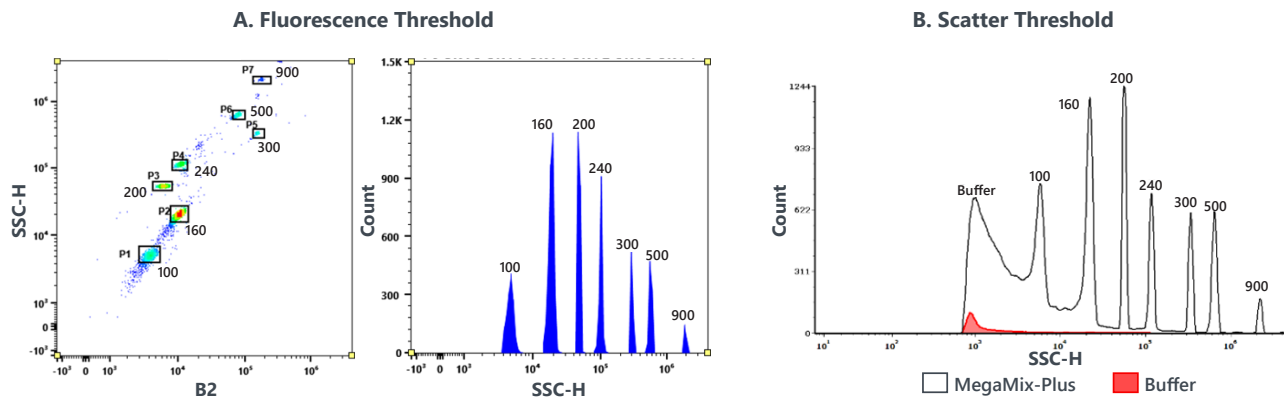


# Enhanced Small Particle Detection on Cytek Aurora and Northern Lights

There is a great deal of interest in characterizing small particles such as bacteria, extracellular vesicles, and viruses by flow cytometry. The common availability, as well as the multiparameter and high throughput capabilities of flow cytometry, make this technology attractive for measuring small particles. However, many of the flow cytometers currently being used are not able to detect biological particles smaller than 600nm, in fact very few can detect biological particles 300nm or less<sup>1</sup>. This lack of sensitivity has led to a bias in the literature toward larger particles, which in the case of extracellular vesicles, represent the smallest percentage of particles<sup>2</sup>. While currently there are no flow cytometers that can detect the entire size range of submicron biological particles<sup>3</sup>, Cytek has developed an Enhanced Small Particle (ESP) detection option for the already popular Aurora and Northern Lights flow cytometers.

## Example 1: MegaMix

A commonly used set of fluorescent polystyrene (PS) beads used to demonstrate an instrument's ability to detect small particles are BioCytek Megamix-Plus. While PS beads have a higher refractive index than biological particles, and therefore have considerably more scatter intensity than biological particles of equal size, they are still used as a tool evaluate sensitivity and resolution. Provided below is an example of this bead set run on an Aurora system with the ESP option with both fluorescence threshold (A) and a violet scatter threshold (B), demonstrating excellent resolution of all the bead populations above the buffer background with both approaches.



Refractive Index (RI)

Scatter Intensity

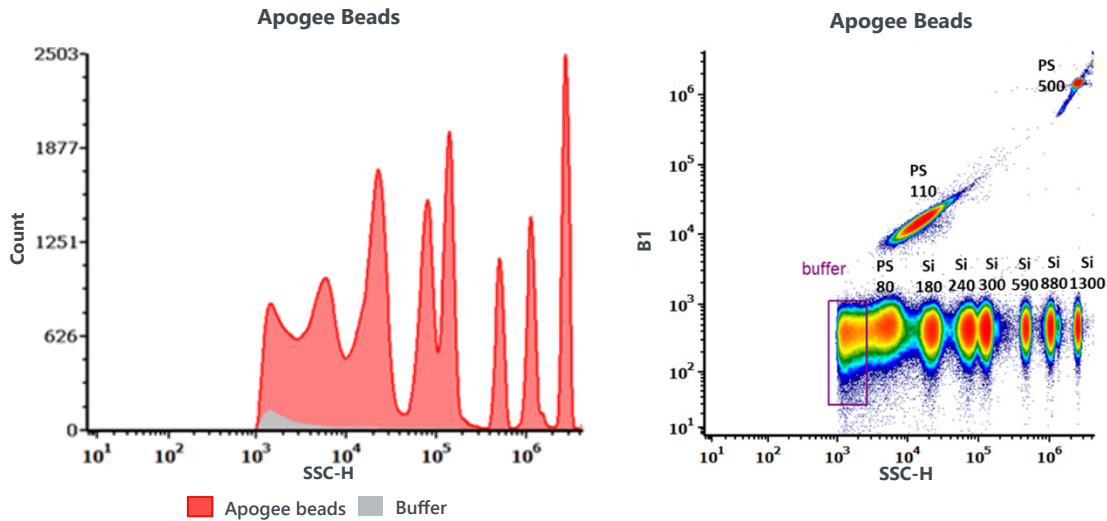
EVs RI 1.37

Si RI 1.43

PS RI 1.59

## Example 2: Apogee Beads

Another commonly used tool to evaluate sensitivity and resolution is Apogee beads. Apogee beads contains both PS and silica (Si). Si has a lower RI (closer to biologicals) than PS, and therefore has less scatter intensity than PS. A new version of Apogee beads (Product 1527 Lot# CAL0145), which has an additional 80nm PS bead added to the previous mixture of 110nm and 500nm PS beads, and 180, 240, 300, 590, 880, and 1300 Si beads, was evaluated. The results are displayed below.

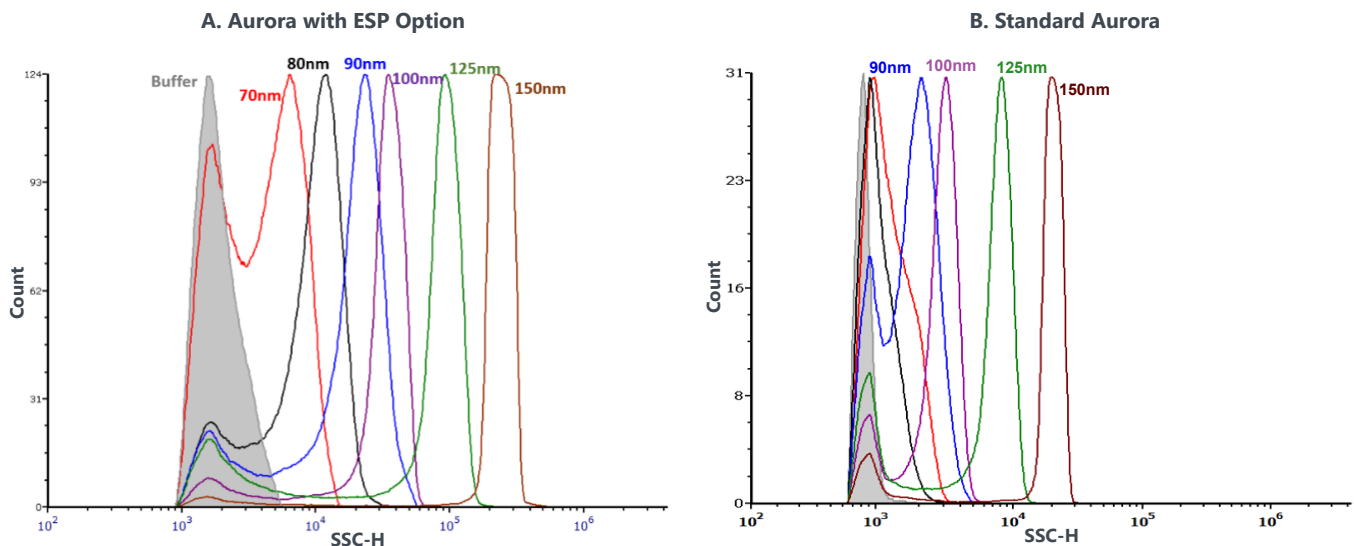


## Example 3: Size Calibration of Using NIST Small Particle Beads

The new violet laser scatter enhancement option increases the sensitivity of size detection for biological particles down to 87.5nm for particles with a RI of 1.39, 97nm for particles with a RI of 1.37, and 117.2nm for particles with a RI of 1.35 (Figure 2). This data is derived from Thermo Fisher Scientific NIST Traceable PS beads (3000 Series Nanosphere™ Size Standards) (Figure 1). Using these beads as calibration standards, arbitrary units (a.u.) of scatter intensity were calibrated into Scattering Cross-Section units ( $\text{nm}^2$ ). From this calculation, one can derive the diameter of particles with RIs comparable to EVs, viruses, and liposomes. Using FCMPass v3 Calibration software developed by Joshua Welsh at NIH/NCI<sup>4,5</sup>, calibration curves were generated from which size of biological sample can be extrapolated. In this calibration protocol NIST Traceable beads in the following sizes were used to generate the graph in Figure 2: 70, 81, 92, 100, 122, 152, 240, 303, and 401nm.

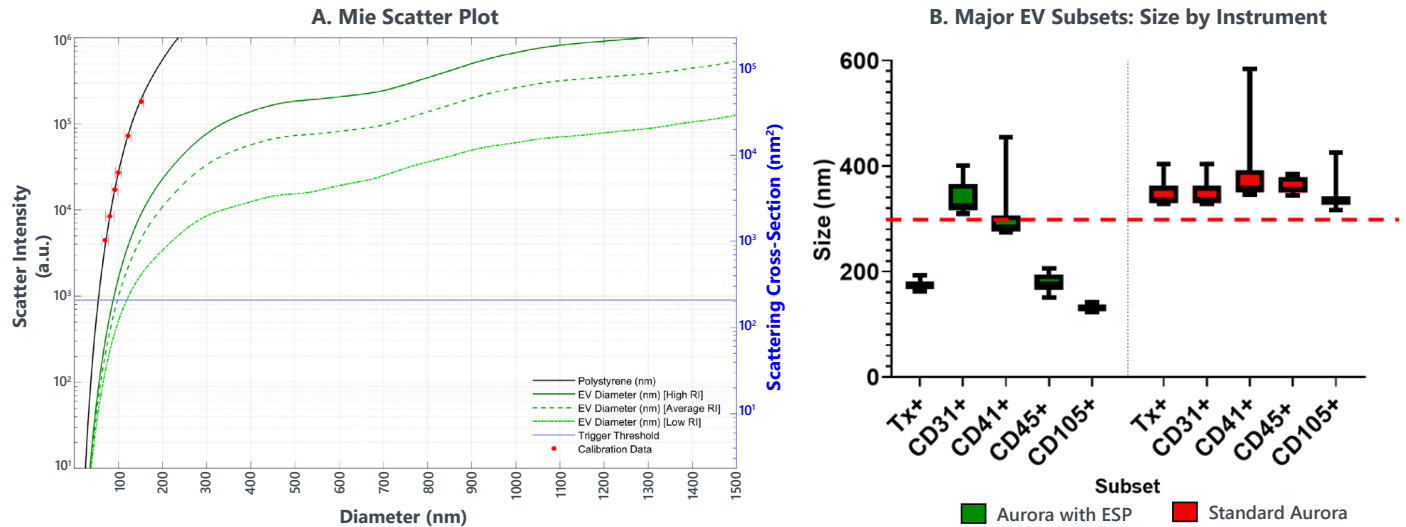
Figure 1: NIST Small Particle Beads

(A) Aurora system with ESP option. 70nm particles are fully resolved above the background and from the 80nm PS bead. (B) Same beads run on a standard Aurora system, showing sensitivity of detection below 100nm.

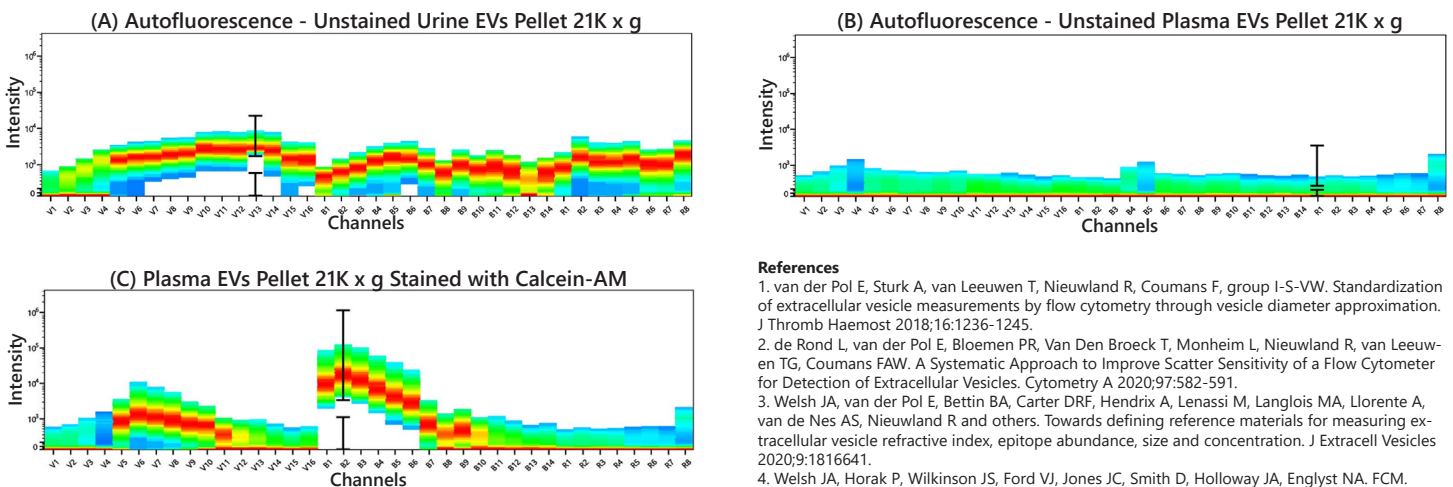


**Figure 2: Scatter Intensity Calibration**

Understanding the Limits of Detection (LoD) for size for biological particles requires calibrating an instrument's scatter intensity a.u. to a more meaningful parameter, such as diameter (nm). This will allow more direct comparisons of data across instruments. FCMPass was used to calibrate an Aurora system with an ESP detection option using NIST traceable size beads. **(A)** Graph showing the Mie theory generated curves for polystyrene beads (Black Line) with actual polystyrene bead data overlaid (Red dot). Blue line represents the Trigger threshold used. The green lines represent the Mie generated curves based on the RI of EVs, with representative curves for low (1.35), high (1.39) and average (1.37) EV RIs. **(B)** Size distributions of subsets of plasma derived EVs derived from the graph in (A), demonstrating the ability to detect smaller EVs with the ESP option. (Data provided by the Lab of Uta Erdbruegger of the University of Virginia).

**Example 4: Spectral Characterization of EVs**

The autofluorescence spectral signature of unstained EVs from **(A)** Urine and **(B)** Plasma isolated by ultracentrifugation at 21K X g. The EVs from urine reveal a unique spectral signature not seen in the EVs from plasma. **(C)** Spectral signature of EVs from plasma stained with Calcein-AM. (Data provided by the Lab of Uta Erdbruegger of the University of Virginia).

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