LATERAL-SHIFT Z-ENCODED MULTIPLE-PARTICLE TRACKING OVER TUNABLE AXIAL RANGES

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The tracking of nanometer scale emitters with high precision is key for unmasking the nanoscale dynamics of relevant systems such as virus trafficking or molecular diffusion at a cellular level. Despite the existence of several optical techniques capable of 10-100 nm localization precision in x-y-z, most methods are limited in axial tracking range to about 3 μm. This is mainly caused by the spreading of light away from focus and the consequent decrease in signal to background [1]. Therefore, the challenge is to design an optical system that concentrates the light into a small region throughout a long axial range, while still maintaining the ability to recognize the z position of the multiple individual emitters with high precision. Here we present a novel optical configuration that enables tracking of multiple particles with 30 nm precision over a tunable axial range up to 12 μm. Our method uses a straight-forward implementation based on the simultaneous acquisition of two wide-field images and an electrically tunable lens (ETL) that is driven faster than the exposure time of the camera detector (EMCCD camera). The first image is formed after passing through the optical axis of the ETL, generating an extended depth of field effect - the system point spread function (PSF) is invariant across the scanned range, and it corresponds to a bright spot from which the position of the particles in x and y can be easily determined. The second image uses a light pathway that is decentered with respect to the ETL optical axis. In this case the PSF corresponds to a bright spot that is laterally shifted with respect to the first one, with a shifting that linearly depends on the z position of the particle (Fig. 1a). Thus, by measuring the lateral distance between the spots acquired in the two images (Δy) we can locate the z position of the emitters (z_p). We provide a theoretical description of the technique that is in good agreement with experiment, and demonstrate tracking of free diffusing beads in water as well as functionalized quantum dots (QDs) in live neurons (Fig. 1b). We believe that the confinement of the PSF and the consequent reduction in the cross-talking between neighboring particles

Fig. 1. a) Principle of the tracking approach. The two light pathways and corresponding images have been false colored. b) Trajectory of a QD diffusing in the soma of a living neuron

offered by our strategy, coupled with its ease of implementation in any wide-field microscope, paves the way for the precise 3D localization and tracking of single-molecules in real time.

[1] M. Duocastella, C. Theriault, C.B. Arnold, "Three-dimensional particle tracking via tunable color-encoded multiplexing", *Opt. Letters* **41**, 863 (2016).