

Supporting Information

Measurement of the particle density for the stock solution of intensity calibration microparticles

In order to accurately measure the absolute counting capability of the micro-flow cytometer it was necessary to know the density of the LinearFlow™ microparticles present in the suspension under analysis. For this purpose, a co-suspension was constructed containing a 50X dilution of the brightest subset of particles from the LinearFlow™ intensity standard calibration kit and a 10X dilution of CountBright™ absolute counting microparticles (Invitrogen, USA). This co-suspension was analyzed on a commercial instrument, specifically a BD LSR II, at the Cornell Biomedical Sciences Flow Cytometry Core Laboratory. The CountBright™ absolute counting microparticles were packaged at an absolute concentration of $1.02 \pm 0.04 \times 10^6 \text{ mL}^{-1}$ according to the manufacturer's specifications. Due to their distinct sizes (the absolute counting microparticles were 7- μm in diameter whereas the linear intensity calibration microparticles were 6- μm in diameter), the two sets of microparticles were distinguishable based on forward scatter, as shown in Figure S-1. The two clusters of differentiation from the scatterplot of Side Scatter vs. Forward Scatter exhibited distinct levels of fluorescence. Specifically, the particles present in GATE 1, which exhibited lower Forward Scatter due to their smaller size, corresponded to the LinearFlow™ fluorescence intensity calibration microspheres as verified by their high fluorescence intensity. The numbers of events counted in each gate are listed in Table S-1. A simple analysis shows that the stock concentration of the LinearFlow™ microparticles was $1.54 \pm 0.06 \times 10^7 \text{ mL}^{-1}$ in the original vial. The measurement was repeated with three replicate co-suspensions, and the error between repeated measurements introduced by variations in pipetting and gating was small compared with the uncertainty, 4%, in the absolute concentration of the CountBright™ absolute counting microparticles.

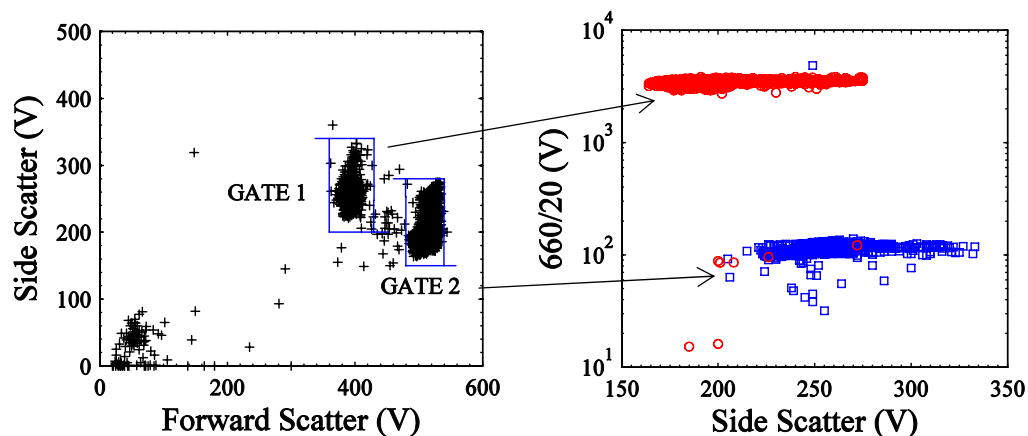


Figure S-1. Left: Scatter-plot shows the scattering measurements (+) for a co-suspension of CountBright™ absolute counting microparticles and LinearFlow™ fluorescence intensity calibration microparticles. Right: Scatter-plot shows the fluorescence intensity of the events from left plot in GATE 1 (□) and in GATE 2 (○). Only the first 4,000 out of 20,000 events are plotted for clarity. These measurements were acquired using a commercial cytometer instrument, specifically the BD LSR II.

Table S-1: Number of CountBright™ absolute counting particles and LinearFlow™ fluorescence intensity calibration particles detected in GATE 2 and GATE 1, respectively, from figure S-1.

	<u>CountBright™ Particles</u>	<u>LinearFlow™ Particles</u>
Gated Events	4,710	14,300
Concentration of particles added to suspension	$1.02 \pm .04 \times 10^5 \text{ mL}^{-1}$	50X dilution from stock
Measured Concentration	Given	$1.54 \pm 0.06 \times 10^7 \text{ mL}^{-1}$