STRUCTURED EXCITATION FOR ENHANCED SINGLE MOLECULE 3D LOCALIZATION

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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, impacting both lateral and axial resolutions. Several alternative localization strategies have been proposed using time varying structured illumination based on shifting 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. Recently, this concept has been revisited to match SMLM wide field approach with different implementations to enhanced lateral resolution [3-5].

We will present a 3D localization strategy based on the modulation of the fluorescence emission using a periodically structured excitation called ModLoc for Modulation Localization [6]. The position of a fluorescent molecule within the moving fringe pattern is encoded in the phase of its modulated emission signal. The camera being slow, the signal demodulation is performed by a specific optical assembly based on a Pockels cell placed in front of the camera. In particular, we will show that the frequency of demodulation must be optimized with the short ON-time of single molecules emitting stochastically during the acquisition. This versatile strategy can be used on PAINT or dSTORM without discarding single molecule events.

We will particularly emphasize the benefit of this approach for axial localization, where a uniform axial precision ~6.8 nm can be reached at several microns in depth. Performances and assets of ModLoc will be discussed. Thanks to the almost isotropic and uniform 3D localization precision, measurements over different depths can be merged to perform 3D imaging over several microns. We will show how ModLoc can be used to improve axial localization in 3D SMLM up to 30 µm in depth.

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