

On-Chip Label-Free Holographic NTA for Biophysical Characterization of Single Particles

Chiara Lombardo¹, Benoit Vignon¹, Andrea Sottini¹, Jaime Ortega Arroyo¹, Romain Quidant¹

¹ ETH Zürich Switzerland,
chiaralo@ethz.ch

Summary:

We present an integrated optofluidic platform combining off-axis holographic microscopy with microfluidics for label-free tracking and characterization of heterogeneous nanoparticles. It extends tracking volumes and uses scattering contrast to simultaneously determine the size and refractive index of freely diffusing nanoparticles. We successfully classified synthetic nanoparticles and characterized biological ones without prior labeling.

Keywords: nanoparticle tracking, digital holography, microfluidics, label-free sensing, single-particle characterization, refractive index sensing

Background, Motivation and Objective

Classifying heterogeneous nanoparticle ensembles is essential for fundamental research and quality control. Conventional Nanoparticle Tracking Analysis (NTA) determines hydrodynamic diameter from Brownian motion. However, standard implementations image only a thin focal slab ($\sim 10\ \mu\text{m}$). Fast-moving particles quickly diffuse out, yielding truncated trajectories and high statistical sizing errors [1]. To address these challenges, we are developing an integrated optofluidic platform combining an off-axis digital holography microscope (Fig. 1a) with a custom two-layer microfluidic chip (Fig. 1b).

Off-axis digital holography overcomes these limitations by recording the interference of scattered and reference light to simultaneously capture intensity and phase [2]. Intensity provides insights into polarizability (related to size and refractive index), while encoded phase information enables numerical axial propagation. This allows volumetric sample reconstruction from a single 2D interferogram. Extending NTA to this holographic configuration (holoNTA) substantially increases the effective imaging volume (Fig. 1c). Capturing longer 3D trajectories allows accurate measurement of the 3D mean squared displacement to extract the diffusion coefficient and derive the hydrodynamic diameter via the Stokes-Einstein equation. Once the diameter is known (Fig. 1d), its scattering cross-section—derived from the reconstructed amplitude—is used to retrieve its refractive index based on Mie theory.

Preliminary measurements happen in a sealed circular imaging chamber (2 mm diameter, 100

μm height) formed by a silicone spacer between two glass coverslips, which suppresses residual flow for drift-free Brownian tracking. This architecture enables rigid optical alignment and complete volume confinement. A two-layer chip with push-up valves enclosing the same $2\ \text{mm} \times 100\ \mu\text{m}$ volume is in development for automated sample exchange via fluidic washing, minimizing reagent use and contamination risks.

Although holoNTA offers superior tracking, the computational burden of volumetric reconstruction typically precludes real-time analysis. This was tackled by introducing a GPU-accelerated, physics-informed detection pipeline.

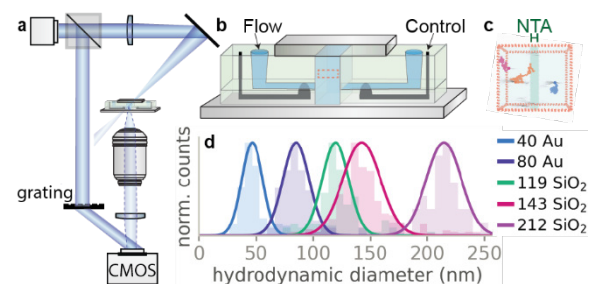


Fig. 1. Optofluidic holoNTA platform. (a) Off-Axis holography microscope. (b) Two-layer microfluidic chip design. The flow layer is used to fill the imaging chamber, which is sandwiched between two glass plates. Push-up valves in the control layer close the imaging chamber. (c) HoloNTA significantly increases the accessible imaging volume compared to the semi 2D conventional NTA. (d) The diffusion trajectories are used to compute the particle's hydrodynamic diameter.

Description of the New Method or System

We developed a custom-built off-axis holographic microscope (Fig. 1a) operating in

transmission dark field, achieved by illuminating at a high angle beyond the collection NA (0.75), at a 488 nm laser wavelength. The system provides a $\sim 100 \times 100 \mu\text{m}^2$ field of view that can be propagated along $100 \mu\text{m}$, resulting in a ~ 1 nL instant imaging volume at 60x effective magnification. A CMOS camera (Basler ace) records interference holograms with rapid exposures (20–100 μs) to freeze the Brownian motion of small analytes.

The acquired holograms are then Fourier transformed and the reconstructed complex field is propagated to 100 planes across a depth of $100 \mu\text{m}$, with GPU acceleration propagating the full 1 nL volume simultaneously. Candidate particles are detected as local maxima of the amplitude contrast across the volume. The candidates are validated by directly fitting the physical PSF model to the complex field [3]. Besides confirming the detection, this step serves to refine the 3D localization and extract the scattering amplitude. Confirmed detections are linked into 3D trajectories with a Kalman-filter-based linker using adaptive statistical gating, globally optimal frame-to-frame assignment, and gap closing. For each trajectory, the mean squared displacement yields the diffusion coefficient and hence the hydrodynamic diameter via the Stokes–Einstein equation. The refractive index of the particles can be retrieved by calibrating the system on known samples via Mie theory.

Results

As proof of principle, 80 nm AuNP were measured at three different concentrations. A total of 2, 8 and 89 AuNP trajectories were retrieved at the three concentrations, corresponding to measured concentrations of 2.2×10^6 , 7.8×10^6 and 5.8×10^7 NP/ml. The retrieved hydrodynamic diameters were 75, 83 and 81 nm respectively, in agreement with the TEM spec of 79 nm (CV 8.9%). Five monodisperse samples were then measured individually (40/80 nm Au, 119/143/212 nm SiO₂, Fig. 1d). Populations whose size distributions overlap significantly still form distinct clusters in the contrast vs diameter map. This capability was proven on a heterogeneous mixture of 40 nm Au, 100 nm PS and 119 nm SiO₂, of which the relative concentrations were not known. Five different 1 nL volumes were measured and resulted in a total count of 1150 trajectories. Mapping the trajectories in the 2D contrast vs diameter plane (Fig. 2a) allowed us to classify each datapoint and retrieve the absolute concentrations of Au, PS and SiO₂, namely $5.1 \pm 1.6 \times 10^7$, $14.3 \pm 1.6 \times 10^7$ and $7.5 \pm 1.9 \times 10^7$ NP/ml. Individual measurements of 40 nm AuNP (N=179) and 80 nm AuNP (N=148) were taken to fit a Mie curve and calibrate the contrast to the corresponding

refractive index (Fig. 2b). The 40 nm AuNPs classified from the mixture (N=201) fall right on the individually measured 40 nm AuNP, and thus on the Mie curve. The medians of the SiO₂ (N=286) and PS (N=524) fall on the Mie bands predicted by literature ($n=1.43$ – 1.48 for SiO₂ and 1.58 – 1.68 for PS) [4]. Measurements were extended to heterogeneous biological nanoparticles (e.g. EVs, liposomes), demonstrating label-free detection and tracking. Ultimately, this integrated optofluidic platform provides a versatile, real-time tool for rapid nanoparticle classification and biophysical characterization.

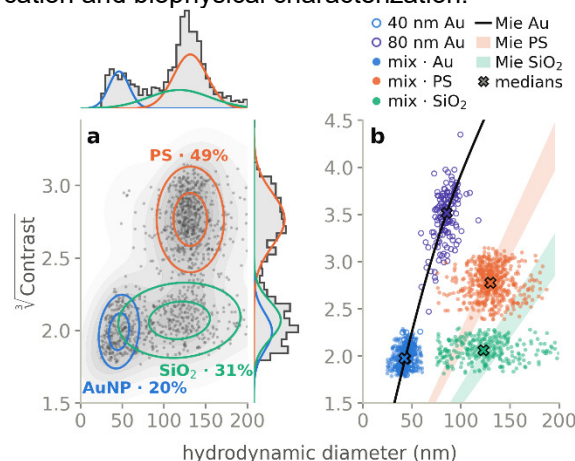


Fig. 2. Single-particle classification of a heterogeneous nanoparticle mixture. (a) The mixture (N=1150 trajectories) in the contrast vs diameter plane. Three populations are resolved, with classified fractions of 20% AuNP, 49% PS and 31% SiO₂. (b) The individually measured 40 nm and 80 nm AuNP fall on the Mie curve for gold; the medians of the PS and SiO₂ populations classified from the mixture fall within the Mie bands predicted from literature refractive indices.

References

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