PHASE ENCODED SINGLE MOLECULE LOCALIZATION MICROSCOPY WITH STRUCTURED EXCITATION

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In Single Molecule Localization Microscopy (SMLM), the position of the emitters is obtained from a fitting of the Point Spread Function (PSF). The localization precision thus strongly relies on the PSF shape, which quickly degrades in 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 with less photons. However, they are designed for single point tracking and rely on the use of a fast monodetector to be time efficient. Therefore, they find applications in single particle tracking or scanned super-resolution microscopy.

In SMLM, a large number of fluorophores need 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 thanks to the centroid detection. 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 discussed, in particular regarding enhanced localization and resistance to aberrations. We will demonstrate how it can be used to improve the axial localization in 3D SMLM in depth, where aberrations are substantial and impair standard PSF engineering methods. We will present 3D images of tubulin network on COS7 cells featuring an almost isotropic 3D localization precision at 30 microns depth that remains constant over the whole capture range (**Fig. 1**).

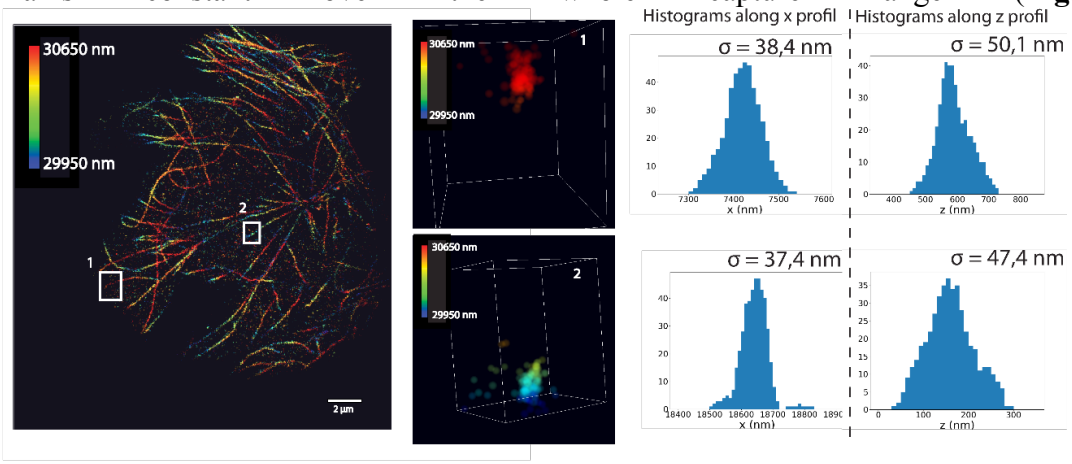

Figure 1: 3D image of tubulin labeled AF-647 at 30 microns in depth in COS7, and typical x,z tubulin profiles

[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] Fort, et al "System and method to measure parameter in a medium", Patent FR1657130A, 07/2016