Spiral phase interferometric scattering microscopy for enhanced 3D particle localization and tracking

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Abstract: Interferometric scattering microscopy (iSCAT) is currently the most powerful technique available for achieving high-sensitivity single particle localization. This capability is realized through homodyne detection, where interference with a reference wave offers the promise of exceptionally precise three-dimensional localization. However, the practical application of iSCAT to 3D tracking has to date been hampered by rapid oscillations in the signal-to-noise ratio (SNR) as particles move along the axial direction. In this study, we introduce a novel strategy based on pupil plane engineering, wherein we use a spiral phase mask to re-distribute the phase of the scattered field uniformly across phase space, thus ensuring consistent SNR as the particle moves throughout the focal volume. Our findings demonstrate that this modified spiral phase iSCAT exhibits greatly enhanced localizability capability. We substantiate our theoretical results with experimental demonstrations, showcasing the practical application of this approach for ultrahigh-speed (50,000 frames per second) 3D tracking of freely diffusing nanoparticles with nanometric precision.
Keywords: Interferometric scattering microscopy, single particle tracking, point-spread function engineering.

1. Introduction

Visualization and tracking single nanoparticles have broad applications in various fields including material sciences, nanotechnology, and biomedical research. Interferometric scattering microscopy (iSCAT) provides exquisite sensitivity to detect very small nanoparticles by measuring linear scattering signals through interference (Fig. 1a) [1]. However, taking advantage of this property for robust 3D particle tracking has to date proven difficult. This in large part is due to the wildly oscillating contrast of the interferometric point spread function (iPSF) in response to particle movement in the axial direction (Fig. 1b) [2]. This property directly leads to undesirable oscillations in the Cramer-Rao lower bound (CRLB) [3], which describes the theoretically best attainable localization precision for a given optical system (Fig. 1c). In particular, the best localizations in the transverse directions are achieved when the interference contrast is highest. On the other hand, the best localization in the axial direction happens to be where the particle contrast drops to near zero, where the signal is quickly varying (Fig. 1c). In other words, the precision which can be reached by standard iSCAT for nanoparticles in the transverse (x_{NP}, y_{NP}), and axial (z_{NP}) dimensions are inconsistent and staggered, preventing precise localization in all three dimensions simultaneously.

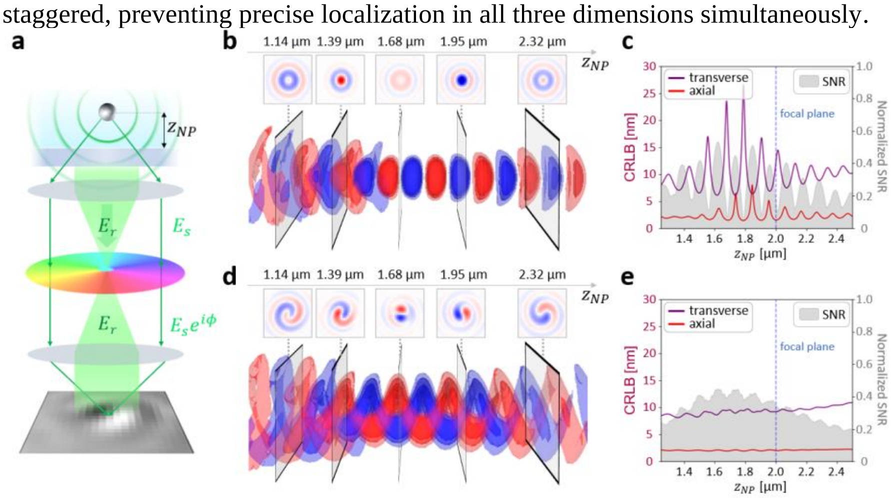

Fig. 1. **Concept of spiral phase iSCAT microscopy**. **a** A phase mask placed in the Fourier plane of the microscope imprints a twisted wavefront onto the light scattered by a nanoparticle, which modifies the particle image. **b** Contrast isosurfaces for standard iSCAT showing oscillating SNR as the particle moves in the axial direction, leading to **c** inconsistent localizability in both transverse and axial directions. **d** The same isosurfaces for spiral iSCAT show a much more consistent SNR, which in turn leads to **e** consistent localizability characteristics in all three dimensions.

In this work, we use pupil plane engineering to significantly improve the suitability of iSCAT microscopy for high-speed, 3D particle tracking. By placing a spiral phase mask in the Fourier plane of an iSCAT microscope, we convert the scattered wave into a vortex beam with a twisted, uniform phase distribution from 0 to 2π about the azimuthal angle (Fig. 1a). As a result, the iSCAT image has continuous visibility as the particle moves throughout the focal volume (Fig. 1d), with the axial position indicated by a twisting structure which evolves from left-handed spiral, to two-lobed, to right-handed spiral through the microscope focal plane. This improved iPSF structure allows for consistent localization performance (Fig. 1e), enabling high-precision, high-speed particle tracking and characterization in three dimensions.

2. Experimental demonstration of spiral phase iSCAT for high-speed 3D single-particle tracking

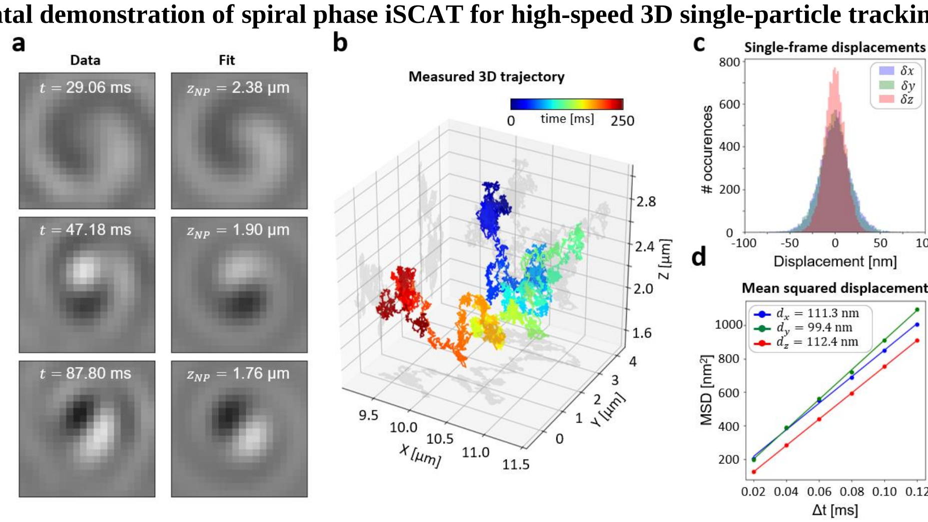

Fig. 2. **Ultrahigh-speed 3D particle tracking enabled by spiral phase iSCAT**. **a** Selected cropped data for a 100-nm diameter polystyrene particle of an iSCAT video at 50,000 Hz (left) and corresponding fits based on a simulated iSCAT model (right). **b** Reconstructed 3D trajectory from 12,500 localizations over 250 ms. **c** Histogram showing frame-to-frame displacements in three dimensions, showing improved localization precision in the axial direction. **d** Mean squared displacement (MSD) curves and associated linear fits to the particle hydrodynamic diameter.

We experimentally demonstrated the improved tracking performance enabled by the spiral phase-engineered iSCAT. We prepared a sample consisting of 100 nm diameter polystyrene nanoparticles diffusing freely in water. We used an iSCAT microscope augmented with a 4f system downstream of the objective lens, with a spiral phase mask projected on a reflection mode spatial light modulator (SLM) placed in the Fourier plane. We captured iSCAT video of 100 nm polystyrene particles freely diffusing in water at a frame rate of 50,000 frames per second. The 3D localization is by first identifying and coarsely locating particles near the focal plane in two dimensions, and cropping to a small window about the particle image. This window is then used in a nonlinear fitting procedure which minimizes the pixelwise least-squared error between a simulated iPSF model and the cropped data. Localizations in neighboring frames are strung together to create a long (10,000s of points) 3D trajectory (Fig. 2b). Analysis of the measured displacements (Fig. 2c) can be used to calculate the particle hydrodynamic diameter with high precision (Fig. 2d).

3. Conclusions

In summary, we introduced a new technique, spiral phase iSCAT, which enables ultrahigh-speed, high-precision tracking of single nanoparticles in three dimensions. This technique utilized point spread function engineering to fully exploit the exquisite sensitivity of iSCAT and achieve reliable 3D tracking with unprecedented sensitivity. We investigated this idea theoretically from the perspective of localization precision and the CRLB, showing that the attainable localization precision is stabilized in both transverse and axial coordinates, and provided experimental results demonstrating the improved performance. This approach will enable a variety of advances in label-free precision characterization of biological nanoparticles such as extracellular vesicles, as well as improved measurement of single particle size and optical properties.

References

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