REVEALING DYNAMICS OF CYTOSKELETAL NON-MOTOR PROTEINS USING INTERFEROMETRIC SCATTERING MICROSCOPY

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Advances of fluorescence-based super-resolution microscopy and nanoscopic tracking techniques helped to reach a spatial resolution below 10 nm with temporal resolution limited to milliseconds timescales [1]. To push the spatiotemporal resolution of fluorescent-based techniques further we are employing interferometric scattering microscopy (iSCAT). The sensitivity of iSCAT was previously proven in detection experiments of small scattering nanoparticles as well as unlabeled single proteins with sub-nanometer spatial and sub-millisecond time resolution [2, 3].

Here we use a high frame rate of iSCAT microscopy in combination with a novel labeling method to track the motion of a single labeled anaphase spindle elongation protein 1 (ASE1) on a microtubule. We achieved spatial resolution better than 2 nm in all three dimensions, and a temporal resolution of 22 μs. With such spatiotemporal resolution, we discover that the ASE1 movement is not, as often believed, directed along a microtubule protofilament and features a more stochastic pattern on microtubule lattice (Fig 1). We distinguish different diffusion characteristics of the axial motion and the perpendicular motion of ASE1. We reveal clear statistical signatures of confined motion and are able to detect sub-millisecond confinements in the trajectories, having the periodicity corresponding to the size of a single tubulin dimer. Our results of high-fidelity ultrafast tracking shades new light not only on unknown choreography and mechanisms of diffusive motion of microtubule-associated proteins but also into the interaction of the ASE1 protein with the microtubule lattice.

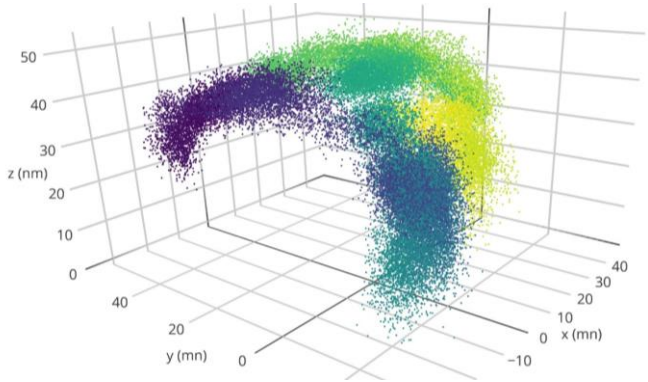

Figure 1 A three-dimensional trajectory of the labeled ASE1 dimer reveals microtubule shape and tubulins position. Time is color-coded from yellow to blue.

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