

Ultrasensitive Nanoparticle Detection with the Delaware Flow NanoCytometer™

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Fig. 1: System. The Delaware Flow NanoCytometer™.

BACKGROUND

Detection and characterization of biological nanoparticles, such as exosomes, extracellular vesicles (EVs), liposomes, and micelles, represents an important next frontier in both research and clinical applications. The main obstacles to nanoparticle analysis in flow cytometry are (i) the small particle sizes and (ii) the short time available for interrogation, which, combined, result in exceedingly small scattering and fluorescent signals. Commercial flow cytometers either modified or tailored for this problem have so far been underwhelming in terms of both speed of analysis and ease of use. Thus, there is a demand for an ultrasensitive flow cytometer that delivers nanoparticle analysis without compromising usability and throughput.

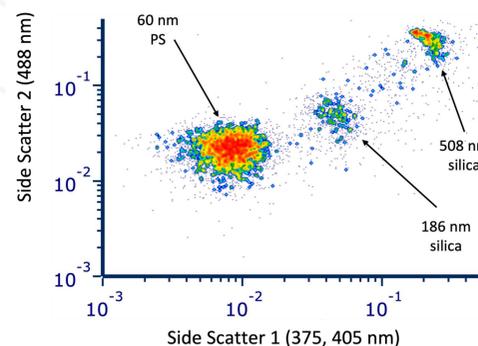


Fig. 2: Sensitivity. Detection of silica (Alpha Nanotech) and polystyrene (Spherotech) nanoparticles on the Delaware.

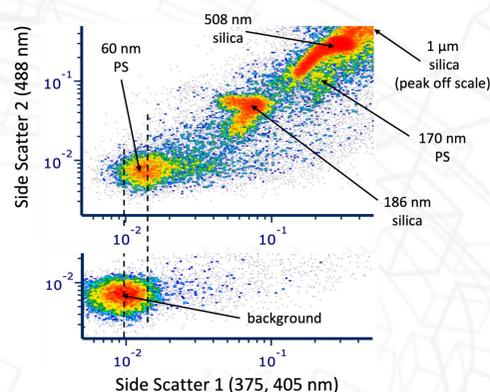


Fig. 3: Resolution. Mixture of Alpha Nanotech colloidal silica nanoparticles and Spherotech polystyrene (PS) nanoparticles detected in a range from 60 nm to 1 μm using the Delaware's two side scattering channels. The 60 nm nanoparticles are resolved above the background.

MATERIALS & METHODS

We have designed, developed, and characterized a new nanoparticle analyzer, the Delaware Flow NanoCytometer™ (Fig. 1), tailored for sensitive detection and characterization of sub-micron particles (biological or otherwise). The Delaware is based on the architecture of our Potomac modular flow cytometry platform (CYTO 2017; CYTO 2021), with design modifications specifically intended to enhance nanoparticle sensitivity. The Delaware has up to five excitation wavelengths (375, 405, 488, 532/561, and 636/640 nm; the version shown here has powers, respectively, of 50, 120, 300, 50 (at 532 nm), and 50 (at 636 nm) mW). The detection module, which—like on the Potomac—has user-selectable filters (Semrock, Chroma, Omega), uses a proprietary high-NA collection lens and offers up to three scattering channels (one forward and up to two side) and up to six fluorescence channels (the version shown here uses three scattering and three fluorescence channels). Ultraprecise sheath flow for superior core stream control is established with our previously introduced Shasta fluidic control system (CYTO 2021). Ultrafiltered sheath fluid (Beckman Coulter IsoFlow) is used in conjunction with additional inline filtering (Sterlitech) to minimize nanoparticle background. The analyzer is operated using our Panama flow cytometry software for user-friendly instrument control and data visualization of nanoparticles, and incorporates our proprietary Cavour always-on flowcell monitoring module.

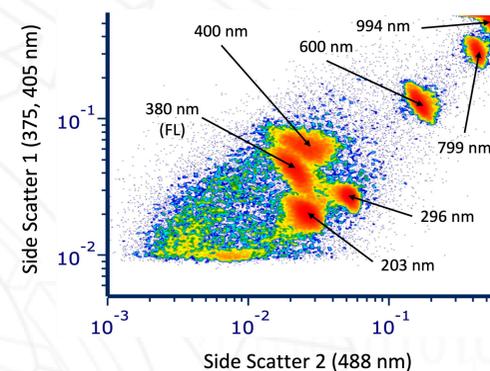


Fig. 4: Dynamic Range. Mixture of Exometry Rosetta™ calibration nanoparticles ranging from 200 nm to 1 μm resolved on the Delaware using two side scattering channels.

RESULTS

The Delaware was characterized using a wide gamut of test samples. Fig. 2 shows silica and polystyrene nanoparticles 60 nm, 186 nm, and 508 nm in diameter resolved using the two side scattering channels (for 375 and 405 nm, and for 488 nm). Fig. 3 shows the same mixture plus 170-nm and 1-μm particles, illustrating the dynamic range at high sensitivity; the 60-nm population is shown as resolved against background. Fig. 4 displays calibration nanoparticles ranging in size from 200 nm to 1 μm, resolved using the two side scattering

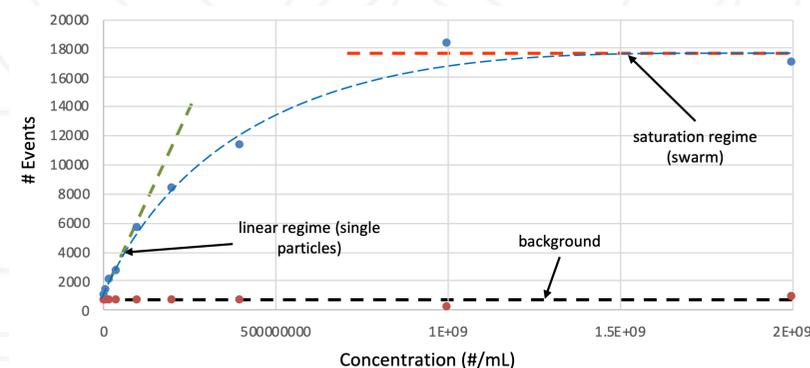


Fig. 5: Linearity. Titration series with Tetraspeck TS100 110-nm nanoparticles. The data shows the transition from "swarm" detection to the linear regime as dilution is increased, demonstrating the Delaware's ability to resolve individual nanoparticles in flow.

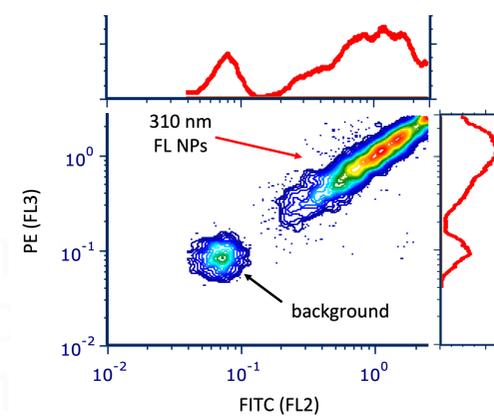


Fig. 6: Fluorescence. Spherotech UltraRainbow 310-nm fluorescent nanoparticles. Fluorescence is shown here as detected on the Delaware in the green (FITC) and orange (PE) channels, well separated from background.

channels. Fig. 5 shows some of the fluorescence detection capabilities of the Delaware. "Rainbow" fluorescent 310-nm nanoparticles were well resolved against background in two fluorescence channels, FL2 (for detection, e.g., of FITC) and FL3 (for detection, e.g., of PE). Fig. 6 shows titration data illustrating the transition from "swarm" detection to the linear regime, where individual nanoparticle (in this case, 110-nm TetraSpeck TS100 nanoparticles) are isolated for interrogation under flow conditions. Similar titration procedures were performed on all samples tested and reported here to ensure that measurements were carried out in the single-particle regime. Fig. 7 demonstrates the Delaware's ability to resolve nanoparticles specifically designed to mimic EV characteristics, in this case Exometry 374-nm Verity Shells organosilica nanoshells, where the sample rises above background. Fig. 8 shows a similar capability, demonstrated with Cellarcus 100-nm Lipo100 lipoprotein shells. For these measurements, the liposomes were first measured intact; then (following best practices) the liposomes were destroyed using Triton-X, a surface-active agent known to disrupt lipid membranes. The bottom graph shows the difference in the measured nanoparticle profiles.

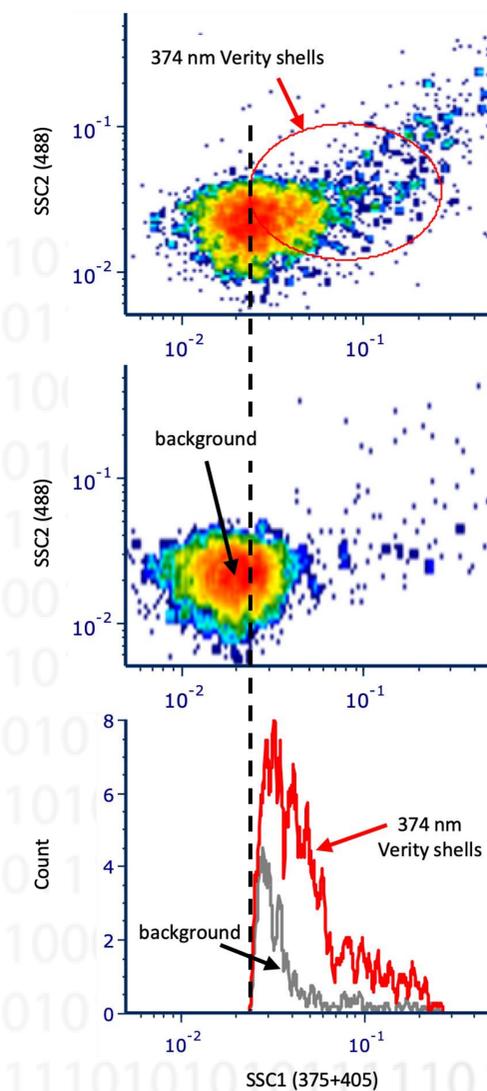


Fig. 7: Shells. Exometry 374-nm Verity Shells™ organosilica hollow nanospheres.

CONCLUSION

We have performed extensive characterization of our Delaware Flow NanoCytometer using, among other samples, silica nanoparticles, polystyrene nanoparticles, fluorescent nanoparticles, organosilica nanoshells, and lipoprotein nanoshells. We have characterized linearity, sensitivity, resolution, dynamic range, fluorescence detection, and ability to detect and resolve EV surrogates. We have

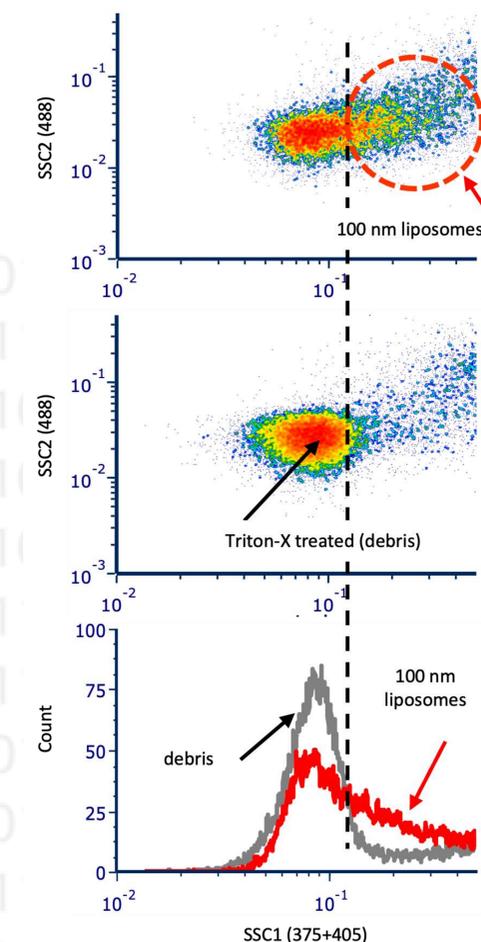


Fig. 8: Liposomes. Cellarcus 100-nm Lipo100™ liposomes (top) resolved on the Delaware. After treatment with surfactant Triton-X, the liposomes are destroyed, producing debris (middle). Histograms of the two samples (bottom).

demonstrated the integration of powerful multicolor excitation sources, an optical system designed to maximize light collection, an ultrastable fluidic control module, and a highly flexible yet intuitive graphical user interface. The Delaware Flow NanoCytometer combines ease of use with advanced nanoparticle sensitivity, down to 60 nm, to offer users a powerful new tool for exosome and EV research.

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The Delaware, Potomac, Shasta, Cavour, and Panama, or use thereof, may be covered in whole or in part by patents in the U.S. and other jurisdictions. A current list of applicable patents can be found at <https://www.kineticriver.com/kinetic-river-corp-patents>.