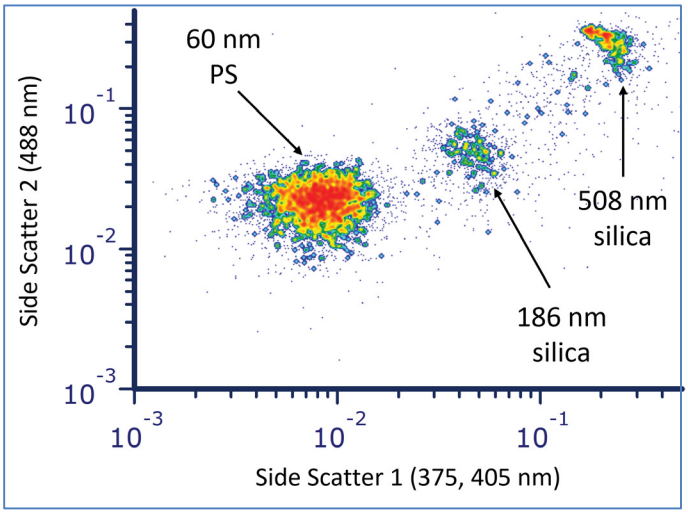
KINETIC RIVER® *Where Light Meets Life®*

Delaware

Flow NanoCytometer™

Tech Notes

Detection and characterization of sub-micron entities, including extracellular vesicles (EVs) and exosomes, represents an important next frontier in both research and clinical applications. These nanoparticles produce exceedingly small scattering and fluorescent signals which standard commercial flow cytometers cannot detect. Even systems designed to address this application have, thus far, fallen short, creating an unmet and growing demand for a nanoparticle analysis system with suitable usability and throughput.



Detection of silica and polystyrene nanoparticles down to 60 nm (PS)

We designed and developed the ***Delaware*** Flow NanoCytometer™ specifically to meet the demanding needs of nanoparticle researchers, providing **sensitive detection** and characterization of biological and non-biological nanoparticles. Based on our modular, customizable *Potomac* architecture, the system incorporates design modifications intended to **enhance nanoparticle sensitivity** without compromising throughput.

The ***Delaware***'s high-power lasers provide up to **five excitation wavelengths** (375, 405, 488, 561, and 640 nm) and a proprietary high-NA collection lens delivers **maximum sensitivity**. The system offers **up to three scattering channels** and **up to six fluorescence detection channels**. The ***Delaware*** features Kinetic River's *Shasta* fluidic control system for ultrastable sheath flow and **superior core stream control**. The *Cavour* always-on flowcell monitor allows you to optimize laser alignment and core stream dimensions in real-time **without removing the cover**. The entire system is operated using our intuitive, easy-to-use *Panama* flow cytometry software for instrument control and data visualization, providing researchers with the flexibility their cutting-edge research requires.

This carefully-crafted instrument has been extensively tested on a variety of materials including polystyrene (down to 60 nm) and colloidal silica (down to 100 nm) nanoparticles, fluorescent nanospheres (100 nm), hollow organo silica beads (374 nm) and lipoprotein shells (100 nm), demonstrating **sensitivity to at least 60 nm** to meet some of the most demanding applications. The ***Delaware*** Flow NanoCytometer combines ease of use with advanced nanoparticle sensitivity to offer users a powerful new tool for exosome and EV research.

The ***Delaware*** – see what you've been missing.

The Delaware*, 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.*

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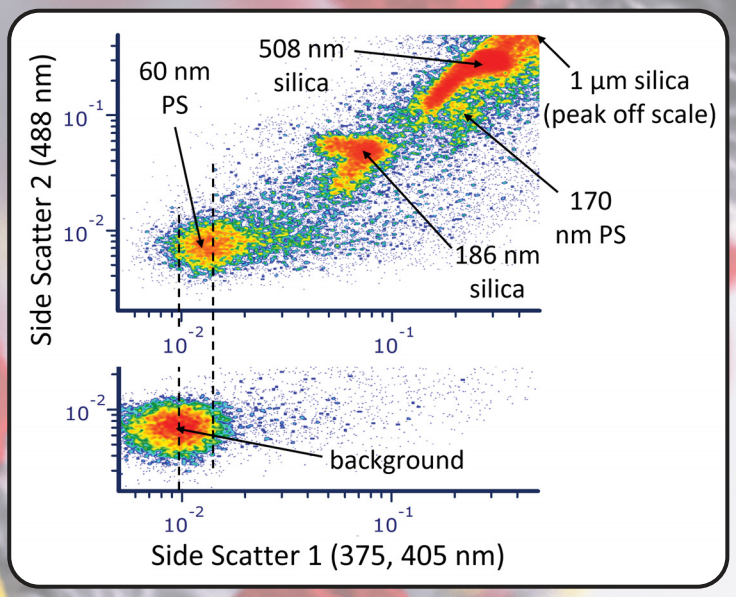
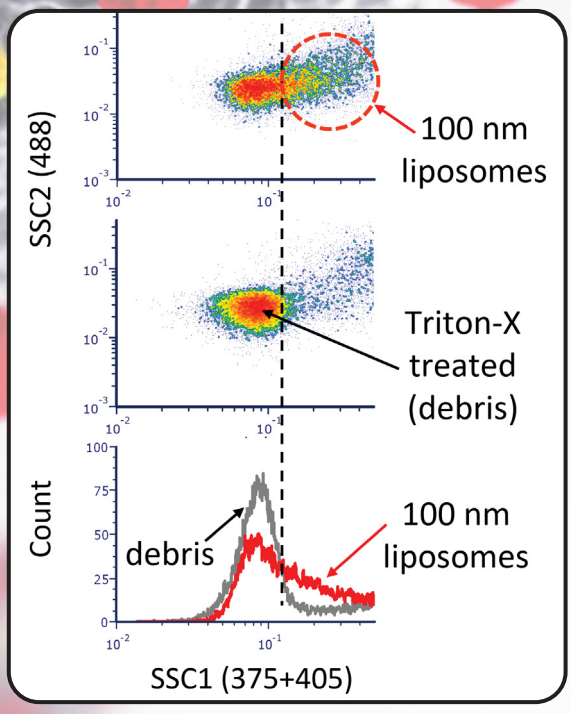
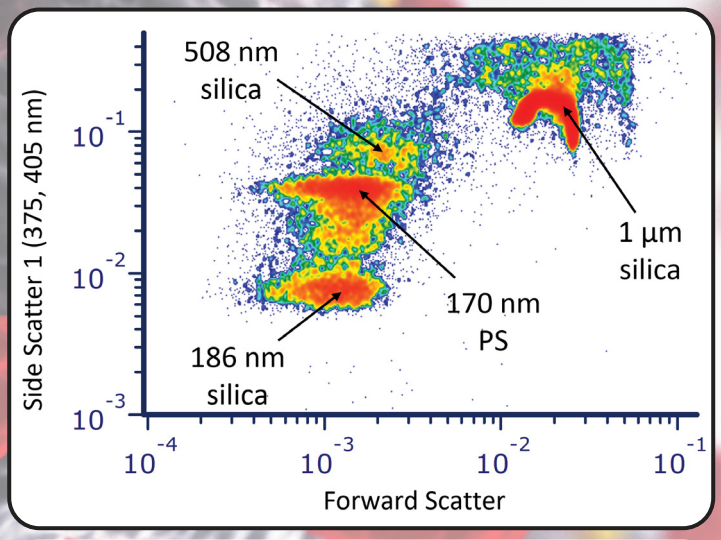


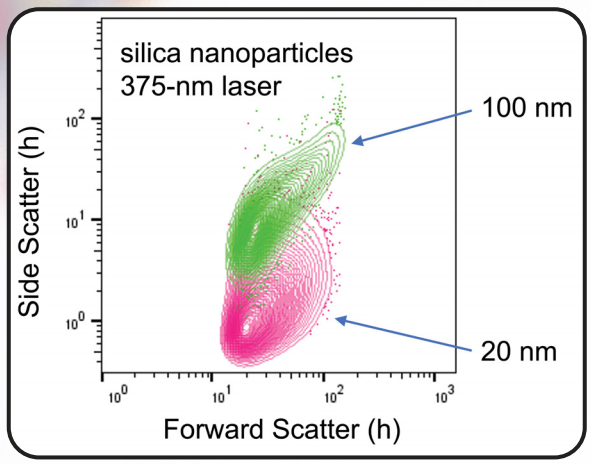
Mixture of colloidal silica nanoparticles from Alpha Nanotech and polystyrene (PS) nanoparticles from Spherotech detected in a range from 60 nm to 1 µm using the *Delaware*'s two side scattering channels.



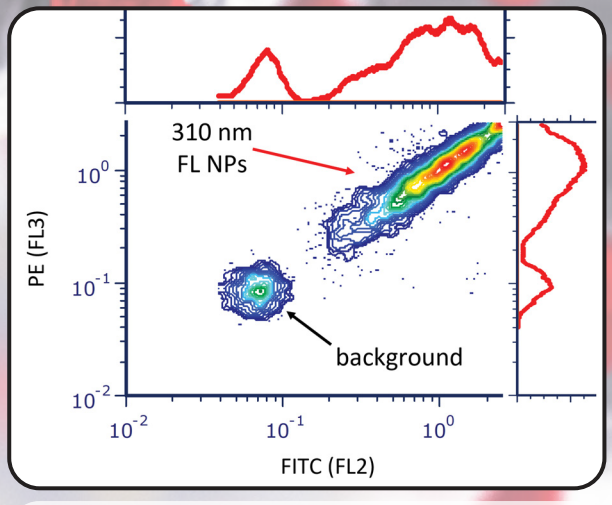
Cellarcus 100-nm liposomes (top). After treatment with surfactant Triton-X, the liposomes are destroyed, producing debris (middle). Histograms of the two samples (bottom).



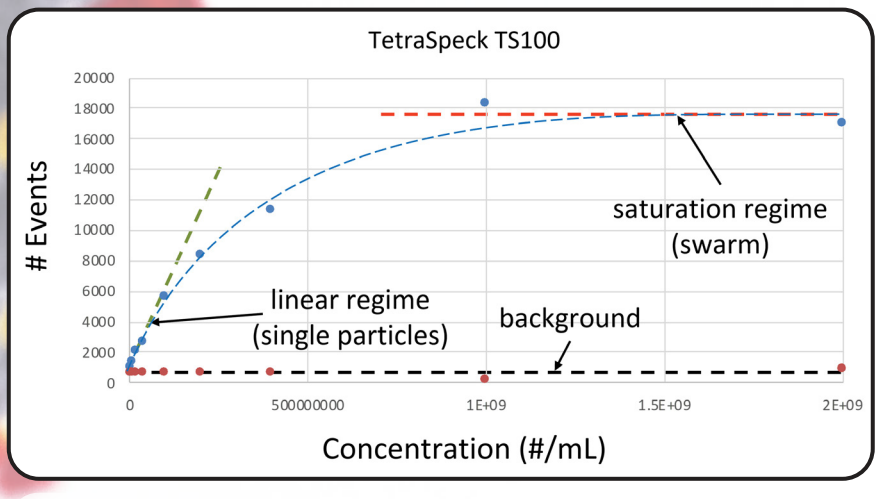
Mixture of silica and PS nanoparticles from 186 nm to 1 µm resolved using forward scattering and 375-/405-nm side scattering.



Colloidal silica nanoparticle detection, demonstrating sensitivity in silica better than 100 nm.



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



A titration series showing the transition from "swarm" detection to the linear regime, demonstrating the *Delaware*'s ability to resolve individual nanoparticles.

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Specifications

Configurations

Basic Configuration	High Sensitivity Configuration	Five-Laser Configuration
2 Lasers 405 nm, 250 mW 488 nm, 200 mW	**3 Lasers** 375 nm, 50 mW 405 nm, 250 mW 488 nm, 200 mW	**5 Lasers** 375 nm, 50 mW 405 nm, 250 mW 488 nm, 200 mW 561 nm, 50 mW 640 nm, 150 mW
Standard Scattering FSC, SSC (405 and 488 nm)	**Ultrasensitive Scattering** FSC, SSC (375, 405, 488 nm)	**Ultrasensitive Scattering** FSC, SSC (375, 405, 488 nm)
2 Fluorescence Channels 525/50 580/23	**4 Fluorescence Channels** 525/50 580/23 615/24 697/58	**6 Fluorescence Channels** 440/40 (optional) 525/50 580/23 615/24 697/58 755/35

Performance

Nanoparticle detection (375-, 405-, and 488-nm excitation; 3 scattering channels):
- 60-nm Spherotech polystyrene
- better than 100 nm Alpha Nanotech colloidal silica

EV surrogates:
- 100-nm Cellarcus lipoprotein shells
- 374-nm Exometry Verity shells

Dynamic range (375-, 405-, 488-nm excitation):
- high sensitivity: approx. 60 nm to 300 nm (PS)
- approx. 100 nm to 1µm (silica)

Installation Requirements

Dimensions and weight:
- 36” x 20” x 23” (W x D x H)*
- 175 lbs. (Five-Laser configuration)*

*excludes monitors, sheath and waste tanks

Environmental:
- 15°–30°C, 60% RH

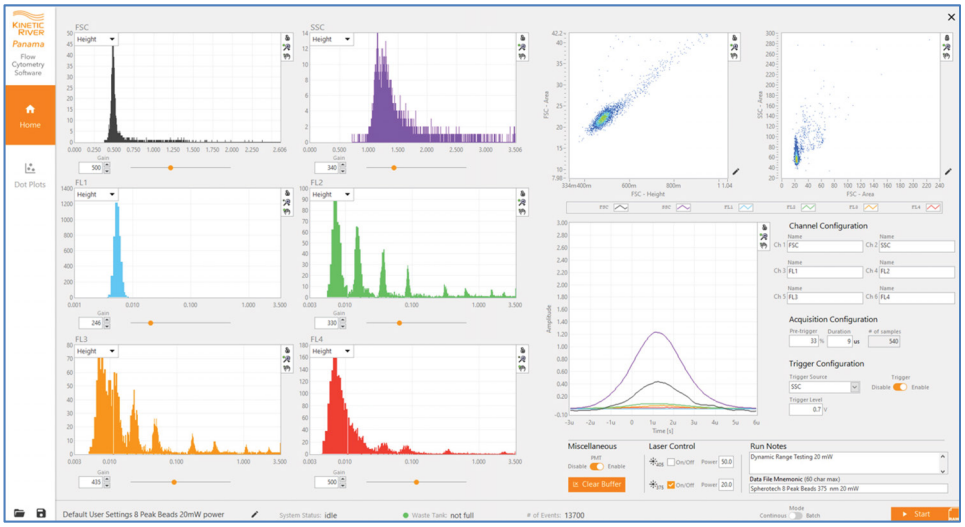
Power:
- North America: 120 VAC, 50/60 Hz, 8A
- Japan: 100 VAC, 50/60 Hz, 8A
- rest of world: 230 VAC, 50/60 Hz, 5A

Fluidics

Dual hydrostatic pressure injection:
- 10-L ultrafiltered sheath fluid capacity
- sample injection speed variable from 0.2 – 20 µL/min

Signal Processing

Data formatting:
- CSV files (directly importable into FlowJo, FCS Express)



The Panama *software for instrument control and data visualization*

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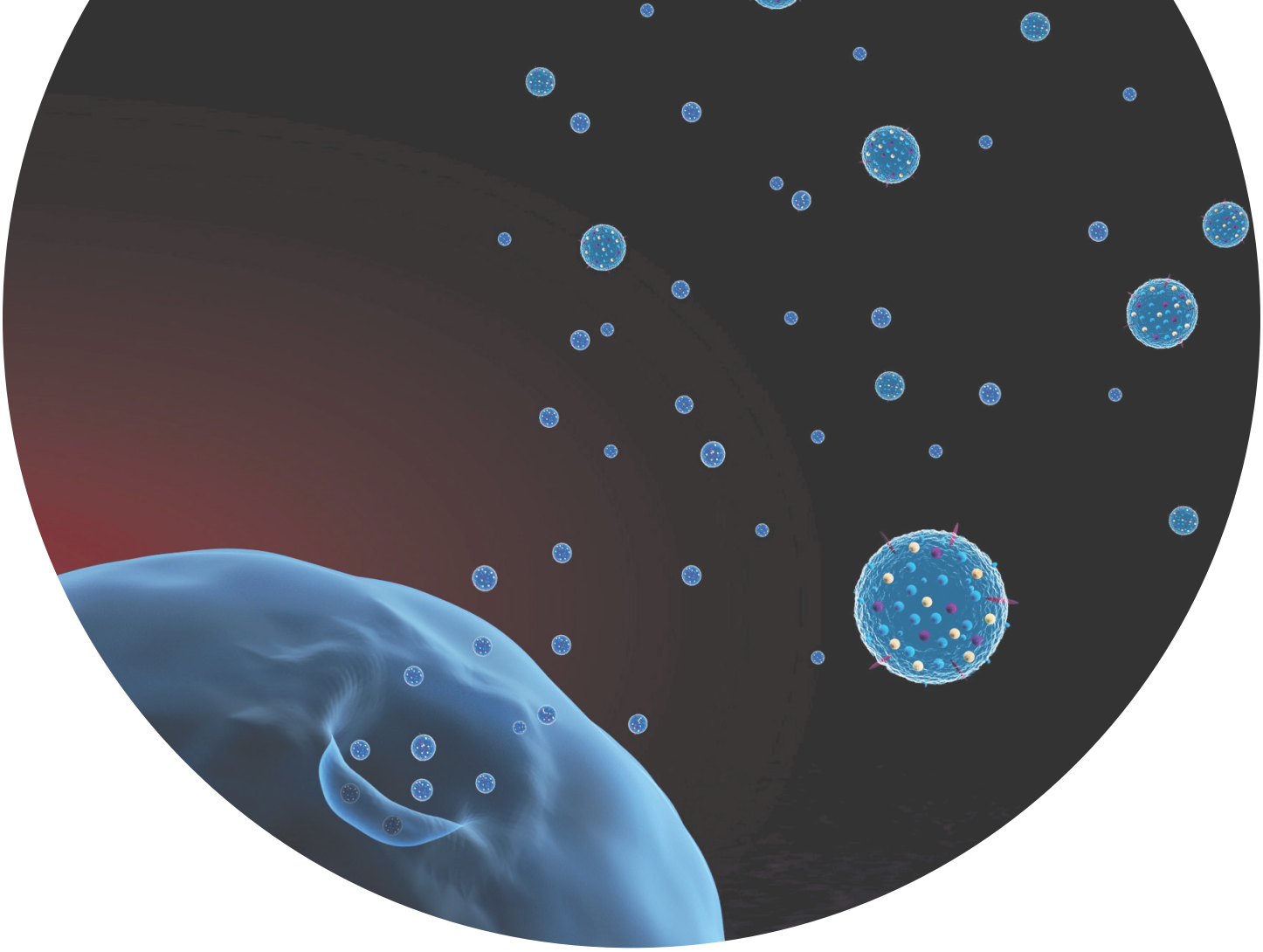

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The *Delaware*, or use thereof, may be covered in whole or in part by patents in the U.S. and other jurisdictions. A current list of applicable patents can be found on our website at https://www.kineticriver.com/kinetic-river-corp-patents/

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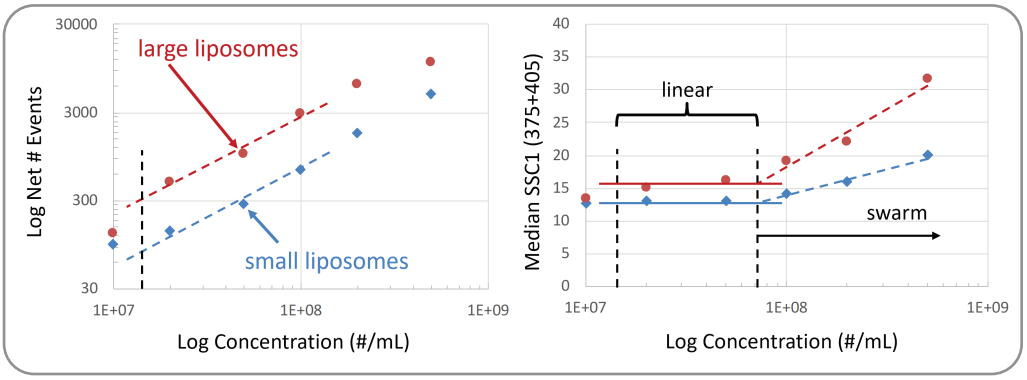
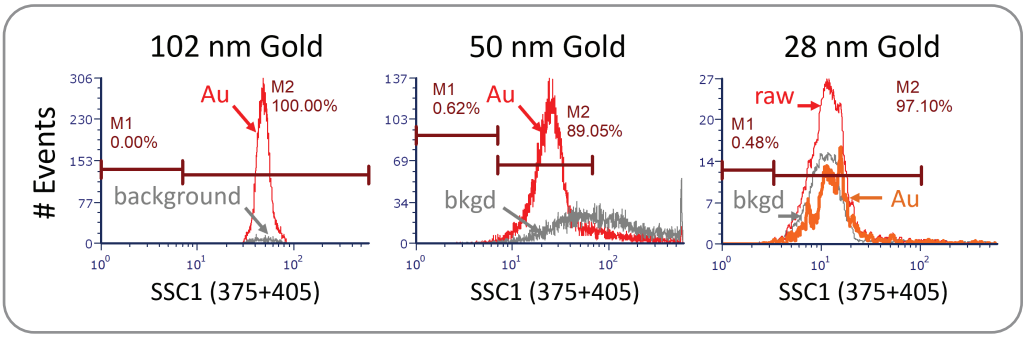
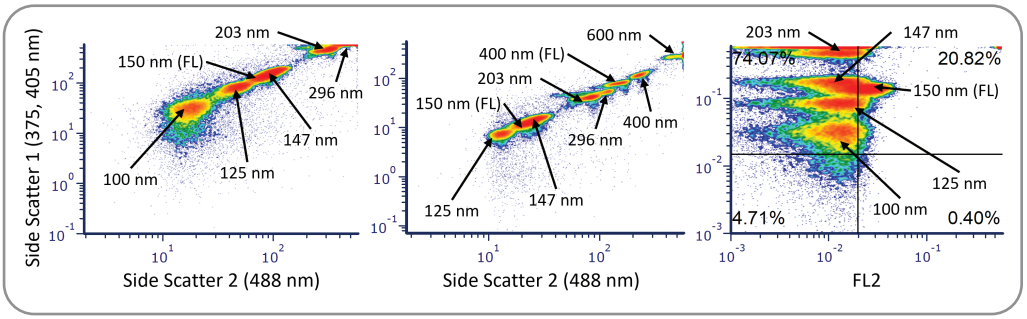
Late breaking data from our *Delaware* Flow NanoCytometer™



The* Delaware *detects 58-nm extracellular vesicles (EVs). Using large (177-nm) and small (58-nm) liposomes from Acoerela (sizing by NanoFCM), the *Delaware* demonstrates linearity in the number of events detected as a function of concentration (left). Side scatter (SSC) measurements (right) show consistent scatter values over a nearly decade of liposome concentration. At higher concentrations, swarming (multiple particles per event, as indicated by increased scattering signal per event) is easily detected.



The* Delaware *detects 28-nm gold nanoparticles. Gold nanoparticles (nanoComposix) were run on the *Delaware*. Histograms of scattering signal from the 102-nm and 50-nm gold particles (red trace in left and center panels) are easily distinguished from background (grey, sheath fluid only). The right panel shows that 28-nm gold particles are also above the detection limit. The 28-nm particles exhibit a histogram peak at a much lower scattering intensity. Raw signal (red) can be discerned from background (grey). The subtracted peak (orange) shows the excess events due to the gold nanoparticles.



The* Delaware *exhibits 22-nm resolution. Using Rosetta calibration nanoparticles (Exometry), 125-nm particles are easily distinguished from the 100-nm particles, as well as the cluster composed of 147-nm and 150-nm particles (left). The center panel shows excellent discrimination of particles from 100 nm through 600 nm. The right panel shows that the 147-nm particles and fluorescently labeled 150-nm particles can be discerned based on the FL2 signal.

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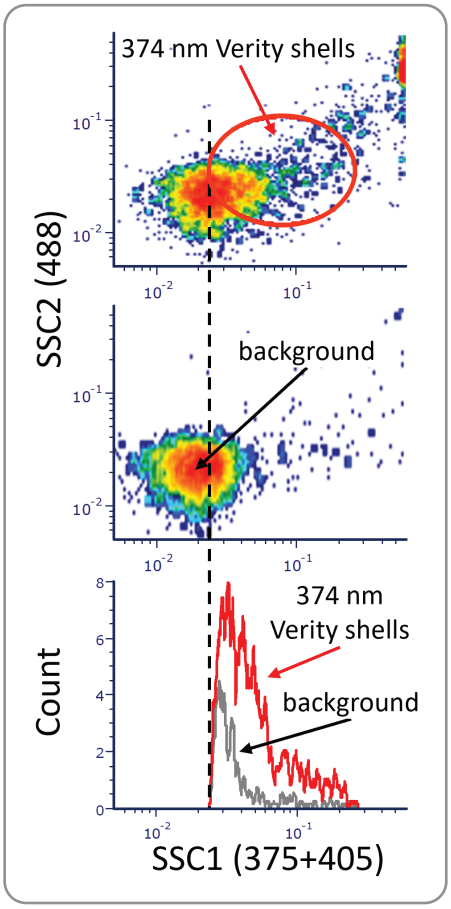
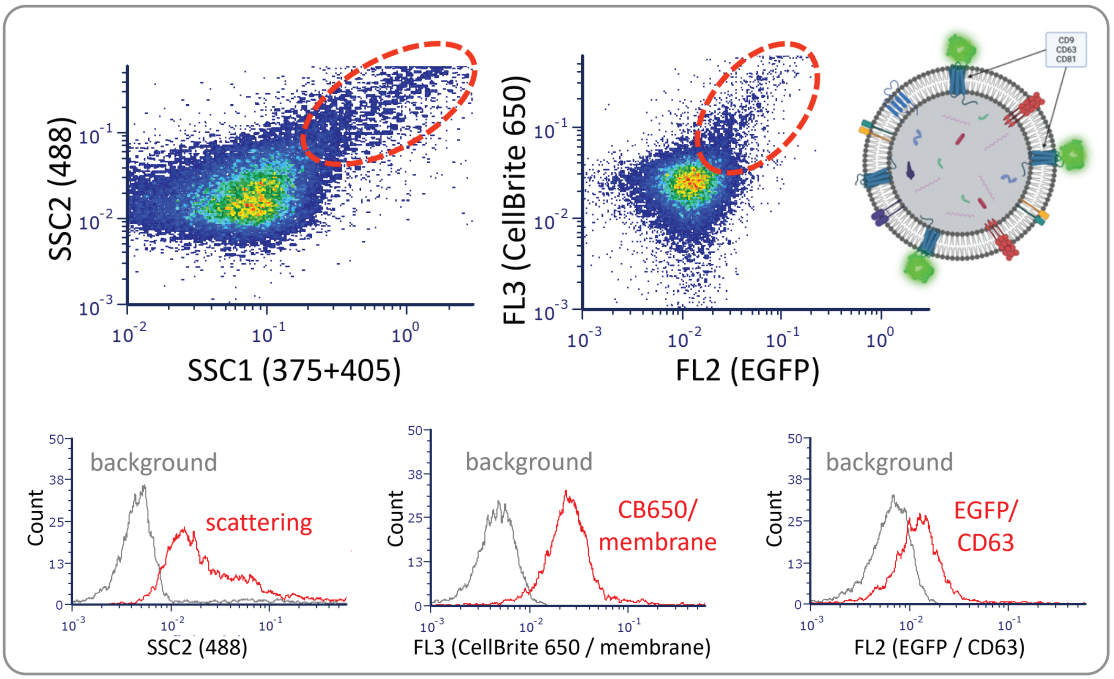


The* Delaware *detects hollow organosilica nanospheres. Dot plots (SSC1 vs. SSC2) show that distinct populations of organosilica Verity Shells (Exometry) can be detected over the background signal (top and middle). Distinct histograms of signal (red) and background (grey) populations are obtained (bottom).



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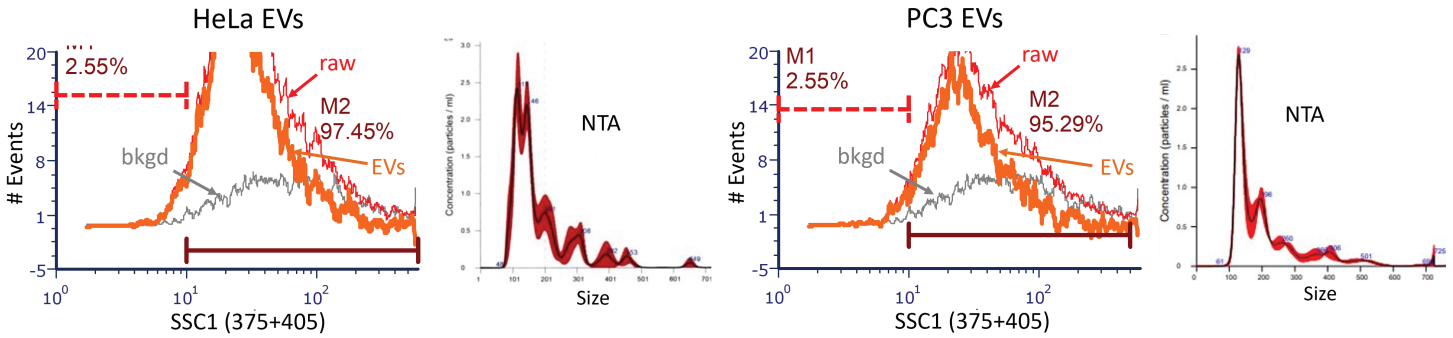
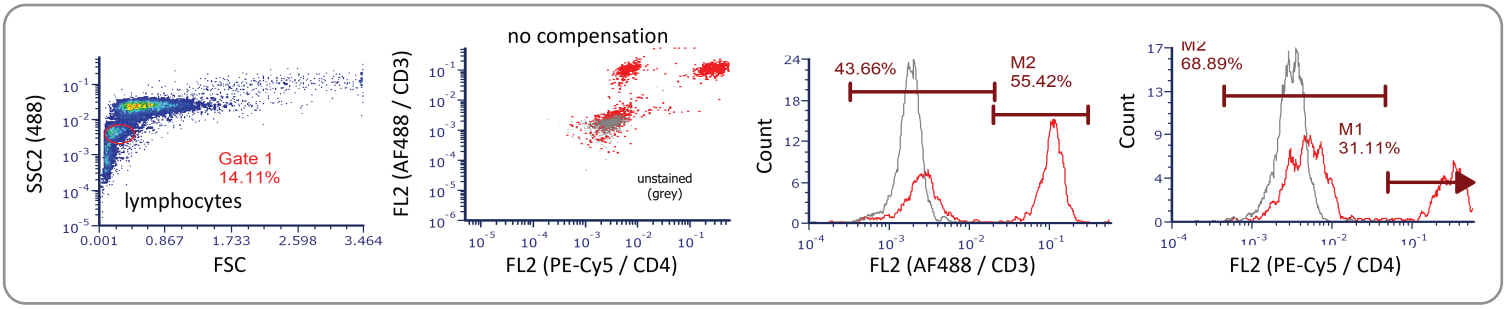
The* Delaware *detects EVs derived from human cells. EVs containing eGFP tagged CD63 (HansaBioMed FLuoEVs) were further stained with Biotium CellBrite Steady 650 membrane dye. EV populations can be discerned from background based on scatter alone (SSC1 vs. SSC2, top left) or fluorescent signals (eGFP vs. CellBrite, top middle). Histograms (bottom) show distinct populations of EVs (red) and background (grey) based on SSC2 or fluorescent membrane stain signal. More importantly, the *Delaware* demonstrates the fluorescent sensitivity required to detect EVs based on CD63 staining alone (bottom right).



The* Delaware *detects EVs derived from human cancer cell lines. EVs isolated from HeLa (left) and PC-3 cells were measured on the *Delaware*. Histograms (1st and 3rd from left) show that raw signal (red) and EV signal (orange, raw minus background), are easily distinguished from background (grey, sheath fluid only). Nanoparticle tracking analysis (NTA, 2nd and 4th from left) shows that the EVs range in size from 50 nm to over 700 nm, but the bulk of the EVs in both populations are under 250 nm. EVs and NTA analysis provided by the Kashanchi lab at George Mason University.



The* Delaware *has full capabilities for analysis of cells in addition to EVs. Using Veri-Cells™ PBMCs (BioLegend), dot plots show that the lymphocyte population can be distinguished based on FSC and SSC characteristics (far left) and that three distinct lymphocyte subpopulations based on CD3 and CD4 fluorescent staining are detectable (2nd from left). In that plot, CD3/CD4 double negative cells overlay with unstained cells (grey). Histograms show the CD3 (3rd from left) and CD4 (far right) positive and negative populations with good separation (high staining index).