Nanoscale dynamic localization of single nanoparticles over an extended thickness at depth in complex (bio)environments

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The nanoscale architecture of living organs is often heterogeneous and tortuous. In the brain extracellular space (ECS), for instance, this complex maze is delimited by cellular walls where ions and signaling molecules diffuse, and its architecture is acknowledged to be important for proper function [1]. However, its precise structure is still mostly unknown. To address this challenge, recent advances in single-particle tracking based on SWIR emitting single-walled carbon nanotubes (SWCNTs) opened the avenue for exploring the ECS at the nanoscale at depth in living tissue [1]. However, in current approaches, the point spread function (PSF) limits the tracking of fluorescent nanoparticles to a narrow depth (typ. <1µm) around the two-dimensional imaging plane, restricting access to the axial information of the structure under study. PSF engineering can overcome this limitation by changing the shape of the PSF through phase modulation of the fluorescence signal [2], but it still needs to be adapted to the SWIR domain and in the context of dynamic imaging at depth. First, we will present single-particle-tracking using a novel design of annular binary phase mask [3] that extends the PSF in the axial direction over several microns. The modified PSF allows two-dimensional imaging of diffusing nanoparticles at depth and in a volume without adapting the tracking algorithm. Second, we will show how three-dimensional tracking of SWCNTs can be achieved over extended depths using a customized double-helix phase mask [4] operating in the SWIR. Applications to the study of the brain ECS structure will then be presented.

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