



Boundary-breaking acoustic focusing cytometry

Introducing the Attune[®] NxT Acoustic Focusing Cytometer—
a high-performance system that's flexible enough for any lab

life
technologies

A Thermo Fisher Scientific Brand

“One of the main projects in my laboratory is focused on the description and functional analysis of an emerging innate lymphoid cell type. These cells are extremely rare both in blood and in tissues. For flow cytometry analysis we use the Attune[®] Acoustic Focusing Cytometer. The high sample rates of the Attune[®] cytometer allow me to reduce centrifugation steps so that we retain more cells and more rapidly detect rare events. We could not have performed these studies with any other instrument.”

Prof. David Cousins
University of Leicester

Rapid, accurate detection of rare events

The Attune® NxT Acoustic Focusing Cytometer is a benchtop analyzer that uses a revolutionary technology, acoustic focusing, to align cells prior to interrogation with a laser for your multicolor flow cytometry analysis. The system offers:

- Unique modular design—one-day field upgradable system with configurations from 1–4 lasers, with the ability to detect up to 14 colors
- Fast detection speed—short acquisition time without loss of data quality enables unique applications such as rare event detection and use of no-wash/no-lyse techniques
- Distinctive acquisition and analysis software—intuitive and powerful for users of all experience levels
- Convenient size—complete setup fits on even small benchtop space

Multicolor analysis in a modular design

With the option to be configured with up to 4 lasers and 14 colors for multiparameter analysis (Figure 1), the Attune® NxT Acoustic Focusing Cytometer was designed as a modular system to fit most experimental design needs and lab budgets. The Attune® NxT cytometer can be designed to accommodate the most common fluorophores and fluorescent proteins used in flow cytometry to match the panels you are currently running (Table 1). Multiple fluorescent proteins can be detected with an optional 561 nm laser (Figure 2). Whether you configure your system now or upgrade later, the Attune® NxT Acoustic Focusing Cytometer can grow with you and your research needs.

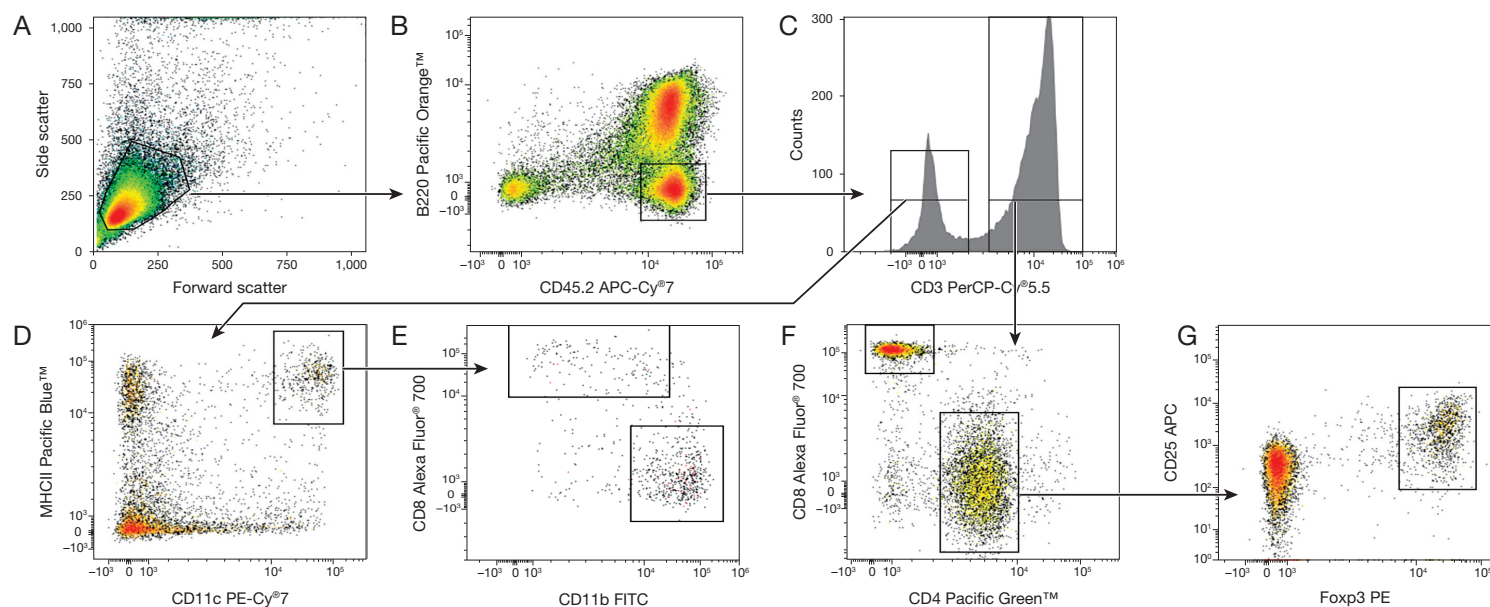


Figure 1. Multiparameter (10-color) analysis of murine regulatory T cells and dendritic cells with the Attune® NxT Acoustic Focusing Cytometer. Lymphocytes were gated using FSC/SSC parameters (A, left) and B220-expressing B cells were omitted from subsequent analysis (A, middle). Within the B220⁺, CD45.2⁺ gate, T cells were analyzed based on their expression of CD3 (A, right). CD3⁺ T cells were separated into two populations based on expression of the co-receptors CD4 or CD8 (B, left). Within the CD4⁺ T cells there is a subpopulation of suppressive regulatory T cells that express the transcription factor Foxp3 and the cell surface marker CD25 (IL-2Ra) (B, right). CD3⁺ cells were separated to show a rare population of CD11c⁺ MHCII⁺, professional antigen-presenting dendritic cells (C, left). Splenic dendritic cells can be subdivided further into CD11b⁺ and CD8⁺ dendritic cell subsets (C, right), each possessing unique antigen presentation properties.

Superior reliability and maximum signal efficiency

The novel design of the optical path helps ensure precise fixed alignment of four spatially separated lasers onto the sample stream enabling consistency in data over time, superior performance, and superior reliability. The instrument can be configured with up to 4 solid-state lasers (405 nm, 488 nm, 561 nm, and 637 nm) with flat top beam profiles to minimize the effects of changes in fluidics or optics, which cause instability and alignment issues leading to instrument downtime. The light emitted from cells in the flow cell is transported with high efficiency to the detection optics through fiber-optic cables with minimal loss of signal. The filters in front of the photomultiplier tubes collect light signals and can be easily interchanged and customized to minimize reagent crosstalk and maximize signals (Figure 3).

What is acoustic focusing?

The Attune® NxT Acoustic Focusing Cytometer uses ultrasonic waves (over 2 MHz, similar to those used in

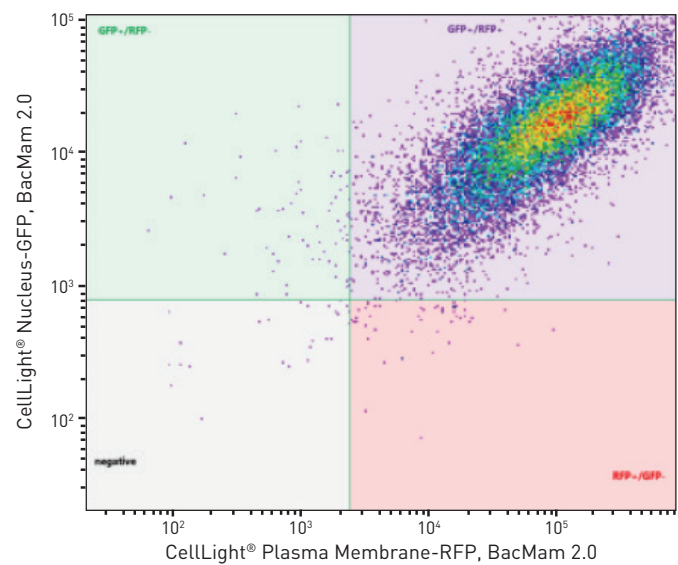


Figure 2. Live-cell fluorescent protein detection. U2OS cells were simultaneously transduced with Celllight® Nucleus-GFP (Cat. No. C10602) and CellLight® Plasma Membrane-RFP (Cat. No. C10608). Cells were analyzed on the Attune® NxT Acoustic Focusing Cytometer using a 488 nm laser and 530/30 emission filter along with a 561 nm laser and 585/16 nm emission filter.

Table 1. Attune® NxT Acoustic Focusing Cytometer fluorophore selection guide.

Laser	Excitation	Emission	Common dye	Fluorescent protein
Violet	405 nm	440/50	Pacific Blue™	ECFP
		512/25	Pacific Green™	
		603/48	Pacific Orange™	
		710/50	Qdot® 705	
Blue	488 nm	488/10	FSC	
		488/10	SSC***	
		530/30	FITC	EGFP, Emerald GFP
		574/26*	Propidium iodide	EYFP
		590/40**	Propidium iodide Fluorescent proteins	EYFP
		695/40	PerCP-Cy®5.5	
		780/60	PE-Cy®7, Qdot® 800	
Yellow	561 nm	585/16	PE	RFP
		620/15	PE-Texas Red®	mCherry, dTomato, DsRed, mStrawberry
		695/40	PE-Cy®5.5	
		780/60	PE-Cy®7	
Red	637 nm	670/14	APC	
		720/30	Alexa Fluor® 700	
		780/60	APC-Alexa Fluor® 750	

*Without a yellow laser present
**With a yellow laser present
***Side scatter can be detected by any laser line (default is blue laser). Violet side scatter is recommended for no-wash/no-lyse applications.

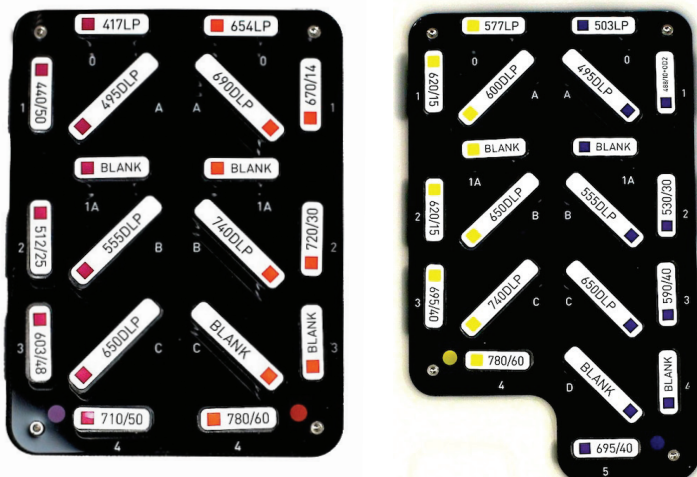


Figure 3. Example filter configuration for the Attune® NxT Acoustic Focusing Cytometer. Filters are easily interchanged to create customized configurations to meet your experimental needs.

medical imaging), rather than hydrodynamic forces, to position cells into a single, focused line along the central axis of a capillary (Figure 4). Acoustic focusing is largely independent of the sample input rate, enabling cells to be tightly focused at the point of laser interrogation regardless of the sample-to-sheath ratio (Figure 5). This, in turn, allows the collection of more photons for high-precision analysis at superior volumetric sample throughput. The Attune® NxT cytometer accomplishes this without high-velocity or high-volumetric sheath fluid, which can damage cells. In addition, volumetric syringe pumps enable absolute cell counting without beads—minimizing cost and sample preparation time. In contrast, cytometers that use hydrodynamic focusing maintain the same sample speed at all flow rates, causing cells to lose focus as the sample core widens to increase flow rate.

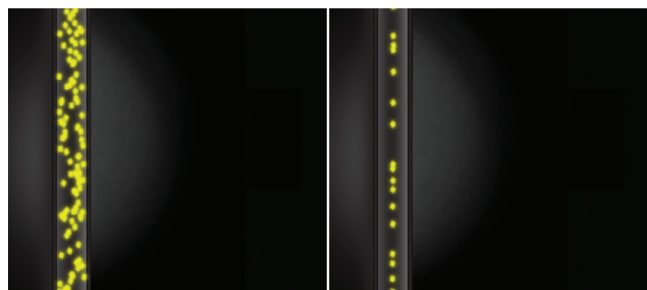
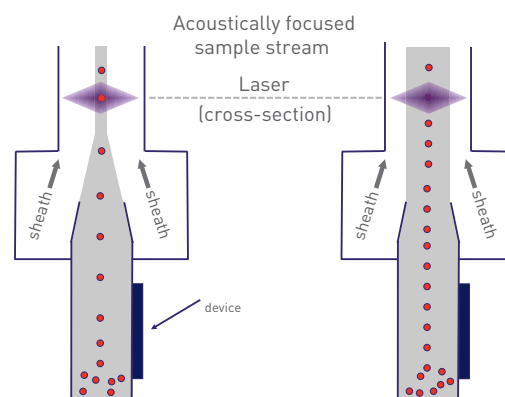


Figure 4. Acoustic focusing in action. Fluorescent microspheres were applied to the capillary system of an acoustic focusing cytometer. Beads flow through randomly without any acoustic focusing (left). With the acoustic focusing, the beads are focused into a single line (right).

A. Acoustic focusing = better precision



B. Traditional hydrodynamic focusing: compromised data quality

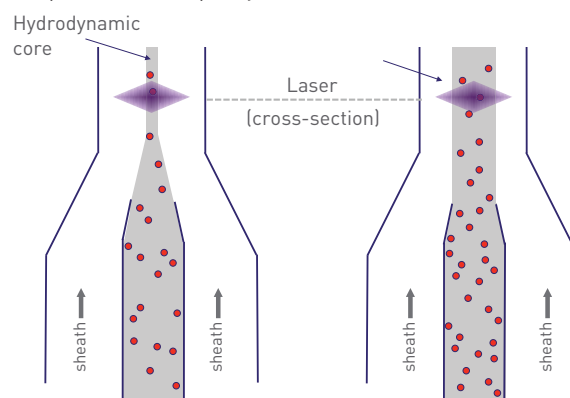


Figure 5. Acoustic focusing vs. traditional hydrodynamic focusing as particles pass through the laser. (A) In acoustic focusing, cells remain in tight alignment even at higher sample rates, resulting in less signal variation and improved data quality. (B) In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream, resulting in increased signal variation and compromised data quality.

Dilute your samples, not your data quality

Washing and lysis of red blood cells (RBCs) can cause significant cell loss and damage. Significantly higher sample collection rates allow the Attune® NxT cytometer to deliver a no-wash/no-lyse protocol to minimize cell loss and simplify sample preparation (Figure 6). This feature is particularly useful for samples that are inherently low in concentration. Dilute samples like cerebrospinal fluid (CSF), stem cells, and any sample with low cell numbers can take a long time to acquire. With the Attune® NxT cytometer, even dilute samples can be acquired quickly and without compromising data. Difficult-to-collect samples like

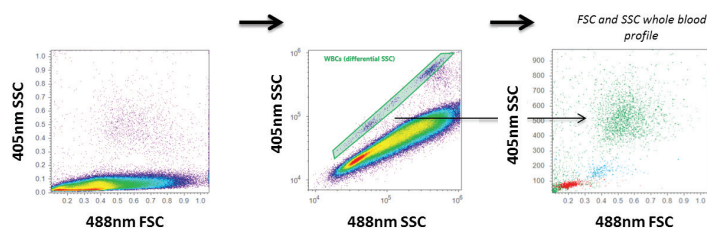


Figure 6. Eliminate sample preparation without compromising data quality. Five microliters of whole blood was stained with antibodies, incubated, and diluted using 4 mL of buffer and analyzed on the Attune® NxT cytometer. 405 nm light is readily absorbed by red blood cells and enables use of the differential side scatter gating shown in the middle panel in which a 405 nm vs. 488 nm side scatter dual parameter plot is used to differentiate the leukocyte and red blood cell populations. From this gated population, a daughter plot of forward and side scatter can be used to identify lymphocyte, monocyte, and granulocyte populations without the need for a fluorescent label.

mouse blood and bone marrow, thin-needle aspirates, or any sample with low cell yield can be stained and then diluted without washing or performing RBC lysis. High sample collection rates make acquisition possible—you can run up to 4 mL in just 4 minutes. Sample loss is minimized in sample preparation, and full panel testing is possible for all precious samples.

Rapid detection of rare events

Analysis of rare cell populations requires the collection of high numbers of events in order to attain a reliable measure of accuracy, leading to long acquisition times. The Attune® NxT Acoustic Focusing Cytometer achieves sample throughput at rates over 10 times faster than other cytometers—up to 1,000 $\mu\text{L}/\text{min}$ and 2×10^7 events per run, enabling rapid detection of rare events with reliable accuracy and without aborting data (Figure 7).

Precision and sensitivity at all sample rates

The Attune® NxT Acoustic Focusing Cytometer enables high sensitivity when you need it most. You will be able to maintain precise alignment, even at high sample rates of up to 1,000 $\mu\text{L}/\text{min}$. The precise alignment provided by acoustic focusing enables researchers to obtain tighter CVs to better distinguish between dim signals and background, resulting in less variation and better signal separation (Figure 8).

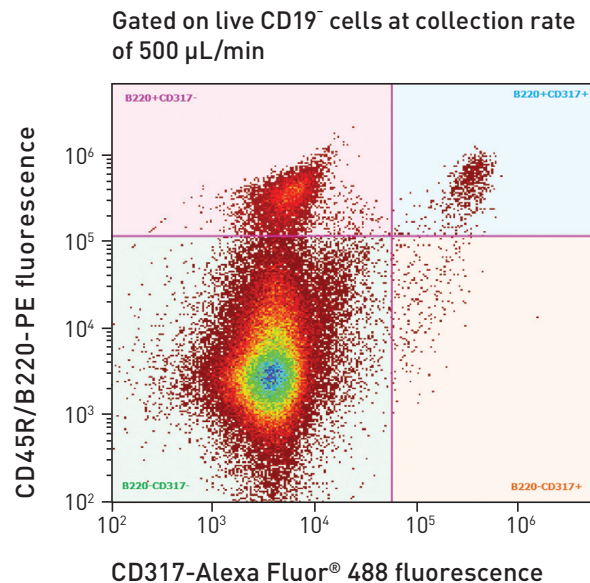


Figure 7. Collection of more than 1 million live cells and detection of a rare population (0.2%) of dendritic cells. Plasmacytoid dendritic cells (pDCs) are a specialized cell population that produces large amounts of type I interferons in response to viruses and are identified using the immunophenotype $\text{CD}19^-/\text{B}220^{\text{high}}/\text{CD}317^+$. Four-color staining of mouse splenocytes included $\text{CD}19$ -Pacific Blue™, $\text{CD}317$ -Alexa Fluor® 488, $\text{CD}45\text{R}/\text{B}220$ -PE direct conjugates, and SYTOX® AADvanced™ Dead Cell Stain. A gate was made on live cells using SYTOX® AADvanced™ Dead Cell Stain, followed by gating on $\text{CD}19^-$ cells. A two-parameter plot of $\text{CD}45\text{R}/\text{B}220$ vs. $\text{CD}317$ was used to identify pDCs. A collection rate of 500 $\mu\text{L}/\text{min}$ was used to acquire 1.3 million total cells with a cell concentration of 7.5×10^7 cells/mL. Plasmacytoid dendritic cells were identified as dual $\text{B}220^+/\text{CD}317^+$ (upper right quadrant) and constitute 0.851% of live $\text{CD}19^-$ cells, which is 0.194% of total splenocytes.

Minimal data variation

Cell cycle analysis is just one example of where it is critical to precisely detect differences in fluorescence intensity between multiple cell populations. With the Attune® NxT Acoustic Focusing Cytometer, minimal variation in results is seen regardless of sample throughput rate. You no longer need to sacrifice throughput for sensitivity (Figure 9).

Software that performs to your specifications

Attune® NxT software is designed to provide powerful data acquisition and analysis using an intuitive, user-friendly interface (Figure 10). Experiments can easily be set up, customized, and saved for future studies. Compensation is automated and can be set up using a guide.

The software is designed to maximize efficiency in performing data analysis, with fast refresh rates for large data sets (up to 20 million events per sample) with the ability to immediately visualize changes on data plots as you make adjustments. The software has unique tools to simplify experimental setup, including reagent selection using the filter configuration manager. This provides guidance for matching the right reagent to the optimized channel on the instrument by selecting reagents from a drop-down menu of prepopulated or customized reagents, which is then applied to plot labels.

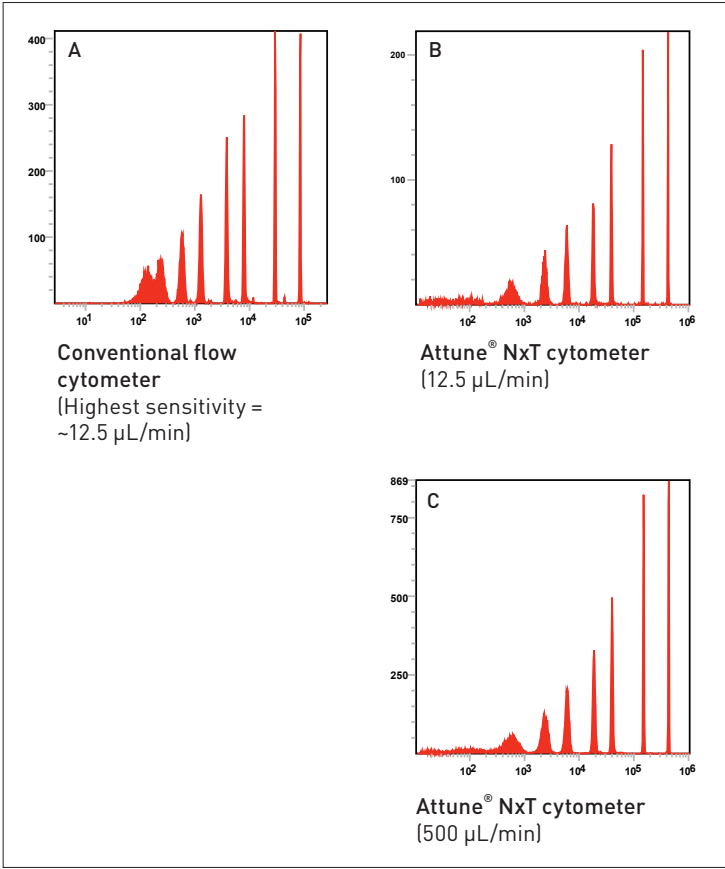
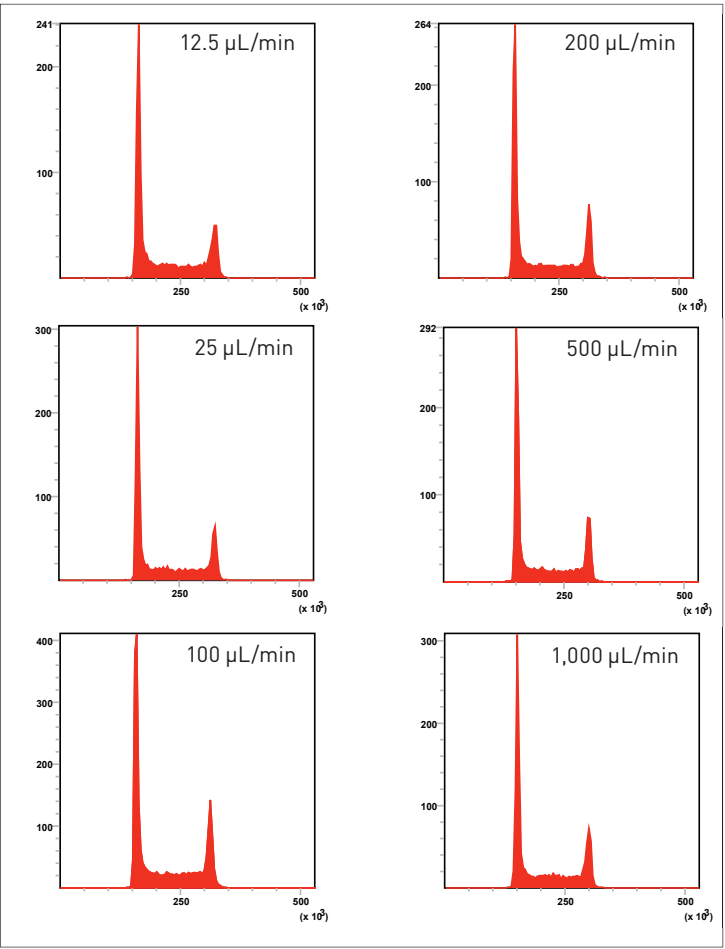


Figure 8. Sensitivity measurements across flow rates. Fluorescent microspheres [Spherotech Rainbow Calibration Particles, 3.2 µm] were run on **(A)** a high-end conventional flow cytometer and **(B, C)** on the Attune® NxT Acoustic Focusing Cytometer using a 561 nm laser and **(A)** 610/20 or **(B, C)** 610/15 emission filters. The conventional cytometer was run using the highest sensitivity setting (~12.5 µL/min). The Attune® NxT cytometer was run at **(B)** 12.5 µL/min, which is equivalent to the traditional flow cytometer and at **(C)** 500 µL/min, which is 40x more sample. The Attune® NxT cytometer showed equal or better results even at the highest flow rates.



Sample rate (µL/min)	12.5	25	100	200	500	1,000
G ₁ CV%	2.99	3.03	2.76	2.94	2.70	2.96
G ₂ /G ₁	1.99	1.99	1.99	2.05	2.05	2.03

Figure 9. Minimal data variation at high sample rates with the Attune® NxT Acoustic Focusing Cytometer. Jurkat cells were fixed and stained with propidium iodide, treated with RNase, and analyzed at a concentration of 1 x 10⁶ cells/mL on the Attune® NxT Acoustic Focusing Cytometer at different sample rates. The left peak in all graphs reflects cells in G₀/G₁ phase, while the right peak reflects cells in G₂/M phase. Regardless of sample rate, the width of the G₀/G₁ and G₂/M peaks and CV% remains consistent for the Attune® NxT cytometer, even at the highest sample rate of 1,000 µL/min.

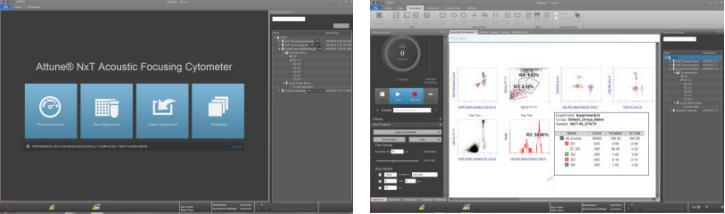


Figure 10. The Attune® NxT software interface.

Service and support

The Attune[®] NxT Acoustic Focusing Cytometer is backed by our worldwide technical support and service programs. We are focused on delivering personalized service from the time our sales representative walks through the door throughout the life of your Attune[®] NxT instrument. The Attune[®] NxT cytometer is fully supported for one year with our extensive service plan, which includes:

- Comprehensive training (2 users per instrument)
- Application and assay support
- Worldwide technical service
- Preventive maintenance

Ordering information

Attune [®] NxT Acoustic Focusing Cytometer			
Lasers	Laser color	Parameters	Cat. No.
4	Blue, red, yellow, violet	16	A24858
3	Blue, violet, yellow	13	A24859
3	Blue, red, violet	13	A24860
2	Blue, yellow	9	A24861
2	Blue, violet	10	A24862
2	Blue, red	9	A24863
1	Blue	6	A24864

For more information,
go to lifetechnologies.com/attune



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