Modulated Single Molecule Localization Microscopy using Homodyne Detection

P. Jouchet[1], C. Cabriel[1], N. Bourg[1], M. Bardou[1], E. Fort[2], S. Lévêque-Fort[1]
[1]Institut des Sciences Moléculaires d'Orsay, Université Paris-Sud, Université Paris Saclay, CNRS UMR 8214, Orsay France
[2]Institut Langevin, ESPCI Paris, CNRS, PSL University, Paris France
Email: pierre.jouchet@u-psud.fr sandrine.leveque-fort@u-psud.fr

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In Single Molecule Localization Microscopy (SMLM), the position of the emitters is obtained from a centroid fitting of the Point Spread Function (PSF). The localization precision relies strongly on the PSF shape which quickly degrades with increasing depth due to aberrations. Several alternative localization strategies have been proposed using time varying structured illumination based on traveling interferences [1] or more recently on triangulation from a zero-intensity point of the excitation beam [2]. These strategies bring significant benefits in particular they achieve a more precise localization precision with less photons. However, they are designed for single point tracking and rely on the use of a fast monodetector to be time efficient. They thus find applications in single particle tracking or scanned super-resolution microscopy and remain limited in field of view for structural imaging.

In SMLM, a large number of fluorophores needs to be localized simultaneously in the image to reconstruct the biological sample structure at the nanoscale. We propose a new localization strategy based on the modulation of the fluorescence emission using a periodically structured excitation [3]. The position of a fluorescent molecule within the moving fringe pattern is encoded in the phase of its modulated emission signal. The use of a camera enables the unfolding of the phase by discriminating fluorophores positioned in different fringes with equal phases. The camera being slow, the signal demodulation is performed by a specific optical assembly placed in front of the camera. The assets and performance of this new localization technique will be shown in particular regarding enhanced localization and resistance to aberrations. We will show in particular, how it can be used to improved axial localization in 3D SMLM in depth where aberrations are substantial and impair standard PSF engineering methods. We will show that it can produce 3D images featuring an almost isotropic 3D and constant over the whole capture range localization precision at 30 microns in depth (**Fig. 1**).

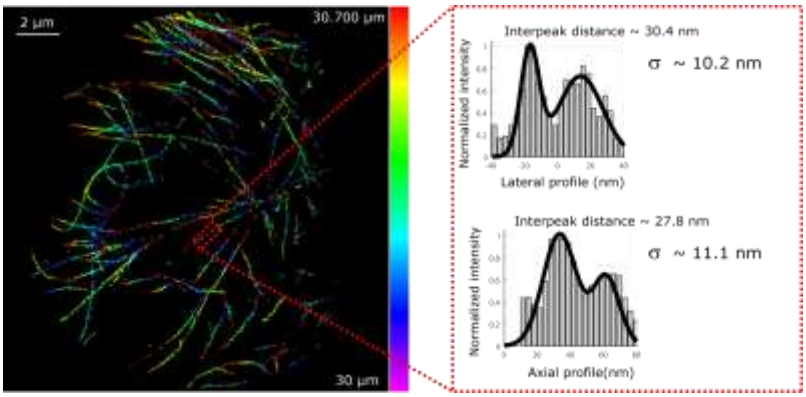

Fig1: 3D image of tubulin labeled AF-647 at 30 microns in depth, and typical tubulin profiles

[1] Busoni et al., "Fast subnanometer particle localization by traveling-wave tracking", *Journal Of Applied Physics* 98, 064302 2005
[2] Balzarotti et al., "Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes", *Science.* 2017 Feb 10;355(6325):606-612
[3] Patent FR3054321-A1