APOGEE FLOW SYSTEMS

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Document #16026

ApogeeMix for Flow Cytometer Performance Assessment

Datasheet for product # 1493

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 latex or silica bead of known size) but same sized particles of different refractive index (η) can give very 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 microvesicles of interest but such particles are not commercially available.
Due to the refractive index difference, latex 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 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].

The *ApogeeMix* product (Cat #1493) is a convenient mixture of non-fluorescent silica beads and fluorescent latex beads with sizes from 110nm to 1300nm which can be used to prepare flow cytometers for the analysis of small biological particles. 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 1.43.

For a more precise calibration for assessment of bacteria and biological vesicle size (refractive index typically in the range 1.36 to 1.40), Apogee's Light Scatter Calibration Module (Cat# 1492) is recommended because it offers scales calibrated for particular refractive indexes, on the light scatter datagrams.

Materials Supplied

The *ApogeeMix* (Cat#1493) contains 25ml of an aqueous mixture of plastic spheres with diameters 180nm, 240nm, 300nm, 590nm, 880nm and 1300nm diameter with refractive index η=1.43, and 110nm and 500nm green fluorescent (blue laser) beads with refractive index η=1.59 (latex). The product is intended to be used to assess a flow cytometer's light scatter and fluorescence performance (both sensitivity and resolution). Shown below are typical data from the *ApogeeMix* analyzed on an A50-Micro flow cytometer (FL1=Green fluorescence).

The fluorescent latex beads may be used to assess the fluorescence sensitivity and to assess the performance of the flow cytometer's optics at a different refractive index.

Approximate particle concentrations for Lot # CAL0046:

Particle Size (nm)	Approximate number per microlitre	Fluorescence from 488nm excitation
110	8000	Green
180	23000	None
240	10000	None
300	9000	None
500	3600	Green
590	2700	None
880	3900	None
1300	3400	None

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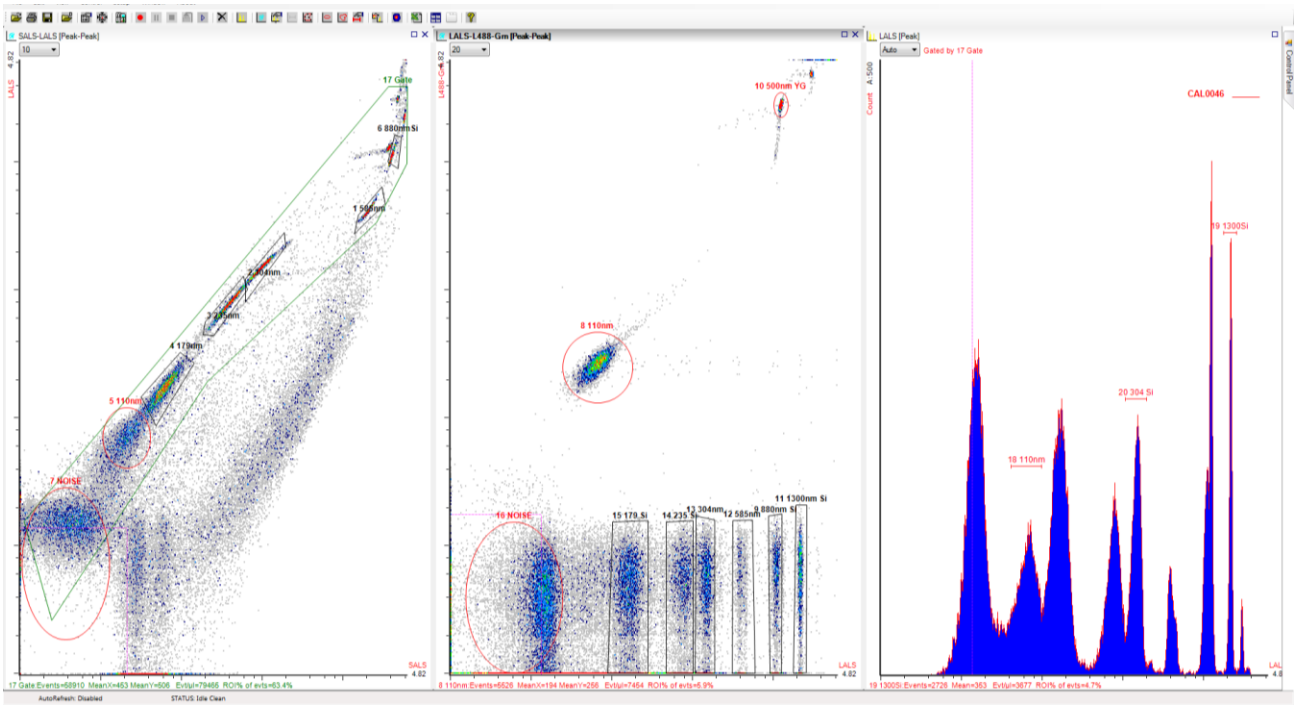
Typical Data from Apogee A50-Micro Flow Cytometer

The resolution of the peaks indicates the flow cytometer's performance. Ideally eight populations should be resolved from each other and resolved from instrument noise:

- 6 populations with refractive index 1.43
  and
- 2 green fluorescent (488nm laser) populations (110nm and 500nm) with refractive index 1.59 (middle graph).



Product Safety

Caution: Product contains 0.05% sodium azide.
MSDS available on request (info@ApogeeFlow.com)

References

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