

ApogeeMix Reference for Flow Cytometer Performance Assessment & Calibration

Datasheet for products #1493 & #1527

Introduction

The complex relationship between particle size and the amount of light scattered at different collection angles makes it difficult to infer particle size from a flow cytometer's light scatter data. A population may be described as scattering an amount of light equal to a reference particle (e.g. a polystyrene or silica bead of known size) but same sized particles of different refractive index (n) give different signal strengths. When comparing data between flow cytometers the difficulties are compounded by differences in light scatter collection angles. Ideally it would be possible to produce stable reference particles of known size and of a refractive index and structure similar to the bacteria or extracellular vesicles of interest but such particles are not commercially available, expensive or relatively unstable.

Due to the refractive index difference, polystyrene beads on their own do not offer an accurate means to assess a flow cytometer's light scatter performance for the study of biological particles. Silica (SiO_2) beads can be used as a better reference particle because silica's refractive index is closer to the refractive index of biological vesicles^{1,2,3,4,5}.

The *ApogeeMix* products (Cat #1493 & 1527) are a convenient mixture of non-fluorescent silica beads and fluorescent polystyrene beads with sizes from 80nm to 1300nm which can be used to prepare flow cytometers for the analysis of small biological particles by providing points of reference. The products differ only in that #1493 does not include the 80nm polystyrene beads. They offer an easy means to assess the sensitivity and resolution of the flow cytometer's light scatter and fluorescence optics and the silica beads offer a means to calibrate a flow cytometers light scatter optics at a refractive index of approximately $n_D = 1.43$ (589nm light).

Calibration to SI Units by Mie Theory Approximation

Attempts to calibrate flow cytometer light scatter signals to SI base units (e.g. nanometres) based on the Mie solution to Maxwell's equations have associated errors:

- they are based on estimations of the light scatter angle ranges collected by the detector and
- biological particles are not homogeneous spheres (Mie theory assumes homogenous spheres)
- the refractive index of biological particles varies
- user error due to the complexity of the process

Calibration to Silica Test Beads, a Better Reference Unit

To eliminate the complexity and errors introduced by Mie Theory conversion of light scatter signals to SI units, flow cytometer light scatter signals may instead be calibrated to silica test bead diameter. Silica test bead diameter is a simple, precise and reliable alternative reference unit accessible to all flow cytometer users. Test beads have well defined characteristics, excellent long term stability and are low cost. The use of silica test bead diameter as a reference unit is more precise (introduces less error) because the relationship between the optical properties of silica test beads and biological particles is more precisely known than the relationship between the optical properties of biological particles and nanometres. The 'ApogeeMix' product offers this simple, precise calibration solution.

A simple and precise practical method to characterise a flow cytometer's light scatter optics is therefore to use test beads of precisely known characteristics as a calibrator to define a scale based on test bead size instead of attempting to convert to SI units.

For situations where a precise calibration to SI units is preferred, ApogeeFlow offers cat#1492 "Light Scatter Calibration Module" which implements a patented process based on particle suspensions of precisely known refractive index (n_D at 25degC in the range 1.36 to 1.42). This practical approach avoids Mie theory approximations and offers scales calibrated to particular refractive indexes, on light scatter histograms. Consult ApogeeFlow for further details.

Materials Supplied

The *ApogeeMix* contains 25ml of an aqueous mixture of SiO_2 spheres with diameters 180nm, 240nm, 300nm, 590nm, 880nm and 1300nm diameter. It also contains 80nm (#1527 only), 110nm and 500nm green fluorescent polystyrene beads. The product is intended to be used to assess a flow cytometer's light scatter and fluorescence performance and to provide useful points of reference for light scatter data from biological particles. Shown below are typical data from the *ApogeeMix* analyzed on a "Micro" flow cytometer (FL1=Green fluorescence). The ApogeeFlow "Micro-PLUS" cytometer offers roughly 30x higher sensitivity.



The fluorescent polystyrene beads may be used to assess the fluorescence sensitivity and to assess the performance of the flow cytometer's optics for particles of relatively high refractive index.

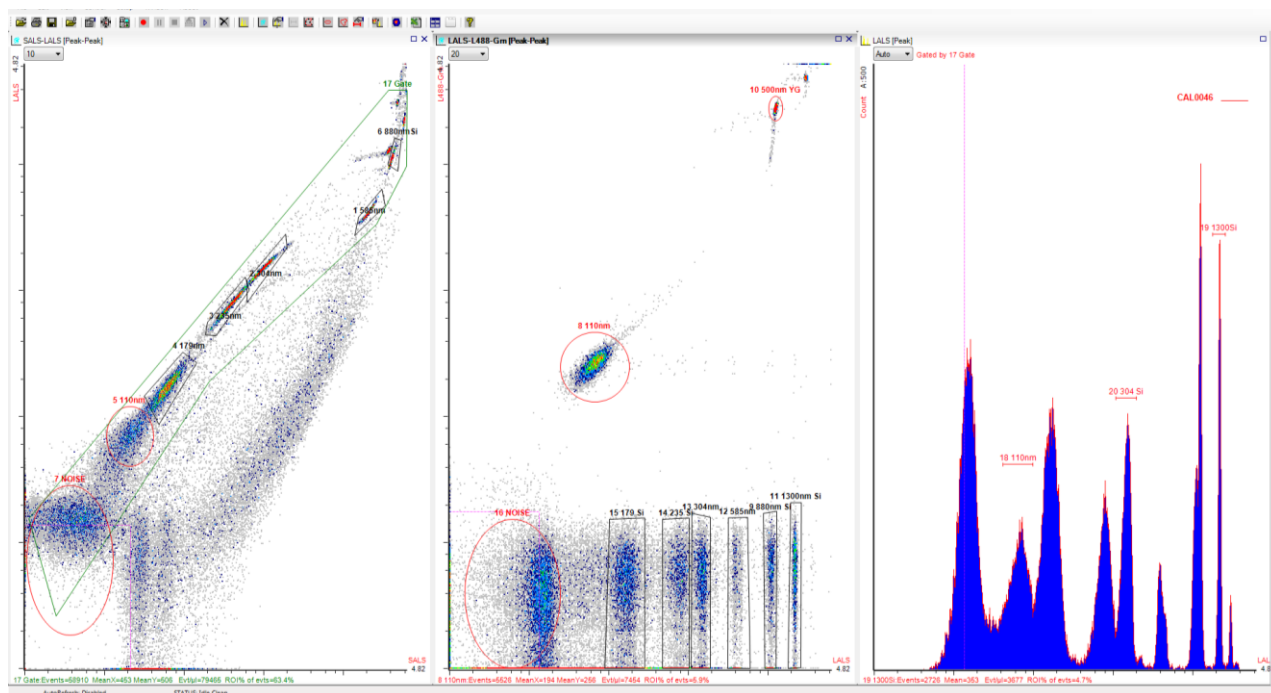
Approximate particle concentrations (lots vary, refer to the certificate of analysis):

Particle Size (nm)	Approximate number per microlitre	Fluorescence
80 (#1527 only)	5000	Green from L405
110	5000	Green from L488 & L405
180	5000	None
240	10000	None
300	9000	None
500	3600	Green from L488 & L405
590	2700	None
880	3900	None
1300	3400	None

Use

Resolution of the populations indicates the flow cytometer's performance. The below image is from #1493 ApogeeMix measured on a standard ApogeeFlow "Micro" Cytometer. A "Micro-PLUS" model offers an extra decade of light scatter sensitivity (next page). For product #1493, eight populations should be resolved from each other and resolved from instrument noise as shown below:

- 6 populations of silica (SiO₂) beads and
- 2 green fluorescent (405nm or 488nm laser) populations (110nm and 500nm) of polystyrene beads (refractive index approximately $n_D = 1.59$, middle graph. Product #1527 also contains 80nm polystyrene beads (green fluorescent from 405nm excitation light – see next page)



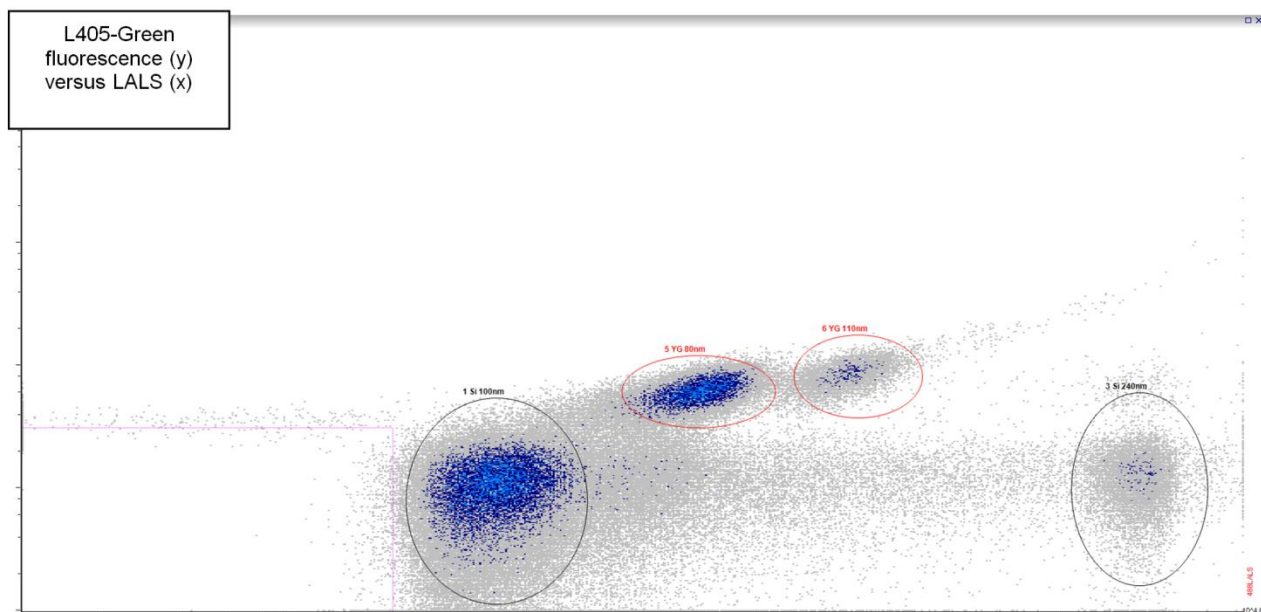
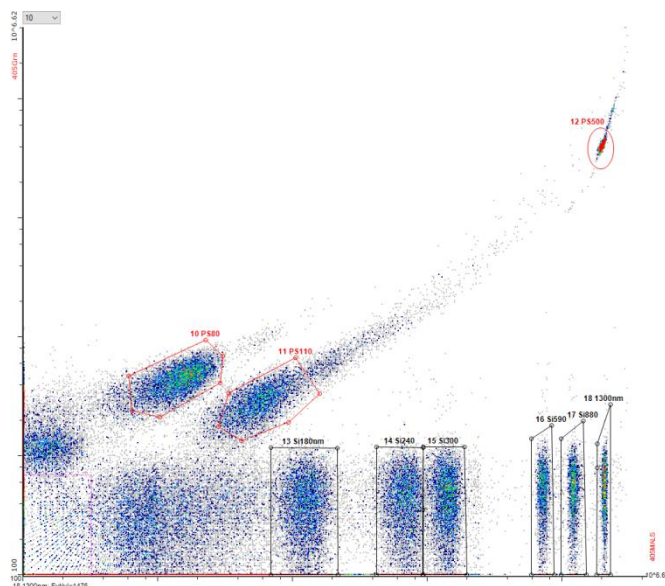
ApogeeMix particle populations provide useful points of reference in light scatter data. For example, the difference in the light scatter signal from a 180nm extracellular vesicle and a 180nm silica test bead is small and, more importantly, *independent of the optical design* (light scattering angle, wavelength) so such beads are a great reference when comparing data between flow cytometers/virometers. The user can define a region of interest ('gate') on a light scatter histogram based on the ApogeeMix test bead populations to capture particles within a particular range of light scatter properties and thus segregate particles within an inferred size range. The user may then state, without the need for complex modelling and approximation, they are measuring particles that scatter amounts of light within a particular silica test bead size range.



The image on the right shows data from cat#1527 including 80nm polystyrene beads ("10 PS80") emitting green fluorescence from a 405nm laser.

Due to the refractive index difference, an 80nm polystyrene test bead scatters about the same as a 150nm extracellular vesicle.

Higher light scatter gain settings (below) show good population resolution. A 100nm silica test bead population (not included in the product) is shown below for additional context. ApogeeFlow offers 100nm silica test beads as product #1517 which scatter less than 80nm polystyrene test beads due to the refractive index difference.



Ordering Information

ApogeeMix products (Cat #1493 & 1527) are available from UK stock. Please email purchase orders and price requests to info@ApogeeFlow.com.

Product Safety

Caution: Product contains 0.05% sodium azide.

MSDS available on request (info@ApogeeFlow.com)

References

1. Journal of Thrombosis and Haemostasis 2011 Jun, 9(6):1216-24
A new microparticle size calibration standard for use in measuring smaller microparticles using a new flow cytometer Chandler, W., Yeung, Wandy, Tait, Jonathan;
2. Water Research 42 (2008) 3757 – 3766
Use of silica microspheres having refractive index similar to bacteria for conversion of flow cytometric forward light scatter into biovolume Paola Foladori, Alberto Quaranta, Giuliano Ziglio
3. J Thromb Haemost 2014; DOI:10.1111/jth.12602 van der Pol E, Coumans FAW, Grootemaat AE, Gardiner C, Sargent IL, Harrison P, Sturk A, van Leeuwen TG, Nieuwland. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing.
4. American Chemical Society 2014 Oct, 2 p.6195-6201 Edwin van der Pol et al
Refractive index determination of nanoparticles in suspension using nanoparticle tracking analysis
5. Journal of Research of the National Institute of Standards and Technology, Volume 119 (2014) <http://dx.doi.org/10.6028/jres.119.029>
The Use of Index-Matched Beads in Optical Particle Counters