Acoustic Focusing Cytometry: Sensitivity and Throughput

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Acoustic cytometry is a new technology that utilizes forces derived from acoustic radiation pressure to focus particles in flow cytometry. This technology exploits the physical differences between particles relative to the background medium, allowing cells to be tightly focused. The ability to focus cells without relying on hydrodynamic forces allows many possibilities outside the scope of conventional flow cytometry. The use of low flow rates are required for sensitive measurements with conventional hydrodynamic focusing, while high flow rates are reserved for less critical measurements. This has created a trade-off of sensitivity and throughput historically accepted with conventional flow cytometry. In contrast, acoustic focusing of particles separates the alignment of cells from a particular flow rate allowing for sensitivity at collection rates much higher than conventional cytometry.

In this study we examined changes in sensitivity with sample flow rate using acoustic focusing cytometry. We performed typical assays using the Applied Biosystems® Attune® Acoustic Focusing Cytometer, such as DNA content cell cycle analysis, immunophenotyping, apoptosis, intracellular antibody labeling, phagocytosis, white blood cell light scatter, and microshpere analysis. Data was collected at all seven collections rates (25µl/min-1000µl/min) and results compared across collection rates for percent positive, percent coefficient of variation, signal to background ratio and/or staining index.

In the DNA content cell cycle testing, conventional hydrodynamic focusing demonstrated a loss of focusing at higher sample rates resulting in higher %CVs and reduction in data quality. In comparison, consistent results and tighter %CVs were obtained across all flow rates using the Attune™ acoustic focusing cytometer. Distinct separation of positive and negative cell populations was demonstrated with the other assays, although a slight drop in staining index was noted at two flow rates. In this study we demonstrate that collection rates up to 1000µl/minute are possible without reduction in sensitivity when using acoustic focusing cytometry.

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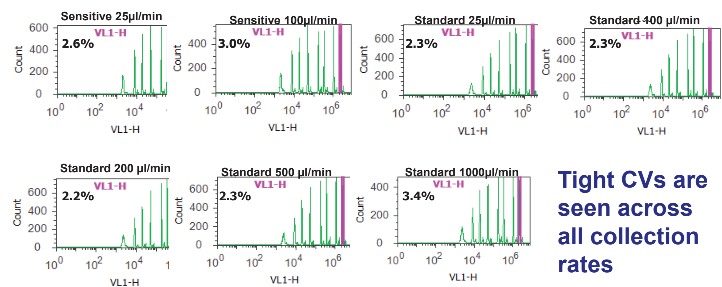
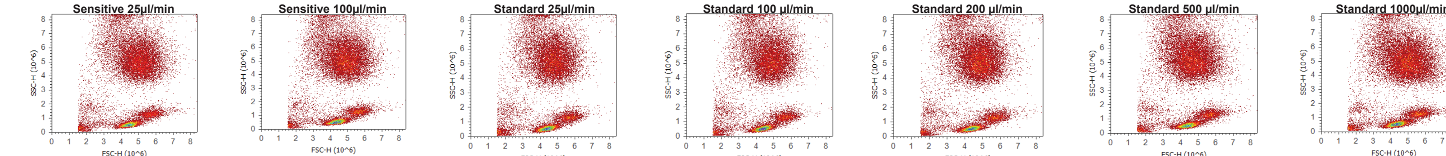
A. Fluorescent Microspheres, 8-peak



Tight CVs are seen across all collection rates

Eight peak fluorescent microspheres (Spherotech 8-peak Rainbow beads) were analyzed at all collection rates on the Attune® Acoustic Cytometer using one fluorescent channel with 405 nm excitation and 450/40 bandpass emission collection optics. Coefficients of variation (CV) for the brightest peak are reported and show little variation across collection rates.

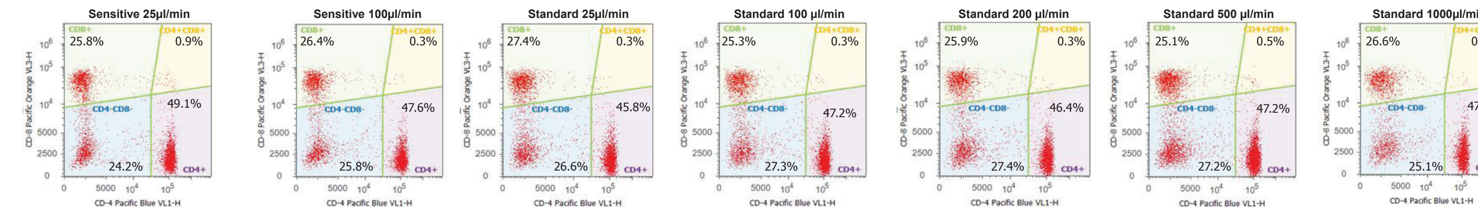
B. Lysed Whole Blood: Resolution of Cell Populations using 405 nm Forward and Side Light Scatter



Ammonium chloride was used with human whole blood to lyse RBCs, and Forward and Side Scatter using 405nm laser light was used to look at resolution of cell populations at all collection rates on the Attune® Acoustic Focusing Cytometer. A consistent pattern was observed at all collection rates.

Consistent scatter
Clear resolution of three blood populations (lymphocytes, monocytes, neutrophils) is possible without change in settings at all collection rates.

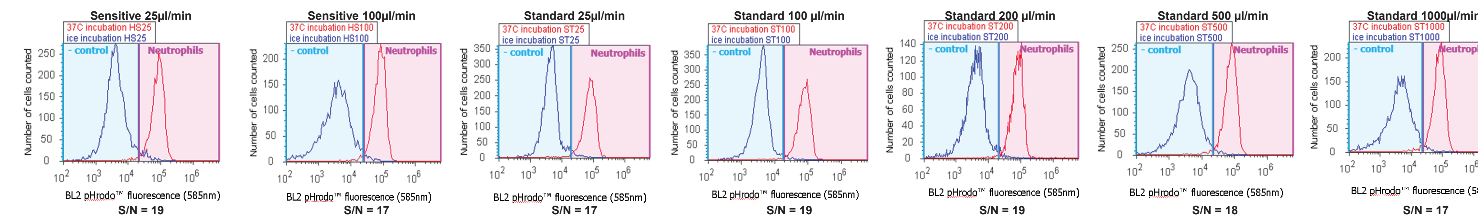
C. Immunophenotyping: CD-4 Pacific Blue™ and CD-8 Pacific Orange™ staining of human lymphocytes



Ammonium chloride was used with human whole blood to lyse RBCs and labeled with anti-human CD4-Pacific Blue™ and anti-human CD8-Pacific Orange™ antibodies. Samples were analyzed using the Attune® Acoustic Focusing Cytometer collecting 5,000 lymphocyte gated events. A dual parameter plot was analyzed for comparison of populations of CD4+CD8-, CD4-CD8+, CD4-CD8-, and CD4+CD8+ cell populations across all collection rates.

Consistent cell populations identified by immunophenotyping over all collection rates.

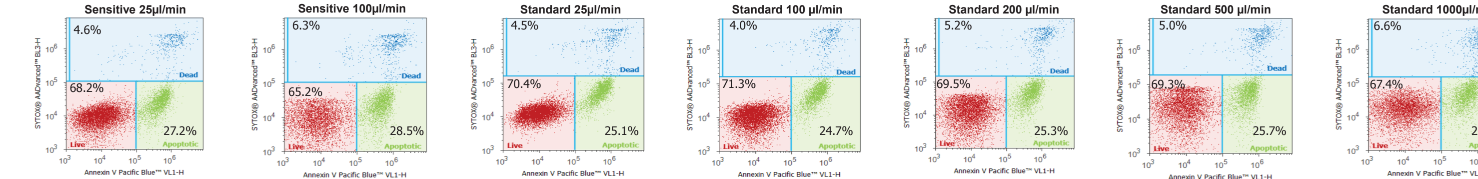
D. Phagocytosis: pHrodo™ *E.coli* BioParticles® Phagocytosis Kit for Flow Cytometry



Whole blood was processed using the pHrodo™ *E.coli* BioParticles® Phagocytosis Kit for Flow Cytometry. Blood was treated with pHrodo™BioParticles® and either incubated on ice or at 37°C for 15 minutes. Following RBC lysis and wash, samples were analyzed on the Attune® Acoustic Focusing Cytometer at all collection rates. The granulocyte population was gated and analyzed for fluorescence. Phagocytosing neutrophils were identified as the population of cells with increased fluorescence upon incubation at 37°C due to phagocytosis of *E.coli* BioParticles® into the acidic phagosome. Phagocytosis was inhibited by incubating other samples on ice. The median fluorescence of the positive population was divided by the median fluorescence of the negative population to obtain a Signal/ Noise (S/N) ratio, which was consistent at all collection rates.

Consistent staining ratio (S/N) over all collection rates.

E. Apoptosis: Annexin V, Pacific Blue™ conjugate and SYTOX® AADvanced™ dead cell stain

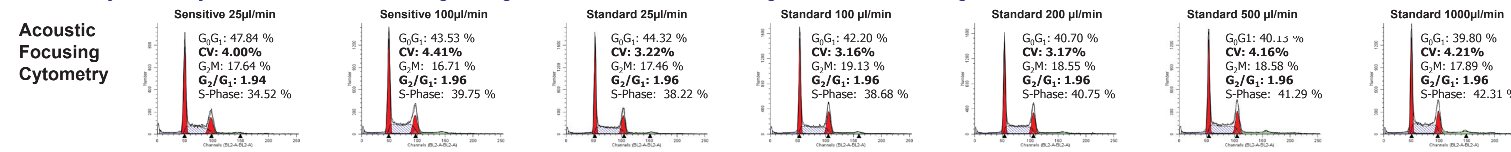


Jurkat cells were treated with 10µM camptothecin for 4 hours at 37°C to induce apoptosis, prior to labeling with Annexin V Pacific Blue™ conjugate and SYTOX® AADvanced™ dead cell stain. Samples were analyzed on the Attune® Acoustic Focusing Cytometer at all collection rates. Live, Apoptotic, and Dead cells were identified and percent of each population was compared across collection rates.

Consistent cell populations identified across all collection rates.

F. Cell Cycle Analysis: No loss of Focusing at Higher Collection Rates Using Acoustic Focusing

Acoustic Focusing Cytometry

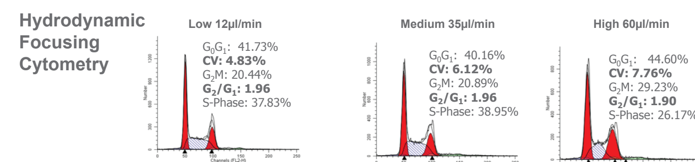


Acoustic Focusing: lower CV & consistent results across collection rates:

Acoustic CV:

3.17% → 4.21% Across all collection rates

Hydrodynamic Focusing Cytometry



Ethanol-fixed Jurkat cells were stained with propidium iodide + RNase. Samples were analyzed on the Attune® Acoustic Focusing Cytometer and on a conventional cytometer, collecting 20,000 total events. Singlet cells were used for analysis of cell cycle using ModFit LT modeling software (Verity House) for unbiased data interpretation.

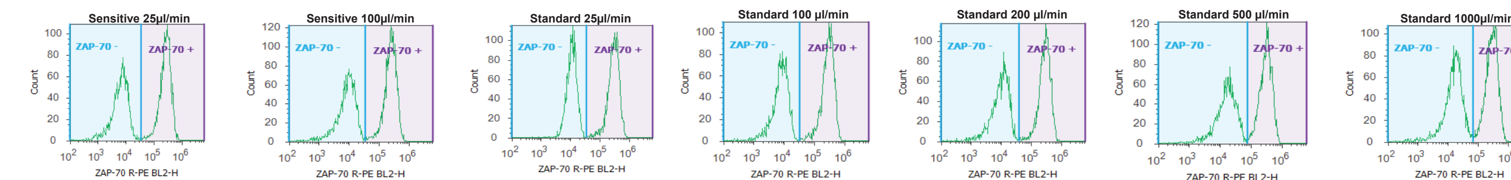
Hydrodynamic Focusing causes a loss of focus at higher flow rates

Conventional CV:

4.83% → 6.12% → 7.76%

low → medium → high flow rate

G. Intracellular Staining: ZAP-70



Fixed and permeabilized lymphocytes from human whole blood were stained for the intracellular antigen ZAP-70. Sample were labeled with surface CD3-Pacific Blue™ antibody and intracellular ZAP-70-rPE antibody, and data was collected across all collection rates on the Attune® Acoustic Cytometer. CD3-negative lymphocytes were identified for ZAP-70 expression. Median fluorescence of positive and negative ZAP-70 cells were used to determine signal:background (S/B) ratio.

Clear distinction of positive and negative populations