

A Nano- and Microparticle Mix for CytoFLEX Size Standardization

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Introduction and Background

The CytoFLEX platform is distinguished by its exquisite sensitivity for size- and fluorescence-based detection. Utilizing VSSC, the CytoFLEX Flow Cytometer is capable of resolving 70 nm-latex and 100 nm-silica nanoparticles. Since most size-based microparticle mixes were not designed to assess nanoparticle detection, their size range is insufficient to adequately standardize the CytoFLEX. In addition, these mixes tend to contain a lot of contaminating particulate in the smaller size range, making more sensitive instruments appear to be noisier.

In order to address these issues, we prepared an improved nano- and microparticle bead mix specifically for the CytoFLEX. The CytoFLEX Sizing Bead mix contains a mixture of fluorescent and non-fluorescent latex and silica NIST-traceable size standards between 80 nm and 2 μm in size. In this poster, we demonstrate the performance of our sizing bead mix using a CytoFLEX-S (N-V-B-R), and compare it with the commercially available ApogeeMix beads.

Materials

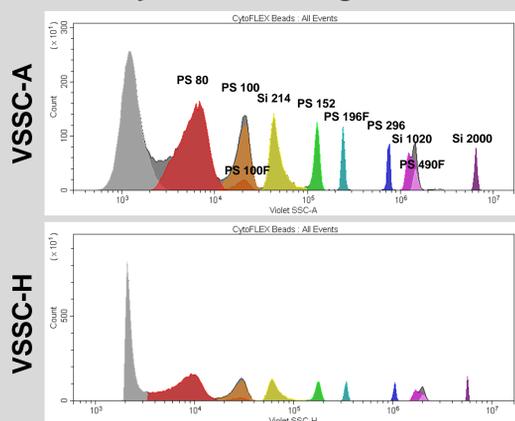
Item	Catalog #	Vendor
CytoFLEX Sizing Bead Mix	Custom	Beckman Coulter
ApogeeMix	1493	Apogee Flow Systems
HPLC Water	WX-0008-1	EMD Millipore
CytoFLEX Sheath	B51503	Beckman Coulter
4-Laser CytoFLEX N-V-B-R	B78557	Beckman Coulter
CytExpert Software v2.0	B49006	Beckman Coulter

Methods

1. The CytoFLEX probe was first cleansed using a standard cleanse panel: Bleach, Detergent, and 2x Water for 2 min each at 240 μL/min.
2. The detector configuration was changed to VSSC, with the 450 filter switched with the 405/10 VSSC filter.
3. The trigger channel was set to VSSC-H, and the threshold level was set to minimize the background noise, yet maintain a little noise for reference right at the threshold of detection.
4. Each bead set was aliquotted into separate 13x75mm tubes.
5. A water control tube was acquired first, followed by the Apogee Beads, and then the CytoFLEX beads.
6. Each tube was acquired for 30 seconds at 10μL/min, and the data were analyzed using CytExpert v2.0.

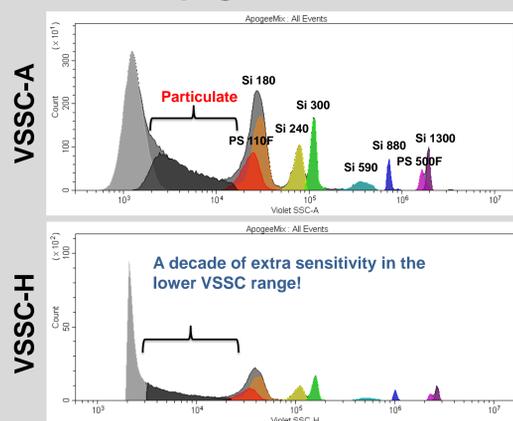
Results

Performance and Resolution by VSSC CytoFLEX Sizing Beads



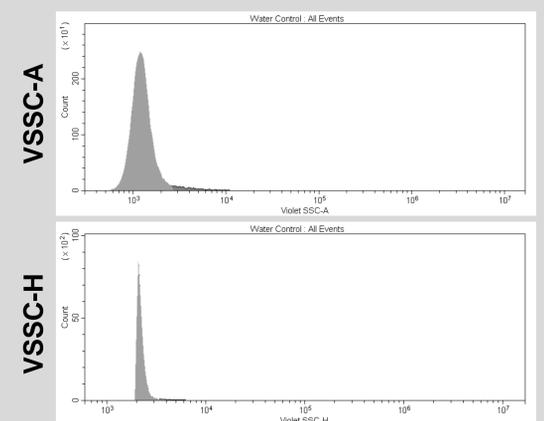
The CytoFLEX Sizing Beads are composed of a mixture of latex and silica NIST-traceable size standards, as well as fluorescent microparticles. They are evenly distributed and spread across the complete VSSC dynamic range for the CytoFLEX.

ApogeeMix Beads



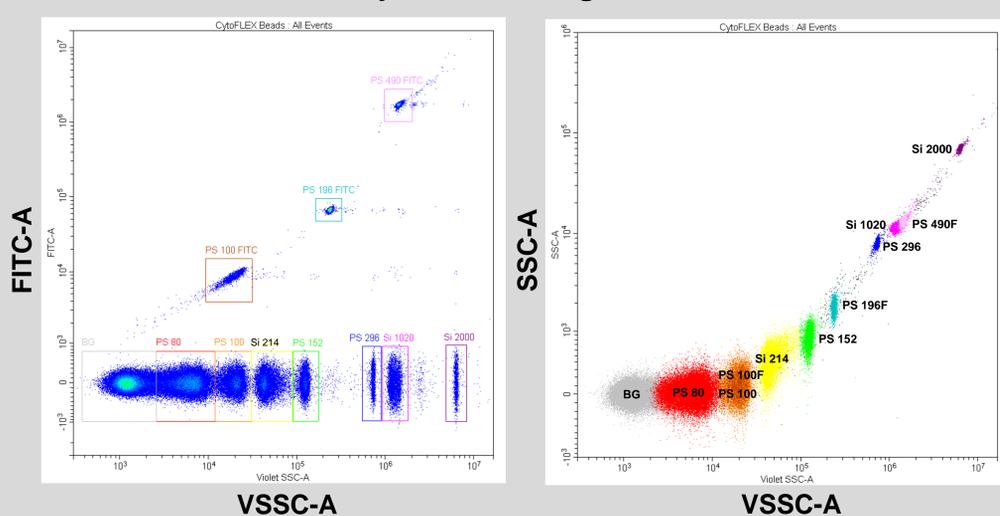
The ApogeeMix Beads are also composed of a mixture of latex and silica beads, including several fluorescent microparticles. These beads contains a significant amount of particulate in the lower size range, they are very unevenly distributed, and have a limited dynamic range.

HPLC Water

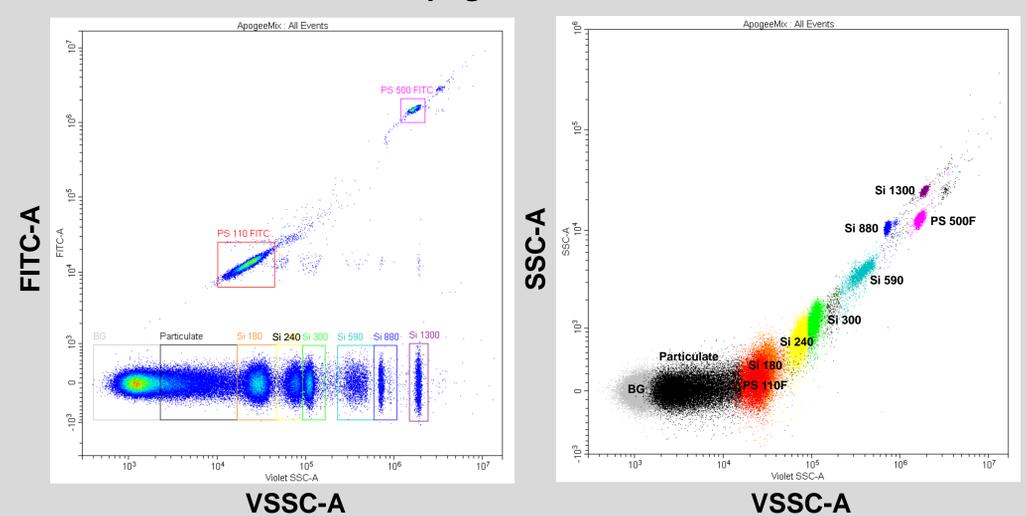


HPLC water was used as a control for instrument cleanliness and particulate contamination. As can be seen above, the instrument was clean and there was minimal background.

Bivariate Scatter and Fluorescence Plots CytoFLEX Sizing Beads



ApogeeMix Beads



Conclusions and Discussion

We found that the CytoFLEX was able to effectively detect and resolve all beads within the CytoFLEX Sizing Bead mix, with the 80 nm-latex beads perfectly resolved above the noise threshold. The ApogeeMix beads were noisier than the CytoFLEX beads, and the smallest bead (110 nm-latex) was located about a decade higher than the noise threshold of the CytoFLEX. Ultimately, since the CytoFLEX is currently one of the most sensitive flow cytometers on the market, the increased range of our CytoFLEX Sizing Beads proved to better address the size-standardization requirements of the CytoFLEX versus the ApogeeMix beads. These sizing beads can be used with any flow cytometer, allowing the user to extend their size standardization into the nanoparticle range (≤ 100 nm).

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