system that uses a cylindrical lens to generate astigmatism, and compare to results obtained using Gaussian model fitting.

4. CONCLUSION: We propose a method and tools to determine a realistic model of a PSF for 3D localization microscopy. The tools include phase retrieval, CRLB computation, single molecule detection and localization by MLE. The method can be applied to any PSF engineering approach using a continuous phase. For an optical system featuring a deformable mirror for PSF shaping, our CRLB tool can be used to optimize the setup. For a given optical system and with suitable calibration, the localization tool should allow state-of-the-art reconstruction of 3D localization images for a wide range of engineered PSF.