Using Violet Side Scatter to Detect and Resolve Nanoparticles on the CytoFLEX Flow Cytometer

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Introduction

The detection of sub-micron particles by flow cytometry becomes increasingly difficult as particle sizes progress smaller than the wavelength of the light being used to detect them. This issue can be compensated for by shifting the interrogation wavelength toward the target particle diameter in order to bring it within an appropriate size ratio for discrimination. The general wavelength range for effective size discrimination is between 1 and 10 times the size of the particle, with wavelengths at either extreme losing the ability to further resolve size differences. At wavelengths beyond the Rayleigh Limit (20x), the system shifts more toward pure elastic Rayleigh scattering off of molecular bonds and induced dipole oscillations. This scattering, as well as particle swarming, creates the lower noise limit, and requires the system to be modified for any further improvement. In this poster, we will demonstrate how to use Violet Side Scatter (V-SSC) on the CytoFLEX flow cytometer to improve nanoparticle detection and resolution.

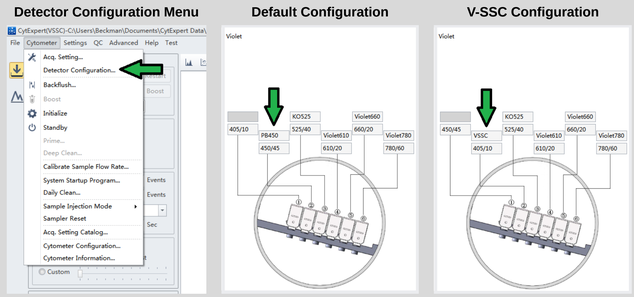
Materials

Item	Catalog #	Vendor
60nm Polystyrene NIST Beads	3060A	ThermoFisher Scientific
80nm Polystyrene NIST Beads	3080A	ThermoFisher Scientific
100nm Polystyrene NIST Beads	3100A	ThermoFisher Scientific
Multimodal Particle Size Standards	MM-010	ThermoFisher Scientific
93nm Silica NIST Beads	147020-10	Corpuscular, Inc
150nm Silica NIST Beads	147030-10	Corpuscular, Inc
200nm Silica NIST Beads	147040-10	Corpuscular, Inc
Whatman Anotop 25 0.02µm Filters	09-9260-13	ThermoFisher Scientific
Water	34877-4L	Sigma Aldrich
CytoFLEX Sheath Solution	B51503	Beckman Coulter
3-Laser CytoFLEX	B53000	Beckman Coulter
CytExpert Software		Beckman Coulter

Method

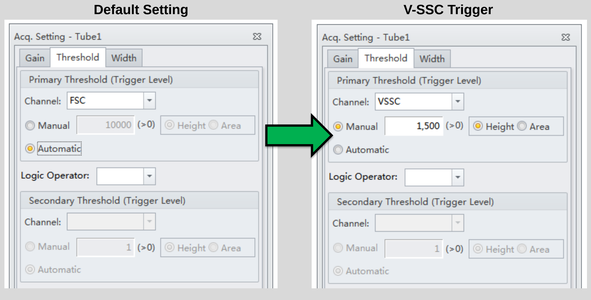
1) Start the CytExpert software and create a new V-SSC configuration, switching Pacific Blue/450nm with the V-SSC/405nm combination. Save this configuration as V-SSC.

Detector Configuration Menu | **Default Configuration** | **V-SSC Configuration**

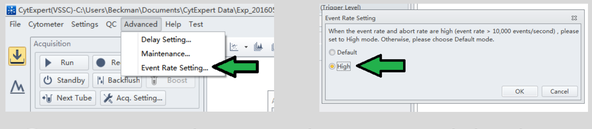


2) Physically switch out the 450nm filter with the 405nm filter in the first detector slot.
3) Start a new CytExpert experiment.
4) Change the Primary Trigger in the Acquisition Settings in order to Threshold on V-SSC Height.

Default Setting | **V-SSC Trigger**



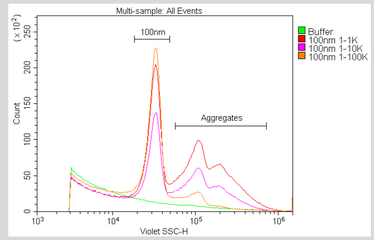
5) Change the Event Rate Setting from Default to High. This will narrow the pulse window and reduce coincidence with any particulate matter.



6) Setup your charts and gates and begin your experiment.
7) Run the sample at the slowest rate possible, and make several dilutions in order to hone in on the maximal detection and resolution, with minimal swarming.
8) Once running, manually adjust the threshold with both buffer alone and the smallest particles that you have in order to optimize the trigger for your sample and gain settings.

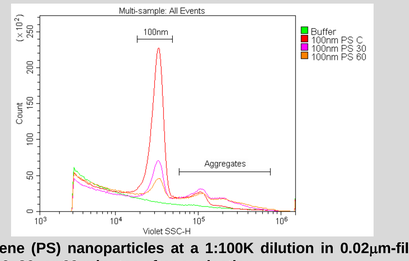
Experimental Considerations

1) If the sample is too concentrated or is run too fast, this will increase aggregation and/or swarming.



100nm Polystyrene nanoparticles in 0.02µm-filtered sheath solution at a dilution of 1:1K, 1:10K or 1:100K from the stock 1% solution. 1:100K is clearly the best.

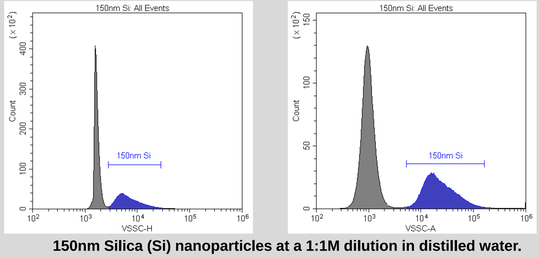
2) The sample should be homogenously distributed. Aggregation can make the resolution of specific populations difficult or impossible.



100nm Polystyrene (PS) nanoparticles at a 1:100K dilution in 0.02µm-filtered sheath solution read at 0, 30, or 60 minutes after sonication.

3) Height is better for discrimination (less swarming detected as larger events), while Area is better for resolution of the thresholded events.

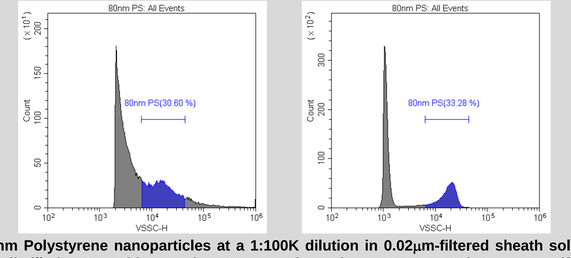
V-SSC Height | **V-SSC Area**



150nm Silica (Si) nanoparticles at a 1:1M dilution in distilled water.

4) The cleaner the sample buffers are of particulate matter the better.

Filtered Sheath Solution | **Distilled Water**

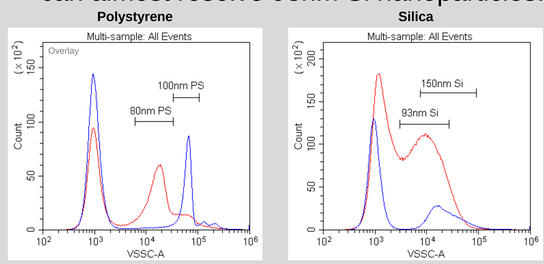


80nm Polystyrene nanoparticles at a 1:100K dilution in 0.02µm-filtered sheath solution vs. distilled water. This experiment was performed on a CytoFLEX that was specifically modified to enhance nanoparticle detection.

Performance

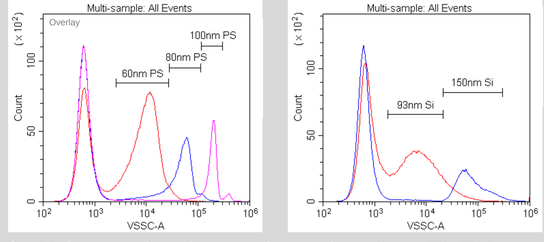
1) Using V-SSC, the CytoFLEX can resolve 80nm PS and 150nm Si nanoparticles. It can almost resolve 93nm Si nanoparticles.

Polystyrene | **Silica**



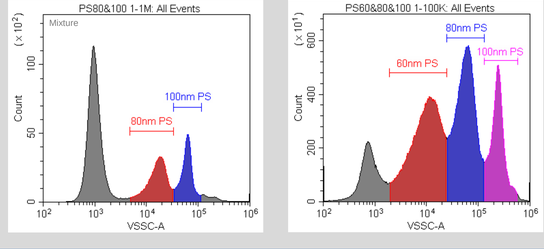
2) With several modifications to specifically enhance nanoparticle discrimination, the CytoFLEX can resolve 60nm PS and 93nm Si nanoparticles using V-SSC.

Polystyrene | **Silica**



3) When mixed together, the CytoFLEX can easily resolve different-sized nanoparticles from each other using V-SSC.

Stock CytoFLEX | **Enhanced CytoFLEX Prototype**



Conclusions

Using V-SSC, the CytoFLEX can easily discriminate and resolve 80nm PS and 150nm Si nanoparticles from noise or each other. Our enhanced CytoFLEX prototype can resolve 60nm PS and 93nm Si nanoparticles.

CytoFLEX is for Research Use Only.

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