Holographic two-photon microscope for real-time 3D single-particle tracking

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In this paper, we present a two-photon microscopy setup to measure intraneuronal transport parameters in a 3D sample in a super localisation regime thanks to the non-linear optical response of nanoparticles (second harmonic generation SHG). We take advantage of a Digital Micromirror Device (DMD) to perform digital holography and change the focus position of the excitation laser. We create a pattern of excitation in the vicinity of the nanoparticle which allows us to super-localise the particle in real time (millisecond regime, with a localization precision of 5 to 10 nanometers) by maximum likelihood approach [1]. The DMD is fast enough to track the nanoparticle during its motion. We also use holograms to correct the wavefront and obtain thus a diffraction-limited spot at the laser focus. The tracking method has been tested on nanoparticles ($BaTiO_3$ nanospheres, ~100 nm diameter) internalized in living cells displaying directional trajectories and typical go and stop phases, as shown in Fig 1 and is described in our article [2]. We aim at completing the intraneuronal transport parameters with the measurement of the rotational movement of vesicles. This additional parameter is useful to understand how the molecular motors are driven along the microtubules [3]. The nanoparticles have different non-linear coefficients depending on their crystalline axis. By rotating the incident polarization and detecting along two orthogonal polarization, we will be able to track the translation motion as well as the rotation of the particle [4] in 3D biological samples (cultures of neurons, brain slices) and in real time.

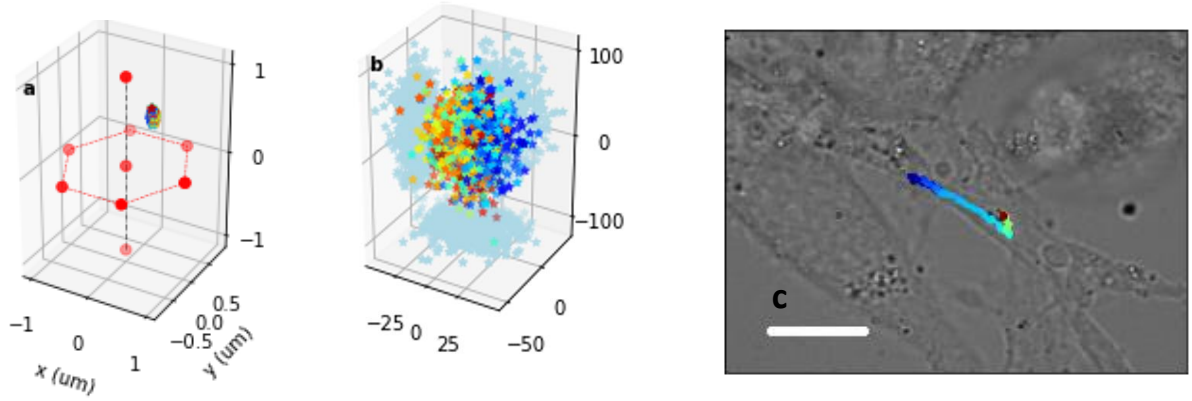

Fig 1. (a) The excitation pattern is represented by the red dots; (b) The zoomed scatter plot shows the dispersion of the estimated position of an immobile nanocrystal: each color is a different measurement, (c) One trajectory observed in living neuro-2A cell. The colormap corresponds to the time and the white line in 10µm.

[1] F. Balzarotti et al., Science 10.1126/science.aak9913 (2016).
[2] Semmer et al. ACS Photonics 2023, 10, 9, 3426–3434
[3] Kaplan et al. Science Advances, 4 :el 602170 (2018)
[4] Mayer et al. Nanoscale, 2013, 5, 8466