NanoView: A Novel Approach to Microparticle Cell Sorting

John Tigges[1], Angie Vandergaw[2], Vasilis Toxavidis[1]

Beth Israel Deaconess Medical Center[1], Propel Labs, Inc.[2]

www.bidmc.org/Research/CoreFacilities/FlowCytometryCore.aspx

INTRODUCTION

There is great interest in both medical and scientific communities in submicron cell-derived particles, termed microparticles or microvesicles. Although competing techniques have been developed, flow cytometry remains the dominant approach. The hurdle in analysis has always been the ability to accurately measure the size characteristics of small particles, especially when only considering scatter properties. Due to advances in microscopy and the ability to identify the existence of <1um cellular particles, flow cytometry instrumentation has been developed to have the ability to identify populations from 400nm to 1um. However, the accuracy of these measurements and the validity of the results are frequently questioned. Therefore, we propose a hardware upgrade that will not only improve accuracy, but will allow for validation of the procedure by recovery of the microparticles.

In this study, we present the results of our independent testing of the new Propel Labs' NanoView forward scatter detector (FSC) integrated onto a Beckman Coulter MoFlo XDP* cell sorter. The NanoView design has improved the optical and electrical systems over the standard FSC diode or PMT for the purpose of extending the detection range down to < 200nm particles. The new optical system design utilizes a custom aspheric imaging lens that has been optimized to collect the scattered light from the core stream and image it onto a pinhole. The collection angles in the FSC direction extend up to 18 degrees, which is double the maximum collection angle of a standard MoFlo FSC detector. The pinhole serves to align the system and remove the stray laser light that has not been generated by the particle of interest and greatly reduces the background light that is received at the detector. The NanoView design has further improved the detection system by replacing the photodiode with a much higher sensitivity PMT detector in the FSC path.

The table below shows that a 100nm particle is more than 1000X dimmer than a 1micron particle. However, by doubling the collection angles, you can **double** the collected light from the smallest particles.

	1.0 µm diameter	0.5 µm diameter	0.25 µm diameter	0.1 µm diameter
Total Intensity from 0 to 50 degrees	1	0.142	0.0179	0.000611
Fraction Intensity from 0 to 9 degrees	75%	46%	28%	22%
Fraction Intensity from 9 to 18 degrees	21%	31%	24%	20%

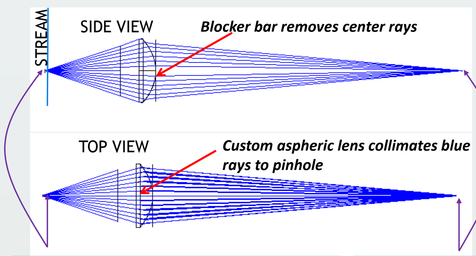
MATERIALS AND METHODS

Optical Design Goal for NanoView

Take the light that is within the 18 degree scatter angle cone of a small particle in a stream and focus it through a 200 micron pinhole in front of the PMT detector.

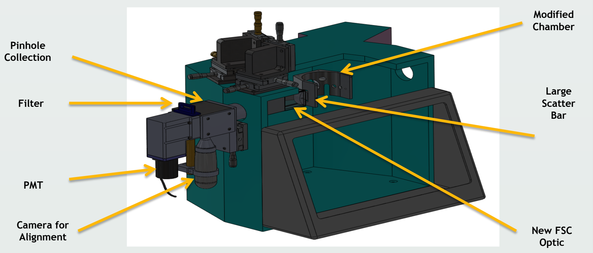
When the system is aligned to look only at the center of the core stream, the pinhole excludes scattered light from other nearby particles and reduces the noise substantially.

NanoView Optical Design and Hardware Design



The blue rays show from a particle the scattered light in the center of the core stream.

The blue rays converge to the 200 micron pinhole.



Gating and analysis.

We will need to construct a protocol to measure microparticles on the NanoView equipped MoFlo XDP cell sorter by first determining the FSC resolution. In order to do this, we will use the different sized-fluorescent beads, Bangs Laboratories Inc. Dragon Green Beads, to adjust FSC and SSC-related settings (voltage and threshold) to recover 192nm, 520 nm, and 780 nm beads. The 520nm and 780nm populations were sorted for purity verification and sizing accuracy.

192nm, 520nm, and 780nm beads are diluted with the PBS/0.1% Tween-20 solution, to a final concentration of 1.29*10^7 beads/ml.

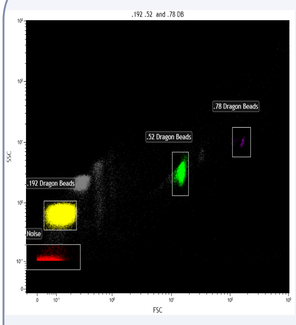
Secondarily, two other Size Range Kits were tested to verify NanoView's functionality. Polysciences Inc. Fluoresbrite Size range Kit I (catalog # 21636) 100nm, 200nm, 500nm, 750nm, and 1um beads; and Beckman Coulter's PCS Control Latex Beads Mixed Kit (PN 6602336) 50nm, 100nm, 300nm, and 500nm were acquired to check for uniformity along different size ranges and materials. Fluorescence was acquired on FL1 to verify bead populations.

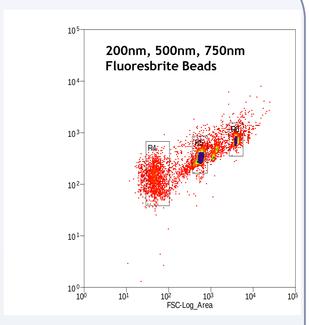
Microbeads were acquired at the following settings using a Coherent Sapphire 488-200 Laser at 100mW and FSC threshold of 0.01%:

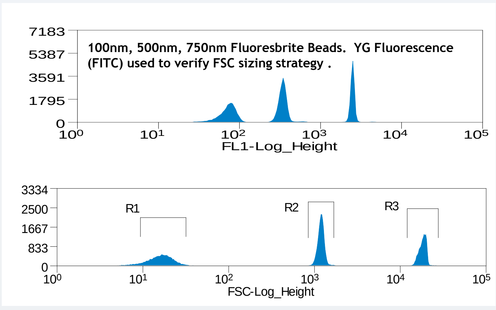
Drop Drive 15V approx. 94K Frequency (dependent on Intellisort II)

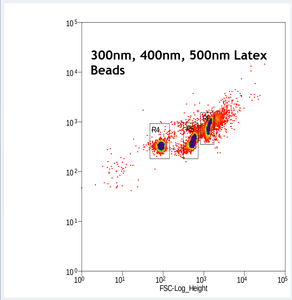
FSC 270V SSC 440V

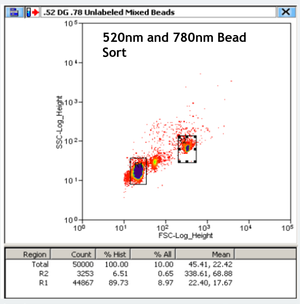
Results



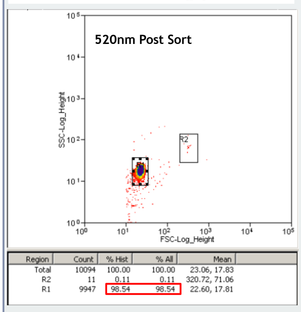




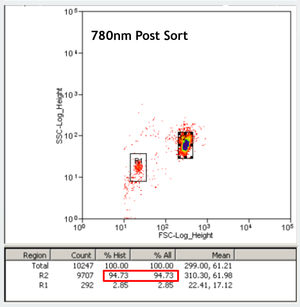




Region	Count	% Hist	% All	Mean
Total	50000	100.00	10.00	45.41, 22.42
R2	3253	6.51	0.65	338.61, 68.88
R1	44867	89.73	8.97	22.40, 17.67

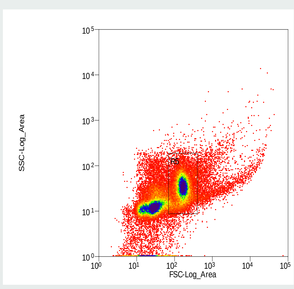


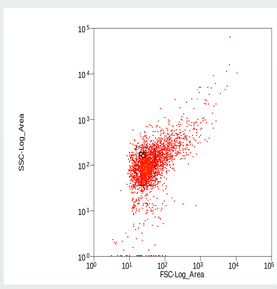
Region	Count	% Hist	% All	Mean
Total	10094	100.00	100.00	23.06, 17.83
R2	11	0.11	0.11	320.72, 71.06
R1	9943	98.54	98.54	22.60, 17.81



Region	Count	% Hist	% All	Mean
Total	10247	100.00	100.00	299.00, 61.21
R2	9707	94.73	94.73	310.30, 61.98
R1	292	2.85	2.85	22.41, 17.12

Liposome Analysis





Liposomes Sub500nm

50nm PCS Control

Conclusions

Many cells, including platelets, endothelial cells, leukocytes, and erythrocytes, shed fragments of their plasma membranes into the circulation. There is increasing evidence that these submicron fragments, termed microparticles, have important physiological roles. Although many techniques have been derived for the identification and characterization of microparticles, flow cytometry is still deemed to be the most accurate and reproducible. With the addition of the NanoView technology to a typical cell sorter, the ability to identify and recover microparticles (<200nm-1um) for further analysis has been simplified and techniques easier to validate. The NanoView sorting system allows for advanced applications with microparticles and will alleviate some of the roadblocks associated with this research area.

In this study, we have shown the NanoView's ability to distinguish a variety of submicron particles ranging from 50nm to 1um. In addition, the ability to sort and recover with high purity has been demonstrated.

Questions or comments: vtoxavid@bidmc.harvard.edu or jtigges@bidmc.harvard.edu.

Please visit www.propel-labs.com for more information.