Flow Post-its
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Small Particle Flow & Refractive Index

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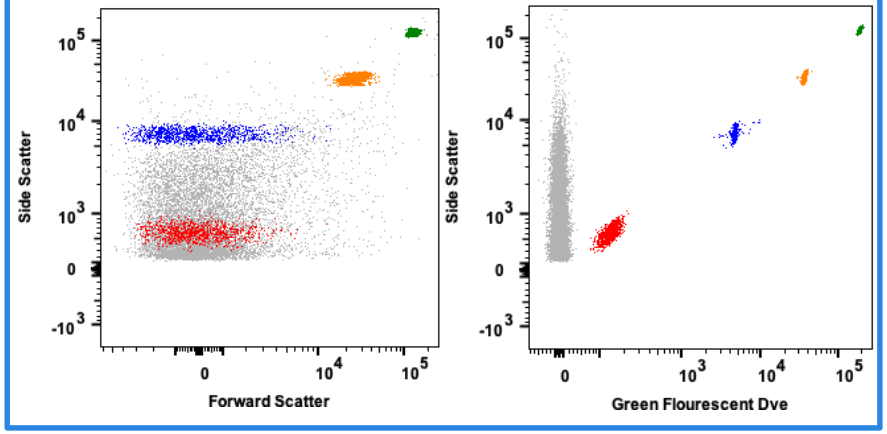
Flow cytometry is a powerful tool, and the ability to gain information in a high throughput manner for samples that are much smaller than "typical" cells is growing in interest. When interested in these **small particle** experiments, researching **extracellular vesicles** or **viruses** for example, it's important to know the limits of your instrumentation and how to **test performance**.

Instrument Performance Test

- A BD FACSAria was set up to test for limits of detection by size using the Sub-micron Particle Size Reference Kit from Thermo Fisher. These *polystyrene* beads are 100nm, **200nm**, **500nm**, **1µm** and **2µm** in size and are fluorescent (488nm ex. 515nm em.).
- Threshold was set to SSC and the voltage lowered to see events when **buffer alone** was run.
- Looking at a FSC/SSC plot overlay, we see that *by scatter* the beads do not resolve from background until 1µm.
- The same data, plotted as fluorescence vs SSC (log), allows for us to resolve the beads from noise as low as **200nm**.



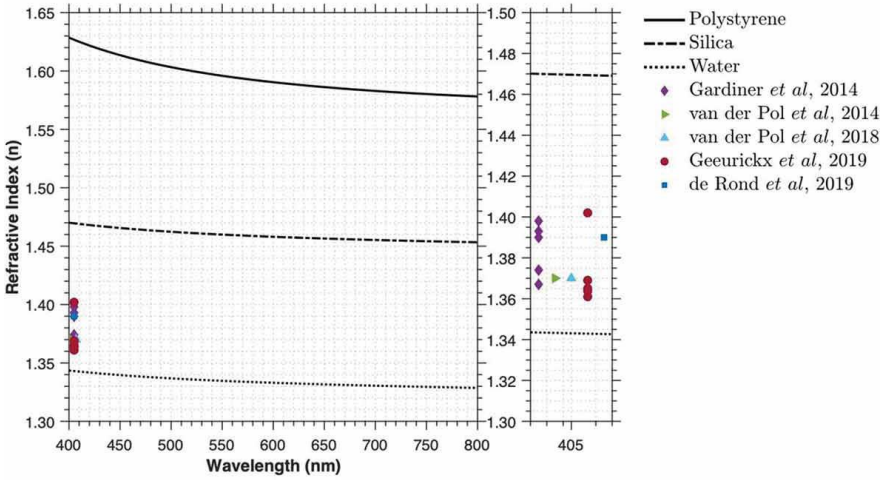


Figure 5. **Dispersion properties of polystyrene, silica and water from wavelengths of 400–800 nm**. Dispersion properties of polystyrene, silica and water were calculated using the Sellmeier equations for published materials [53–55]. Median refractive index (geometric mean in case of Gardiner *et al*) measurements for different EV sources were acquired from the literature [22,23,34,40,41].
Welsh JA et al. (2020), doi: 10.1080/20013078.2020.1816641

The difference of **refractive index** (RI) between the particle of interest and the sheath fluid plays a critical role in resolution of populations by scatter in flow cytometry.

As seen above, **polystyrene has a much higher RI than reported values for extracellular vesicles**. **The ability to resolve 200nm polystyrene beads *does not* equate to the ability to resolve 200nm extracellular vesicles due to differences in scatter.** Alternative materials, such as hollow organosilica beads or viral standards, should be considered when testing performance due to lower RI.

Suggested reading for extracellular vesicle flow cytometry: Nolan, J. (2015) doi: 10.1002/0471142956.cy1314s73
Coumans, FAW et al. (2017) doi: 10.1161/CIRCRESAHA.117.309417

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