



## Micro-PLUS Flow Cytometer

### Technical Specifications

ApogeeFlow's 'Micro-PLUS' is a high speed cytometer optimised for extreme small particle applications: extracellular vesicles, virions and nanoparticles.



Configurations:	Up to 9 fluorescence colour detectors Up to 3 light scatter detectors Up to 4 lasers Optional Autosampler for 96 well plate format Volumetric sample system gives absolute counts for every region of interest
Speed:	>100,000 events/sec Up to 45 samples per hour
Particle sorting:	Optional 'Kairos' sorting module, a novel fluid-based particle sorting module.
Typical Applications:	Extracellular vesicles Virions > 100nm (immunotherapy vectors, vaccine development) Nanoparticles Protein aggregation Bacteria
Sensitivity:	Light scatter sensitivity: 100nm silica beads (70nm polystyrene beads) Fluorescence sensitivity approximately 50 MESF FITC, 25 MESF PE. For any flow cytometer, the minimum measureable number of fluorescent molecules is heavily dependent on the sample (e.g. unbound dye), choice of laser, choice of photomultiplier detector, optical filter choice and method of noise measurement.
Resolution:	10nm (polystyrene beads – see data below) by light scatter
Size:	W32 x H50 x D48cm (W50 x H50 x D48cm with Autosampler)
Weight:	25 to 35kg depending on configuration
Power consumption:	Less than 250 Watts
Installation:	Requires two power sockets (110VAC or 230VAC) rated for at least 5A 10 to 30°C operating environment No external cooling requirements. Ventilation space at rear at least 10cm



- Sample Fluidics:** Volumetric sample injection  
Adjustable sample aspiration volume, 50-400 $\mu$ l standard, option for up to 900 $\mu$ l  
Software selectable sample flow rate from 0.7 to 100 $\mu$ l/min  
Sample concentrations up to 10<sup>9</sup> per ml
- Sheath Fluidics:** Refillable sheath fluid tank. Optional closed loop ultra-pure sheath fluid system.
- Waste Fluidics:** No special requirements.  
Bleach may be used in waste tank if desired  
Performance insensitive to waste tank height
- Electronics & Data Management**  
Fast electronics with PCIe interface able to measure more than 100,000 events/sec  
Threshold software selectable on all channels with 'AND' or 'OR' combination logic  
Pulse height & area measurements on all channels  
Trigger width measurement for each particle  
Event time stamp for each particle  
22 bit data from a 26 bit data acquisition path  
Internal PC with Windows 64 bit operating system  
Optional external desktop or laptop PC (LAN connection)
- Optics:** High numerical aperture light collection optics (immersion lens)  
Up to 4 lasers: 375nm, 405nm, 488nm, 532nm, 552nm, 561nm, 635nm (contact Apogee for more options)  
High sensitivity photomultiplier detectors (choice of sensitivities available)  
Fluorescence filter blocks may be changed without realignment  
Custom optical filter blocks  
Fluorescence optics capable of detecting from 400 to 800nm  
Large (side) and small (forward) light scatter photomultiplier detectors  
  
Optional medium angle light scatter detector  
Optional deep red detector (#3491) to extend the fluorescence range up to 900nm
- Software:** Apogee Histogram software with Work List sample management  
FCS 3.1 file format  
Histogram resolution software selectable from 32 to 65535 with zoom feature  
Display modes: linear and/or logarithmic gain selection for each parameter  
Unlimited number of histograms and cytograms  
Amorphous regions of interest (gates)  
Gating using AND, OR and NOT logic  
RATIO parameter configurable for any 2 detectors  
Autocycle module  
Gain and subtraction settings adjustable during and after acquisition  
Export of statistics from multiple files to Microsoft Excel  
Export of histogram data in '.csv' format  
Quick copy to clipboard feature for datagrams  
Overlaying histograms for easy data comparison  
Work List window for sample queuing and sample to sample data comparison  
Automatic fluidic shutdown option  
Automatic power off option
- Publications:** Refer to <http://apogee-flow.com/references.php>



## Micro-PLUS Performance

### ApogeeMix Beads (Cat#1493)

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  $n=1.43$  (silica), and 110nm and 500nm green fluorescent (blue laser) beads with refractive index  $n=1.59$  (polystyrene). 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* analysed on a Micro-PLUS flow cytometer (FL1=Green fluorescence).

Particle Size (nm)	Fluorescence from violet or blue excitation
110	Green
180	None
240	None
300	None
500	Green
590	None
880	None
1300	None

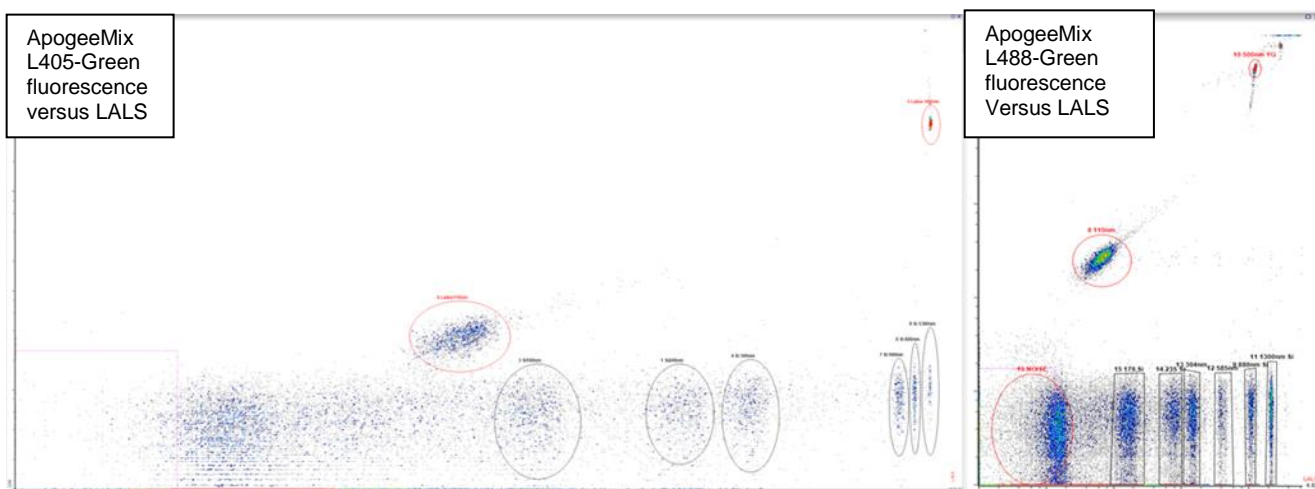
The fluorescent polystyrene beads may be used to assess the fluorescence sensitivity and to assess the performance of the flow cytometer's optics at a different refractive index.

The Micro-PLUS is comfortably able to resolve all populations in the ApogeeMix and offers more than a logarithmic decade (about 30x) separation between the 110nm polystyrene beads and instrument noise (left image) by light scatter. For Micro-PLUS models Apogee offers product #1517, a mixture of 80nm polystyrene and 100nm silica test beads which both scatter significantly less than the 110nm polystyrene beads in the ApogeeMix.

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 (silica) and
- 2 green fluorescent populations (110nm and 500nm) with refractive index 1.59 (polystyrene).

The left image below shows green fluorescence (vertical axis) from a violet laser (left image) using high gain light scatter settings and the right image shows green fluorescence from blue laser excitation (lower light scatter gain).

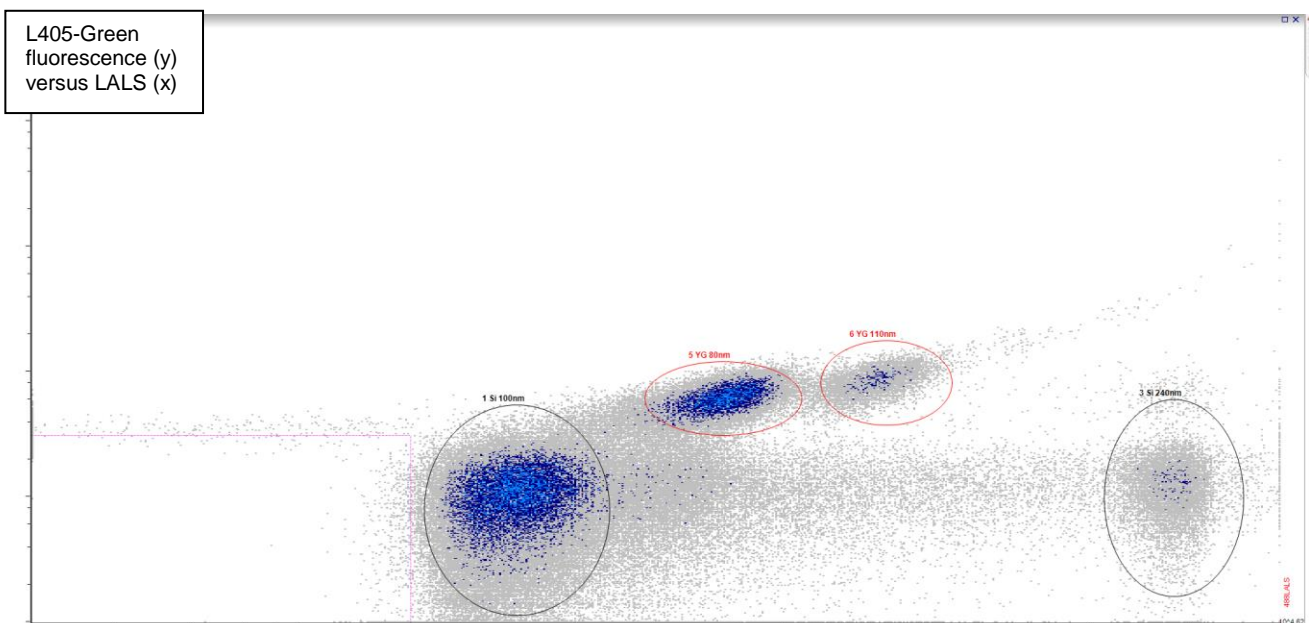




## Light Scatter Sensitivity

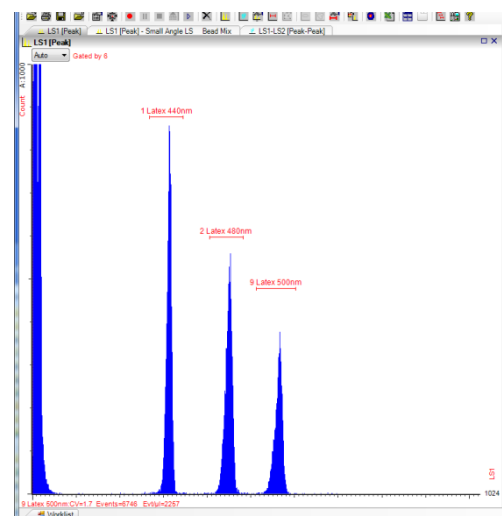
The Micro-PLUS is the first commercially available flow cytometer with the ability to resolve 100nm silica beads from noise by light scatter. From left to right, the data below shows a suspension of 100nm silica (non-fluorescent), 80nm polystyrene beads (fluorescent), 110nm polystyrene beads (fluorescent) and 240nm silica beads (non-fluorescent). The 80nm polystyrene beads scatter more brightly than 100nm silica beads due to their higher refractive index.

In the graph below, logarithmic green fluorescence is plotted vertically (Y) and logarithmic large angle light scatter (LALS) is plotted horizontally (X).



## Light Scatter Resolution

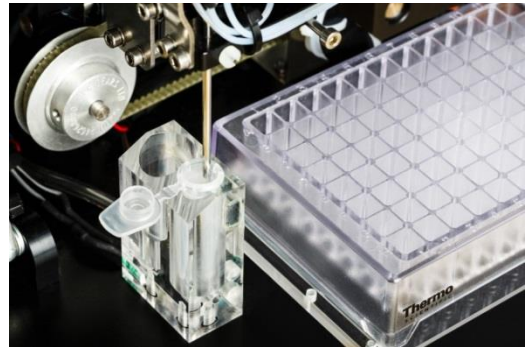
The Micro-PLUS is capable of exceptional resolution of particles of bacterial size and smaller. This histogram shows resolution of 440nm, 480nm and 500nm polystyrene beads by light scatter (linear axis), indicating the ability to resolve particle size differences less than 10nm.





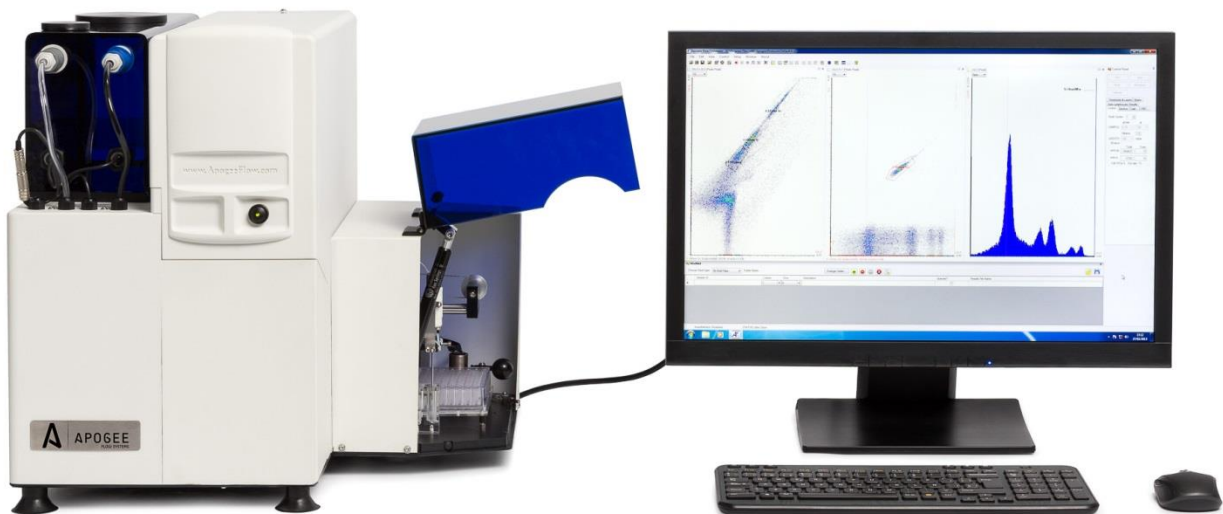
## Optional Autosampler for 96-well Plates (Cat# 2259)

Apogee's flow cytometers can be fitted with an automatic sampling robot to sample from 96 well format plates or racks of tubes. It is compatible with deep well or standard plates. Autosampler operation is managed via the Histogram application Work List. A separate holder for individual microcentrifuge tubes allows the operator to run individual samples.



### Features:

- 'Suck & spit' capability to re-suspend samples before aspiration from the well/tube
- Optional 'Plate shake' agitator to re-suspend samples before aspiration from the well/tube
- Email notification when the Work List is finished or to notify the operator of a fault condition (e.g. empty sheath fluid tank).
- Automatic fluidic shut-down and power off option when the Work List finishes
- Up to 50 samples per hour. With cleaning cycles and longer sample acquisition times, typically 3 to 4 hours per 96 well plate. Auto-shutdown function allows 'walk away' operation.
- Ability to auto-load different settings for each sample



- The Histogram Software offers a 'Work List' to manage sample data. In addition to the sample location, sample identifier and description, statistics columns may be added so that a table of results can be easily compiled. Results are saved in csv format.

