Correlative microscopy arrays for nanoparticle standards characterization

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Correlative microscopy combines complementary images from multiple instruments in a synergistic analysis of nanoscale structures and properties. However, the accurate correlation of disparate images at random locations across a wide field is challenging, limiting measurement throughput and statistical validity. Major challenges include microscopy position errors and sample preparation issues.[1,2] Meeting both challenges would enhance correlative microscopy for materials science, biology, and nanotechnology.

To solve this problem, we introduce a correlative microscopy array, featuring a square array of nanoscale fiducials that we design for compatibility with multiple microscopy methods and suspension deposition processes. We fabricate prototype arrays in silicon (100) using electron-beam lithography and reactive ion etching, forming robust and reusable imaging substrates. We etch the fiducials into the substrate to reduce interference with suspension deposition and to present a different feature polarity in comparison to nanoparticles on top of the substrate, facilitating image interpretation. Fiducial arrays provide reference positions for magnification calibration and aberration correction for each instrument, enabling programmatic registration of image data with high throughput and reliability.

We apply a prototype array in an initial study of the dependence of fluorescence intensity on the spherical diameter of polystyrene nanoparticles, which commonly serve as size standards and may serve as intensity standards in benchmark tests of nanomedicine and nanoplastic measurements. However, the intensity–size trend is poorly understood and subject to bias due to sizing errors,[3] requiring correlative metrology to obtain a reference trend. Accordingly, we perform correlative fluorescence microscopy (Figure 1a,b) and atomic force microscopy (Figure 1d,f) on single nanoparticles, with corresponding corrections for intensity non-uniformity and height non-linearity to improve accuracy. For an initial sample of 15 nanoparticles, we find reasonable agreement of the particle size distribution in a comparison of manufacturer specifications resulting from transmission electron microscopy and our measurement results from atomic force microscopy (Table 1). A power-law approximation of the intensity–size trend gives an apparent exponent of approximately 2.5, not yet accounting for exponent attenuation bias due to sizing uncertainty. Building on this initial dataset (Figure 1c,e), we aim to extend our novel methodology across a broad range of nanoparticle samples.

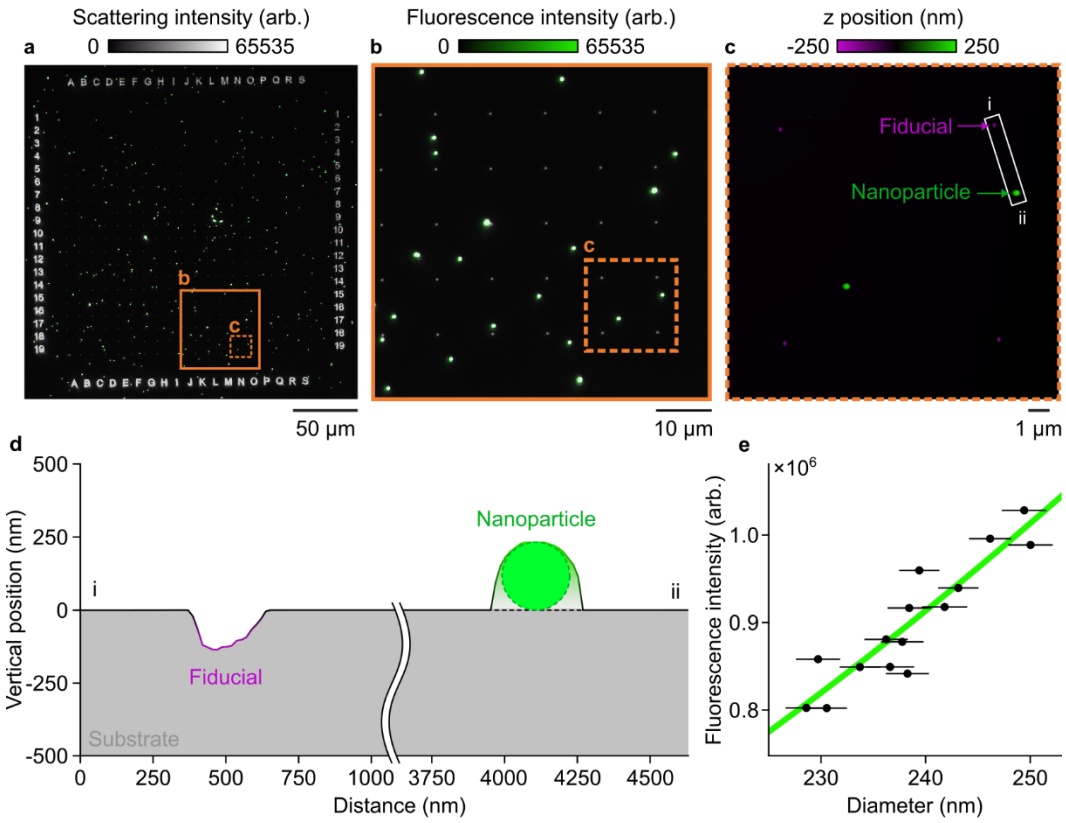

Figure 1. Correlative microscopy arrays for nanoparticle standards characterization. (**a**) Composite optical micrograph combining (white) darkfield and (green) fluorescence imaging modes of the same optical microscope, showing spherical polystyrene nanoparticles on a correlative microscopy array. Peripheral letters and numbers label fiducial positions. (**b**) Composite micrograph showing a region of interest, corresponding to the orange square in panel (a). (**c**) Atomic force micrograph showing a region of interest, corresponding to the orange dashed square in panels (a) and (b). (**d**) Atomic force micrograph profile showing the different vertical-position polarities of a fiducial and a nanoparticle from (c). In our initial analysis, we model the nanoparticle image as a sphere (dotted circle outline), accounting for the convolution artifact of the probe tip, with the spherical height corresponding to the nanoparticle diameter. (**e**) Scatter plot showing preliminary data for the dependence of fluorescence intensity on spherical diameter. Diameter uncertainties are 68% coverage intervals corresponding to the uncertainty of scanner non-linearity calibration, which is one component of sizing uncertainty. A comprehensive evaluation of sizing uncertainty is ongoing. Intensity uncertainties are smaller than the data markers. The solid green line shows the best-fit trendline of a power-law model, with an apparent exponent of approximately 2.5. This value is subject to an attenuation bias that requires a measurement error model to correct.

Table 1. Initial comparison of particle size distributions from different microscopy methods

Transmission electron microscopy mean ± standard deviation	**Atomic force microscopy** mean ± standard deviation
240 nm ± 10 nm	239 nm ± 2 nm

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