SUB-MILLISECOND TRACKING OF CELLULAR NANOSCALE DYNAMICS IN 3D

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KEY WORDS: single particle tracking, live-cell imaging, trajectory, multiplane localization, fluorescence nanoscopy, virus transmission

Recent advances in super-resolution fluorescence microscopy have enabled three-dimensional imaging of cellular features at the nanometer scale under live-cell conditions [1]. However, observing nanoscale dynamics often requires sub-millisecond time resolution, especially for diffusive processes, where particle displacement can exceed 100 nm/ms. Unfortunately, image acquisition in super-resolution microscopy is several orders of magnitude slower.

We have developed a novel particle tracking microscope capable of observing fast 3D trajectories of sub-diffraction sized intracellular objects [2]. The instrument combines scanning-free biplane detection [3] with optimized beam steering, yielding 300 μs time resolution and nanometer localization precision. We were able to demonstrate 3D tracking of single fluorescent particles at speeds of up to 150 nm/ms over several seconds and large volumes. Focused excitation of only the particle of interest, while avoiding confocal pinholes, guarantees maximum detection efficiency and minimal laser irradiation. Combined with the absence of sample movement, this ensures high live-sample compatibility.

Here, we show a recent improvement of the setup using a deformable mirror for axial scanning, which enhances response times and eliminates mechanical coupling to the sample. Additionally, we present new data from experiments with living cells. We have tracked actin-driven "surfing" of GFP-labeled HIV-like particles on the membranes of infected cells, a process involved in retroviral cell-to-cell spread [4]. Fast 3D particle tracking has the potential to contribute to the understanding of the underlying mechanism and the evaluation of possible inhibitory drugs.

Figure: (a) A fluorescent bead is moved by programming a step function (black) into a piezo translation stage. The tracking result (grey) demonstrates the speed and accuracy of the instrument [2]. (b) 3D trajectory of a GFP-labeled virus particle undergoing actin-driven motion on the membrane of a living host cell (unpublished data).