Boring beads or surprising standards?
A lateral nanoflow assay reveals flummoxing fluorescence

K.-T. Liao,[†, ‡, §, ‖] A. C. Madison,[†, ‖] A. L. Pintar,[†] B. R. Ilic,[†] C. R. Copeland,[†] and S. M. Stavis[†, *]

[†]National Institute of Standards and Technology, Gaithersburg, Maryland 20899, [‡]University of Maryland, College Park, Maryland 20742 [§]Food and Drug Administration, Silver Spring, Maryland 20993, [‖]Equal contribution, [*]samuel.stavis@nist.gov

Colloidal nanoparticles of polymeric materials and organic chemicals are at the vanguard of commercial nanotechnology, with diverse applications ranging from instrument calibration to therapeutic delivery. Simultaneously, environmental contamination from nanoplastic byproducts is of grave concern, with potential hazards of chemical sorption and tissue penetration. Quantifying and correlating the property distributions of colloidal samples are fundamental to optimizing product quality and to assessing byproduct hazards, but such measurements remain challenging. To meet this challenge, we develop a lateral nanoflow assay that integrates complex nanofluidic replicas, optical localization microscopy, and novel statistical analyses (Figure 1).[1, 2] We apply our sample-in-answer-out system to quantify the steric diameters and fluorescence intensities of single nanoparticles of amorphous polystyrene that sorb and carry hydrophobic fluorophores, which are both commercial products and model nanoplastics. We find that such samples are surprising standards rather than boring beads, yielding a dramatic conclusion to an introductory presentation at EIPBN 2018,[3] with original analysis and broad impact.

In our disposable devices, hydrodynamic interactions automate the advection and dominate the diffusion of colloidal nanoparticles. Through steric interaction with the silicone structure, the diameter distribution of reference nanoparticles probes the unknown limits of the replica function of analytical separation (Figure 1). Readout is by optical microscopy, and the integration and calibration of device and microscope improve the accuracy of quantifying intensities, filtering data, localizing objects, and referring positions of size exclusion to nanofluidic depths across an ultrawide field to achieve high throughput (Figure 2). A comprehensive statistical model approaches the information limit, discriminates between data types due to size exclusion and surface adsorption (Figure 1), and reduces single micrographs to a diameter histogram (Figure 3). In comparison to the reference diameter distribution from transmission electron microscopy, the experimental distribution moments are accurate to within a mean error of 2 nm, which is comparable to the diameter uncertainty of single nanoparticles in our assay. A Bayesian statistical analysis reveals a fundamental structure–property relationship (Figure 4). Fluorescence intensity scales with steric diameter to the power of 4.1 ± 0.5 at 95 % coverage, confounding basic concepts of surface adsorption or volume absorption. Distributions of fluorescivity—an intrinsic property that we isolate and define as the product of the number density, absorption cross section, and quantum yield of an ensemble of fluorophores—are ultrabroad and asymmetric, limiting any dimensional and chemical inference from intensity, particularly in ensemble analyses. This flummoxing fluorescence resets expectations for optimizing products, understanding byproducts, and applying standards that involve fluorescent nanoplastics.

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[1] K.-T. Liao and A. C. Madison *et al.*, *arXiv*, 2101.03881 (2020).
[2] K.-T. Liao *et al.*, *Lab on a Chip*, **18**, 139-152 (2017).
[3] K.-T. Liao and S. M. Stavis, *EIPBN* (2018).

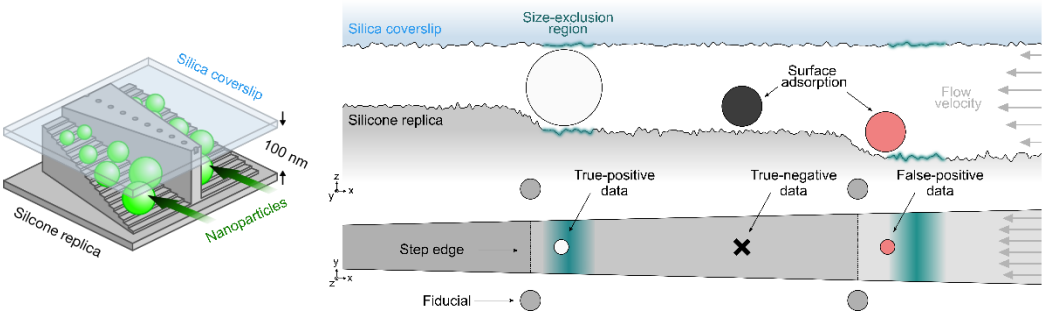

Figure 1. A model of device–particle interactions yields three types of data for statistical analysis.

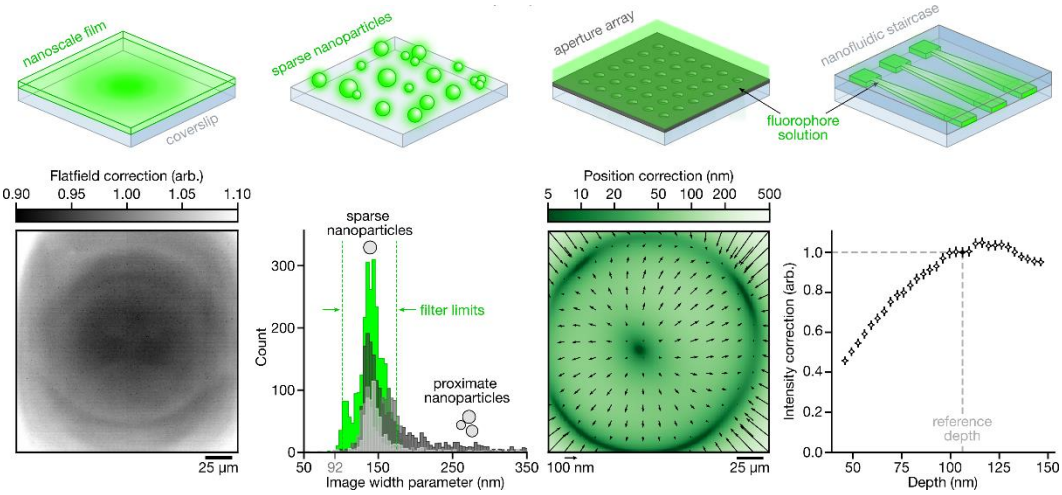

Figure 2. Standards and calibrations improve measurement accuracy across an ultrawide field.

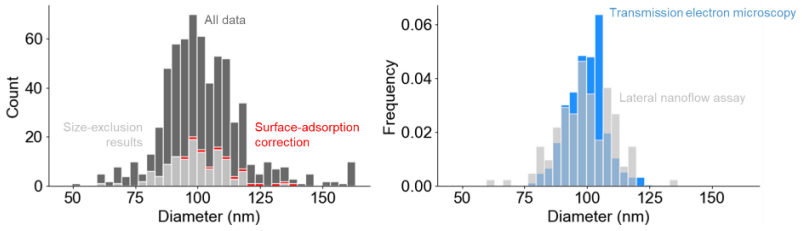

Figure 3. Reduction and correction of data yields sizing accuracy to within a mean error of 2 nm.

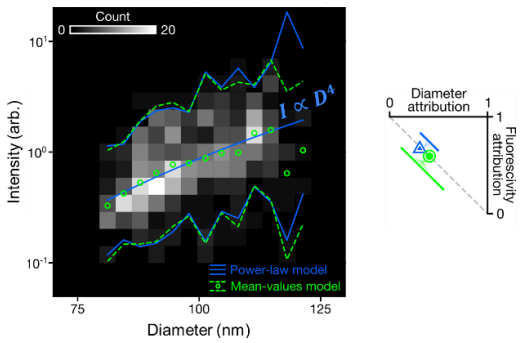

Figure 4. Bayesian statistical analysis reveals flummoxing fluorescence intensity.