High-Speed Imaging and Tracking of Very Small Nanoparticles by Coherent Brightfield (COBRI) Microscopy

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Brightfield optical microscopy has been used extensively in many scientific fields including biology because of its simple and reliable configuration. However, brightfield microscopy has not been considered as a sensitive imaging modality. For small objects, they scatter weakly and give little optical contrast under the brightfield microscopy. Recently, by using a laser as the illumination light source in a brightfield microscope, we demonstrate coherent brightfield (COBRI) microscopy that greatly improves the sensitivity and speed of brightfield microscopy [1]. Due to the high temporal coherence of a laser, the contrast of nano-sized particle is enhanced under COBRI microscopy. Moreover, the high spatial coherence of the laser facilitates versatile beam shaping and thus enables high-quality, high-speed imaging. Importantly, COBRI microscopy is essentially a wide-field interferometric imaging technique which provides shot-noise limited sensitivity for detecting very small nanoparticles. Using COBRI microscopy and back-pupil engineering, we are able to directly visualize single 10-nm gold nanoparticles and track their motions at 1,000 frames per second (fps) with a spatial precision ~10 nm. Such sensitivity is sufficient for seeing weakly scattering nanoparticle with a scattering cross section of 10^{-16} cm^2. Endogenous biological nanoparticles, e.g., virus particles and cell vesicles can be easily observed. For example, we monitor single native vaccinia virus particle attaching to the plasma membrane of a live cell in a continuous manner at an ultrahigh speed of 1,000,000 fps (corresponding to a temporal resolution of 10 μs) [1]. From such high-speed and high-precision measurements, we unveil rapid and local virus-membrane interactions. Furthermore, COBRI microscopy also allows us to see intracellular transportation of small cell organelles at ultrahigh spatiotemporal resolutions [2]. Finally, we have also developed image post-processing methods that selectively remove the undesired scattering background (due to the stray light, imperfect alignment, or even the large cell structures) [3]. Successful background estimation and correction improve both the detection sensitivity and localization precision of single-particle tracking. We believe COBRI microscopy will open the door to study nanoscopic dynamics in live cells with high spatiotemporal resolution.

Reference

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