



discover innovation
without compromise

Introducing the

Attune® Acoustic Focusing Cytometer

A break in tradition. A **breakthrough** in flow cytometry technology.

The Attune® Acoustic Focusing Cytometer utilizes sound waves to align cells in a sample stream prior to passing through a laser (Figure 1). Cell alignment is maintained in the precise center of the sample stream even as the sample stream widens with an increased rate of sample introduction. In addition, cells remain within the optimal focal point of the laser beam regardless of the rate at which they pass (Figure 2A).

- Cells remain in the focal plane regardless of sample rate
- Time that cells spend in front of the laser can be optimized
- Cell sample acquisition rates are over 10 times faster than those of traditional cytometers

In contrast, to align cells prior to their passing through the laser, traditional flow cytometry utilizes the hydrodynamic focusing created by the differential in flow rates of the outer sheath fluid and the inner core of the sample stream. As the sample introduction rate increases, the sample core stream widens and cells become less focused. When the sample introduction rate is increased, the probability of cells passing through the optimal focal point of the laser beam decreases, resulting in increased variability of signal (Figure 2B).

How will acoustic focusing impact your results?

Maintaining cells in tight alignment in the laser focal plane regardless of sample introduction rate can lead to significant improvement in data quality by:

- Decreasing cell-to-cell variation (lower CV)
- Improving rare-event detection
- Allowing the collection of more events in a single run
- Increasing sensitivity to dim signals

Key Reference:

Ward M, Turner P, DeJohn M, and Kaduchak G. Fundamentals of Acoustic Cytometry. *Current Protocols in Cytometry*, 1.22.1–1.22.12, July 2009.

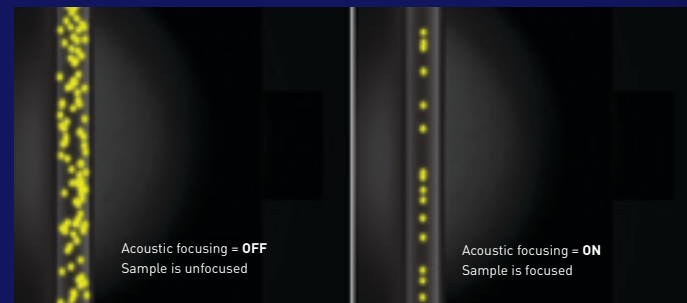


Figure 1. Acoustic focusing in action. Fluorescent microspheres were applied to the capillary system of an acoustic focusing cytometer. Beads flow through randomly without any acoustic focusing (left). With the application of acoustic focusing, the beads are focused into a single line (right).

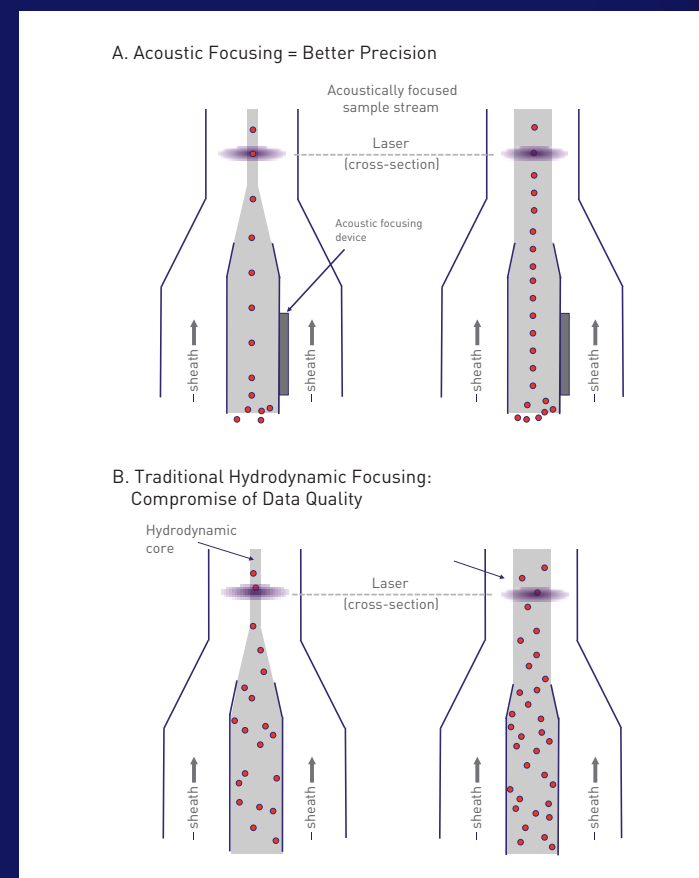


Figure 2. Acoustic focusing vs. traditional hydrodynamic focusing. (A) In acoustic focusing, cells remain in tight alignment even at higher sample rates. With this tight alignment, cells pass through the laser beam at its optimal focal point, resulting in less signal variation and improved data quality. (B) In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream. The speed at which cells pass through the laser is not changed, and is determined by the speed of the sheath fluid flow. Cells are distributed throughout the sample core stream because of reduced differential pressure between sample stream and sheath stream, resulting in reduced cell focusing. Cells are not in tight alignment as they pass through the laser beam, resulting in increased signal variation and compromised data quality.

Attune® Acoustic Focusing Cytometer

Designed for Performance

- Higher sample introduction rates without impacting sensitivity
- Better clarity through precise data, amazing peak separation
- Absolute cell counts
- Powerful analytical software
- Minimal bench space requirements

Enabling Better Results

We know that your samples for flow cytometry are a valuable investment of resources and time. At the end of the day, you want to get the best data possible from your flow cytometer. The Attune® Acoustic Focusing Cytometer uses the new acoustic forces to precisely align cells within the instrument, resulting in less signal variability and better data clarity. By uncoupling cell alignment from hydrodynamic forces and sheath flow, the Attune® Acoustic Focusing Cytometer can also run samples at rates around 10 times greater than other cytometers with minimal fluid consumption. Combining the latest advances in acoustic focusing technology with powerful analytical software gives you complete control of your results.



Results You Can Trust: Cell Cycle Analysis

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® Acoustic Focusing Cytometer, minimal variation in results is seen regardless of sample throughput rate. You no longer need to sacrifice throughput for sensitivity (Figure 3).

- Minimal variation even at high sample rates (Figures 3 and 4)
- Less variability in results (see changes in %S phase from Table 1)
- No sacrifice of sensitivity for speed

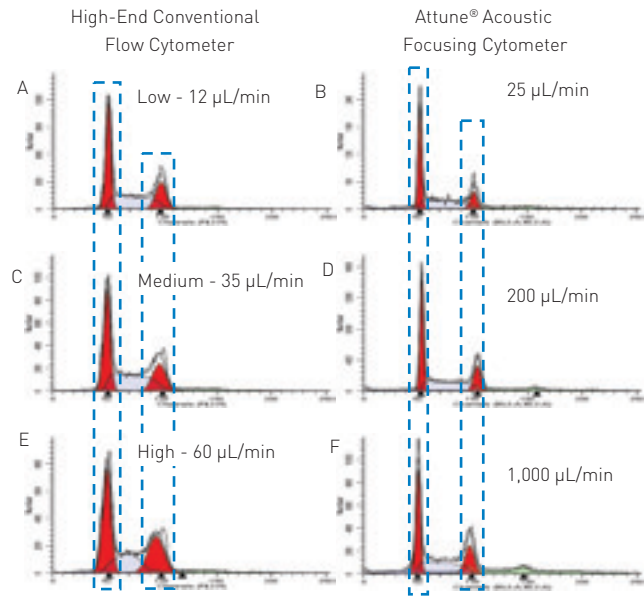


Figure 3. Minimal data variation at high sample rates with the Attune® Acoustic Focusing Cytometer. Jurkat cells were fixed and stained with propidium iodide, treated with RNase, and analyzed at a concentration of 1×10^4 cells/mL on a high-end instrument that uses hydrodynamic focusing, and on the Attune® Acoustic Focusing Cytometer at different sample rates. The left peak in all graphs reflects cells in G_0/G_1 phase, while the right peak reflects cells in G_2/M phase. Note that as sample rates increase on the instrument that uses hydrodynamic focusing, the width of the G_0/G_1 and G_2/M peaks increase, whereas for the Attune® cytometer the peaks are relatively stable, even at the highest sample rate of 1,000 $\mu\text{L}/\text{min}$. These results are quantitated in Table 1.

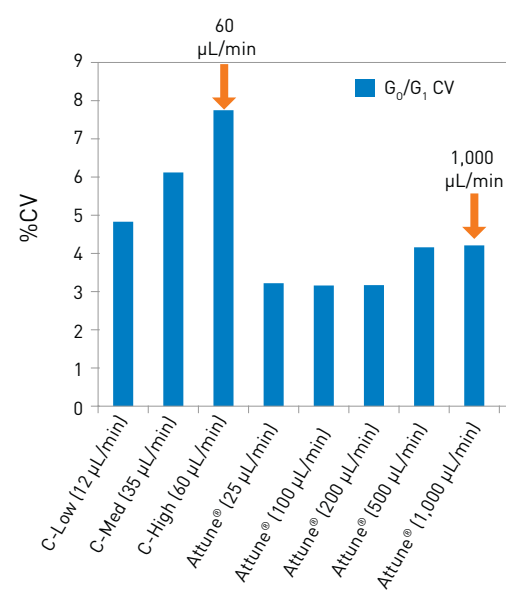


Figure 4. Percent CV of G_0/G_1 at different flow rates on the Attune® cytometer and a competitor (C) at different sample rates. Note the minimal change in variability (%CV) for the Attune® Acoustic Focusing Cytometer, even at a high sample rate.

Table 1. Cell cycle analysis on the Attune® Acoustic Focusing Cytometer vs. a high-end conventional cytometer.

	Sample Rate	% G_0/G_1	%S*	% G_2/M	G_2/G_1 Ratio	%CV
High-End Conventional Cytometer	12 $\mu\text{L}/\text{min}$	41.73	38.73	20.44	1.96	4.83
	35 $\mu\text{L}/\text{min}$	40.16	38.95	20.89	1.96	6.12
	60 $\mu\text{L}/\text{min}$	44.60	26.17	29.23	1.90	7.76
Attune® Acoustic Focusing Cytometer	25 $\mu\text{L}/\text{min}$	45.20	40.29	14.51	1.94	3.22
	100 $\mu\text{L}/\text{min}$	42.81	38.72	18.74	1.94	3.17
	1,000 $\mu\text{L}/\text{min}$	40.25	38.55	21.20	1.94	4.21

*Note the disparity of results in the percentage of cells in S phase, with the conventional cytometer. Such disparity can lead to inaccurate assessment of cell cycle data.

Fast and Faster: Rare-Event Detection

Analysis of rare cell populations (stem cells, minimal residual disease, tetramers, and NKT cells, to name a few) requires the collection of high numbers of events to attain a reliable measure of accuracy, leading to long acquisition times. The Attune® Acoustic Focusing Cytometer allows rapid detection of rare events at rates that are 10 or more times faster than with a traditional cytometer (Table 2). In addition, the Attune® Acoustic Focusing Cytometer is designed to collect up to 20,000,000 events per run, allowing researchers to detect those rarest of rare events.

- Acquire sample over 10 times faster than with conventional cytometers (Table 2)
- Absolute counting capability—no more expensive counting beads (Figure 5)
- Acquire up to 20,000,000 events per sample data file

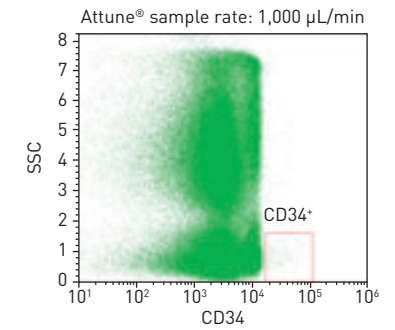


Figure 5. Identification of $CD34^+$ cells from peripheral blood. Peripheral blood from a normal donor was stained and run on the Attune® Acoustic Focusing Cytometer at a flow rate of 1,000 $\mu\text{L}/\text{min}$ with a stop gate set at 500,000 total cells. Two hundred and fifty $CD34^+$ cells (red box) were identified within the population of cells after 4 minutes and 28 seconds of analysis.

Table 2. Rare-event detection and cell counting at highest instrument sample introduction rates.

Instrument	Sample Flow Rate ($\mu\text{L}/\text{min}$)	Acquisition Time	Percentage of $CD34^+$ Cells	$CD34^+$ Cells/ μL , Direct Measurement
Attune® Acoustic Focusing Cytometer	1,000	4 min, 28 sec	0.05%	0.05
High-end conventional cytometer	60	13 min, 46 sec	0.02%	N/A

Sensitive, Accurate Results: Cell Proliferation by Dye Dilution

Successful proliferation analysis by dye dilution requires sensitive instrumentation and an extremely bright dye to accurately distinguish fluorescently labeled cells from autofluorescence after several cell divisions. The combination of the Attune® Acoustic Focusing Cytometer and Molecular Probes® CellTrace™ Violet dye allows the identification of up to 10 population doublings following stimulation. Better yet, since CellTrace™ Violet emission is collected off the violet laser of the Attune® cytometer, it is fully compatible with Green Fluorescent Protein (GFP)–expressing cells for further multiplexing capabilities.

- Resolve population peaks that are close together (Figures 6A and 6B)
- Simplify multiplexing with GFP cells (Figure 6C)

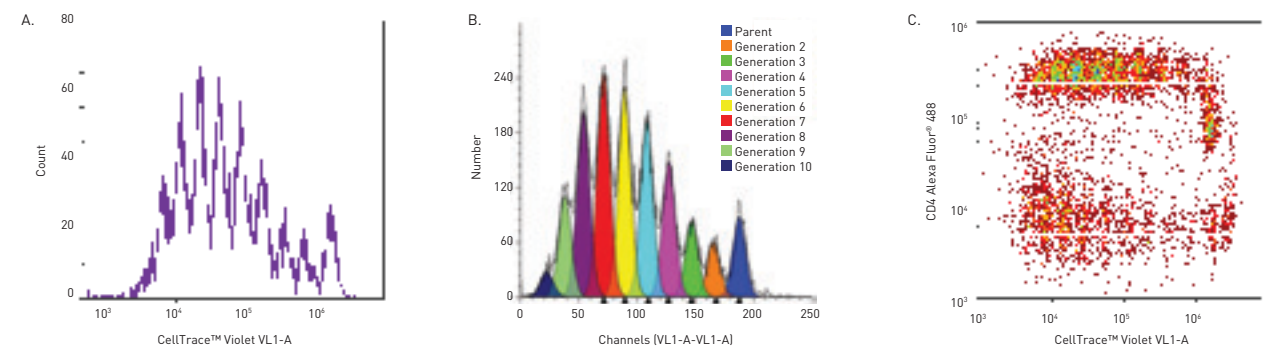


Figure 6. Ten cell divisions identified with the Attune® Acoustic Focusing Cytometer and the Molecular Probes® CellTrace™ Violet Cell Proliferation Assay. Human peripheral blood mononuclear cells were isolated from whole blood, stained with CellTrace™ Violet (Invitrogen Cat. no. C34557), and stimulated to proliferate in culture. Cells were stained with mouse anti-human $CD4$ Alexa Fluor® 488 prior to analysis on the Attune® Acoustic Focusing Cytometer at a flow rate of 25 $\mu\text{L}/\text{min}$. (A) Histogram of fluorescence intensity with each peak representing one subsequent generation of proliferating cells. (B) To provide statistics about each generation of cells in a population, the fluorescence histogram was further analyzed with proliferation modeling software (ModFit LT™, Verity Software House). The location of each generation of cells is represented by a unique peak color. (C) Two-dimensional plot allowing the simultaneous analysis of cell proliferation between $CD4^+$ and $CD4^-$ cell populations.

Reliable, Unambiguous Results: Immunophenotyping

The Attune® Acoustic Focusing Cytometer shows excellent segregation of populations in immunophenotyping experiments (with up to 6 colors) (Figure 7).

- Six-color detection with minimal compensation
- Strong signal separation for more data clarity
- Less need for difficult tandem dyes

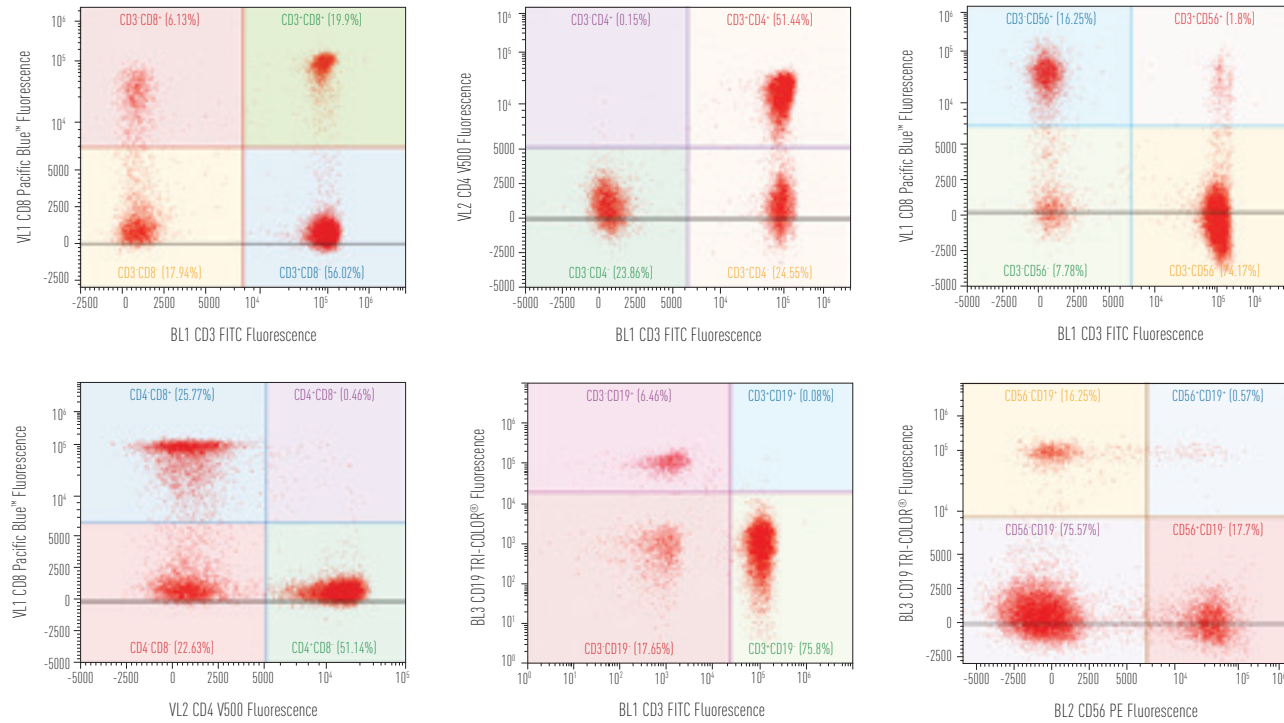


Figure 7. Six-color immunophenotyping analysis on the Attune® Acoustic Focusing Cytometer. Normal human blood cells were labeled for six-color immunophenotyping with the following directly labeled mouse anti-human antibody conjugates: CD45-Pacific Orange™, CD3-FITC, CD8-Pacific Blue™, CD56-R-PE, CD19-TRI-COLOR® (all from Invitrogen), and CD4-V500 (BD Biosciences). Gating was performed on CD45-positive lymphocytes to generate these bivariate plots.

Dim Signals? No Problem: ZAP-70 Staining

Whether analyzing routine or difficult-to-detect, low-abundance antigens like ZAP-70 (Figure 8), the Attune® Acoustic Focusing Cytometer will give you high-quality results right on your benchtop.

- Clear resolution of difficult-to-detect signals
- Simplified detection of intracellular antigens
- Facilitated cell signaling studies

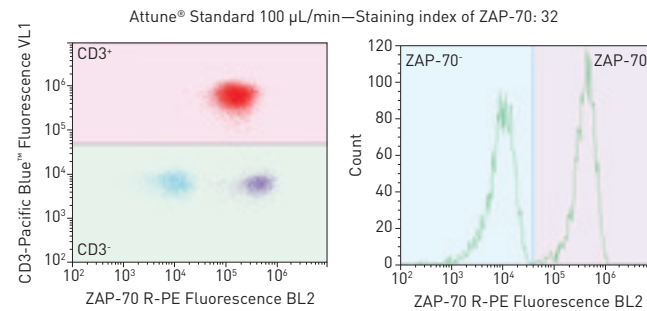


Figure 8. Identification of ZAP-70 positive cells with the Attune® Acoustic Focusing Cytometer. CD3-z associated protein (ZAP-70) is normally expressed primarily in T cells and natural killer (NK) cells. Dual parameter analysis (gated on lymphocytes) was performed for CD3 and ZAP-70 at collection rate of 100 µL/min. A lymphocyte gate was used on the forward and side scatter dual plot to define the cells that are illustrated in the CD3 vs. ZAP-70 dual parameter plot. A region was made on the CD3-negative cells to gate for the ZAP-70 histogram. Markers placed on the ZAP-70 negative and positive populations were used to calculate the staining index.

More Choices, More Power to Multiplex

The Attune® Acoustic Focusing Cytometer comes equipped with 405 nm (50 mW) and 488 nm (20 mW) lasers and three emission channels per laser. This configuration allows up to six-color analysis in addition to forward and side scatter data collection and absolute cell counts without the need for counting beads. Combined with the power of Molecular Probes® fluorescence detection technologies (Table 3), the Attune® Acoustic Focusing Cytometer is ideal for the study of many cell analysis protocols, including:

- Cell cycle
- Rare-event detection
- Cell proliferation
- Immunophenotyping
- Intracellular staining
- Multiplexing with GFP-expressing cells
- Cellular signaling
- Apoptosis
- Phagocytosis

Table 3. Fluorophore selection guide for the Attune® Acoustic Focusing Cytometer with standard filter set.

		Default Filter (nm)	Filter Range (nm)	Fluorophores	Other Fluorescent Dyes	
Excitation Laser	Violet (405 nm)	Blue	450/40	430–470	Pacific Blue™ Alexa Fluor® 405	PO-PRO™-1
						DyeCycle™ Violet
		Green	522/31	507–537	Qdot® 525 Horizon™ V500	Fixable Violet Dead Cell Stain
						CellTrace™ Violet
		Orange	603/48	579–627	Pacific Orange™ Qdot® 605	Calcein Violet
						SYTOX® Blue
	Blue (488 nm)	Green	530/30	515–545	Alexa Fluor® 488 Fluorescein	FxCycle™ Violet
						Calcein
		Orange	575/24	563–587	PE	Fluo-3/Fluo-4
						TO-PRO™-1
		Red	640LP	>640	PE-Alexa Fluor® 610 PerCP PE-Alexa Fluor® 700 PE-Cy® 5.5 TRI-COLOR® PerCP-Cy® 5.5 PE-Cy® 7 PE-Alexa Fluor® 750	GFP
						JC-1/DiOC ₂ (3)
					Fixable Aqua Dead Cell Stain	
					Fixable Yellow Dead Cell Stain	
					Fixable Green Dead Cell Stain	
					PI	
					Fura Red™	
					DyeCycle™ Orange	
					JC-1/DiOC ₂ (3)	
					pHrodo™	
					SNARF® (low pH)	
					PI	
					JC-1/DiOC ₂ (3)	
					Fixable Red Dead Cell Stain	
					7-AAD	
					SNARF® (high pH)	
					SYTOX® AADvanced™	
					DyeCycle™ Ruby	

Attune® Cytometric Software

Attune® Cytometric Software is designed to provide powerful user-defined analysis using an intuitive interface for simplified experimental analysis (Figure 9). Templates can be built around specific applications and saved for consistent experimental design. Compensation is automated or user-defined, and can be set up using a compensation guide. Utilities such as quick-save, drag-and-drop, and copy-and-paste provide rapid manipulation with commonly used functions. Experiments can be easily set up with automated settings that can be completely customized and saved for future experiments.

No Software Licensing Fees

The Attune® Cytometric Software can be downloaded without licensing fees. The software can be added to any desktop or laptop computer at your institution without any additional costs. Results can easily be analyzed at your convenience at your own computer, allowing the next user to run experiments on the Attune® cytometer.

Instrument Controls

- Instrument status
- Control sample flow rate
- Select sensitivity level
- Run startup/shutdown protocol
- System performance tracking by date

Visualization Tools

- Plot overlay or side-by-side graph comparison
- Standard or custom gate shapes
- Histograms, dot plots, density plots
- Customize statistics
- Statistics in Microsoft® Excel® format
- Copy and paste plots into reports (Microsoft® Word or PowerPoint®)

Analysis Setup

- Save protocol templates
- Automated or user-defined compensation
- Raw data capture
- Data display in Linear, Log, or Lin/Log scales
- Set universal compensation across multiple data sets

File Format Compatibility

- Save data and images in PDF format for reports
- Data format in standard FCS 3.0 for compatibility with third-party analysis tools (i.e., FlowJo, ModFit LT™, FCS Express)



Instrument Performance Tracking

The Attune® Cytometric Software provides automated baseline calculation and performance test functions with minimal user interactions. Performance tracking is facilitated through the reports function in the software.

Instrument performance tracking is critical for collecting and analyzing accurate experimental data. The Attune® Performance Tracking Beads are designed for use with the Attune® Cytometric Software to automatically characterize, track, and report performance measurements of the Attune® Acoustic Focusing Cytometer (Figure 10). The beads are used to define a baseline and conduct daily measurements of the cytometer. Vials contain enough beads for 50 measurements, and specific information for each lot is downloaded to the Attune® Cytometric Software prior to use.

Performance tracking for the Attune® cytometer includes a comprehensive set of procedures to monitor the daily performance of the instrument. The performance tracking process involves:

- Running performance tracking beads
- Monitoring changes in the coefficient of variation and in the associated PMT voltage
- Tracking linearity of instrument performance
- Evaluating the detector sensitivity and background over time
- Automatic setting of laser delay

Instrument performance is affected by the lasers, optics, and fluidics of the instrument. For optimal instrument performance, it is critical to follow the manufacturer's recommended schedules for maintenance.

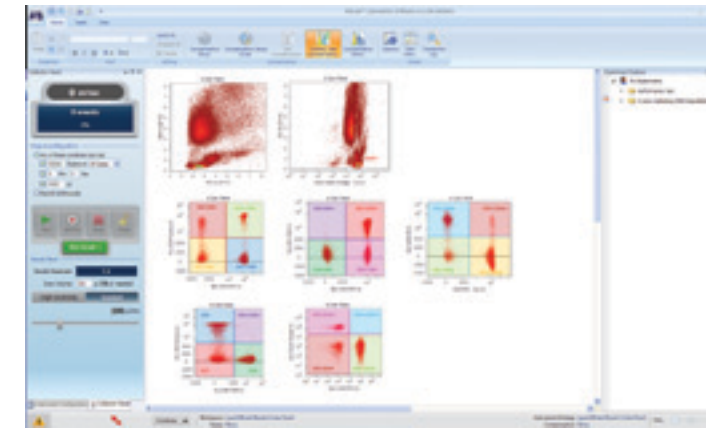


Figure 9. Example of Attune® Cytometric Software. The instrument control panel on the left side of the screen is used for experiment setup and control of laser selection, optics, and fluidics (flow rates). The main frame of this screen shows data analysis plots, while the right panel shows sub-file organization.



Figure 10. Example of Levey-Jennings plot showing Attune® cytometer performance over time. This report is generated using data from performance tracking beads.

$$F = -\nabla \left\{ \frac{4}{3} \pi a^3 \left[\beta_0 \frac{\langle p^2 \rangle}{2} f_1 - \frac{3}{2} \left(\frac{\rho_0 \langle v^2 \rangle}{2} \right) f_2 \right] \right\}$$

Attune® Acoustic Focusing Cytometer FAQs

How does this instrument differ from other flow cytometers?

The Attune® Acoustic Focusing Cytometer differs from traditional flow cytometers in that it uses acoustic forces (sound waves) to focus cells into the center of the sample stream rather than using hydrodynamic forces from a surrounding sheath stream. What does this mean for the user? The speed at which cells transit the interrogation chamber (laser path) can be slowed down in high-sensitivity mode, providing excellent sensitivity to dim signals. In addition, because the cells are focused without dilution into larger-volume sheath fluid, the sample can be run at up to 1,000 µL/minute, nearly 10 times faster than most other cytometers. Thus, the user can dilute precious samples to conserve their consumption.

What applications are supported by the Attune® cytometer?

Most standard applications for flow cytometry have been tested on the Attune® cytometer, including live/dead cell discrimination, cell cycle analysis, cell proliferation assays, basic phenotyping (up to 6 colors), rare-event detection, and detection of phosphoproteins. Many standard cell types have been tested, including Jurkat cells, HL60 promyoblast cells, Ramos B cells, U266 myeloma cells, mouse splenocytes, HeLa human cervical carcinoma cells, bovine pulmonary artery epithelial (BPAE) cells, 3T3 mouse embryo fibroblast cells, CHO (Chinese hamster ovary) cells, and human whole blood cells. Cells up to 100 µm in diameter have been run without clogging, and the minimum particle size reliably detected is 1 µm.

What is Attune® Focusing Fluid?

Attune® Focusing Fluid is a buffered, azide-free support/carrier reagent optimized for transporting particles through the Attune® Acoustic Focusing Cytometer. The focusing fluid provides the balance of total flow solution through the interrogation chamber and aids in maintaining system cleanliness. The solution contains a surfactant to help clean the fluid lines and to minimize bubble formation.

How much Attune® focusing solution will I consume?

Because it uses sound waves instead of sheath fluid to align cells, the Attune® Acoustic Focusing Cytometer will generally use less than 1 L of focusing solution after 8 hours of continuous operation (depending on acquisition conditions).

Can the results obtained from the Attune® cytometer be analyzed with other software? What format can be used to export data?

Yes. The file format is FCS 3.0, which is compatible with most standard analytical software. Data can be exported in tab-delimited, Microsoft® Excel®, or comma-delimited format.

Can the software be installed on other computers within my institution?

Since the applicable software license allows the installation of the software on any any desktop or laptop computer at your institution, the Attune® Cytometric Software can be installed on any computer at no additional cost, enabling a broad network for analysis.

What is the maximum number of events that can be recorded per sample run?

Attune® Cytometric Software is enabled to record up to 20 million events per sample run, which gives researchers performing rare-event analysis a powerful tool to achieve significant numbers and high confidence in the data.

How is the Attune® Cytometric Software distributed, and how are upgrades supported?

Attune® Cytometric Software is included with your purchase and can be downloaded at www.appliedbiosystems.com/attunesoftware. Upgrades of major versions that significantly improve features and performance may require purchase.

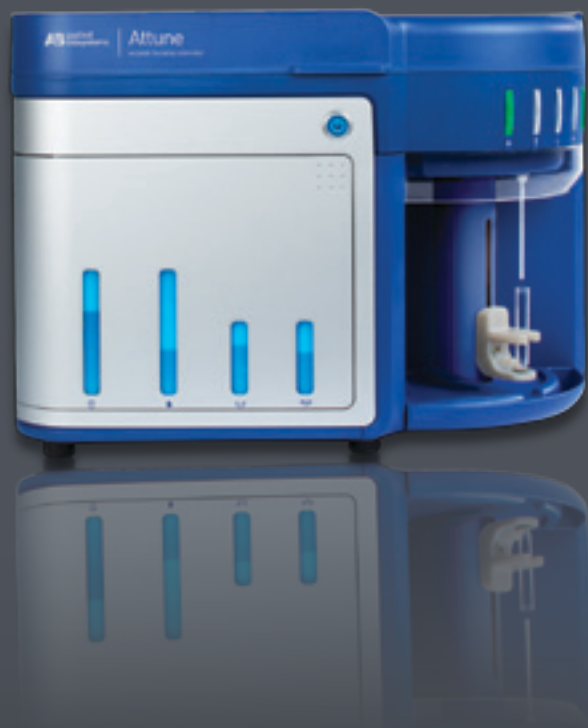
What service and support are available with the Attune® cytometer?

The Attune® Acoustic Focusing Cytometer is backed by Applied Biosystems' well-established worldwide technical support and service programs. The Attune® cytometer is fully supported for one year with our extensive service plan, which includes:

- Comprehensive training
- Application and assay support
- Worldwide technical service

Extended service plans are also available.

For more information detailing available service plans, visit www.appliedbiosystems.com/attunecytometer.



System Attributes

Physical Characteristics

Footprint (H x W x D): 16 in/40 cm x 23 in/58 cm x 17 in/43 cm

Weight: 64 lb/29 kg

Operating temperature: 15–30°C

Operating humidity: <80% noncondensing

Electrical requirements: 100–240 VAC, 50/60 Hz, <300 W

Optics

Excitation lasers: 405 nm (50 mW) and 488 nm (20 mW)

Fixed laser alignment

Standard Emission Filters

BL1: 530/30 nm

BL2: 574/26 nm

BL3: 640 nm LP

VL1: 450/50 nm

VL2: 522/31 nm

VL3: 603/48 nm

Fluidics

Sample rates: 25–1,000 µL/min

Calibrated delivery volumes for volumetric analysis

Standard and sensitive laser transit times

Sample loading volume: 100 µL–4 mL per run

Fluid storage within instrument with level sensing

Nominal fluid consumption of 1 L per day

Designed to accommodate 17 mm x 100 mm to 8.8 mm x 45 mm tubes

Computer

Minitower Windows® XP dual-processor, with 24-inch flat-panel monitor

4 GB RAM

1 Tb disk space

Keyboard, mouse, mouse pad included

Optional second monitor

Electronics

Data acquisition of up to 20,000 events/sec

Resolution of at least 6 decades

User-adjustable PMT voltage

Adobe® Flash® upgradeable firmware

Performance

Height, area, and width measurements on all scatter and fluorescent channels

Sensitivity: Mean equivalent soluble fluorescence

FITC <100, PE <50

Service

Supported by Applied Biosystems service with 48- to 72-hour response, depending on region

1-year warranty with purchase of new instrument

AB Assurance is our premium repair plan designed to maximize instrument performance and ensure availability of critical systems. AB Assurance will help keep your laboratory running smoothly with preventive maintenance and fast response should one of your instruments require repair. This plan is ideal for laboratories that depend on their instruments and need to reduce the risk of production downtime.

Key Features:

- Planned maintenance visit(s)
- Priority phone and email access to instrument technical support
- Phone and email access to application technical support

User Interaction with Instrument

Visual display of system status on instrument

Instrument startup and shutdown ≤15 min

User-changeable, keyed filters

Fully automated compensation or manual mode

Regulatory

Research Use Only; CE, UL, RoHS compliant

Attune® Cytometric Software

Software controls acquisition, analysis (width, height, and area measurements), and instrument

Output file format: FCS 3.0

Live gating with automatic saving

No licensing fees

User and administrator log-in

Capable of collecting 2 x 10⁷ events per sample

Automated and manual compensation

Less Hazardous Waste

The Attune® Acoustic Focusing Cytometer offers a “greener” solution because it uses very low sheath fluid volumes, thereby generating significantly less biohazardous waste. Competitors' instruments will generate up to 20 L of biohazardous waste per day, whereas the Attune® cytometer will generate less than 1 L of biohazardous waste per day.

Less Hazardous

The Attune® Acoustic Focusing Cytometer was engineered to meet the European Union's Restriction of Hazardous Substances (RoHS) Directive (Directive 2002/95/EC), eliminating lead, cadmium, mercury, hexavalent chromium, polybrominated biphenyls (PBBs), and polybrominated diphenyl ethers (PBDEs).

The Attune® Acoustic Focusing Cytometer Fits Any Lab

Size

- Small footprint—fits on all standard lab benches
- Aseptic work—fits into standard tissue culture hoods

Fluidics

- Low sheath use makes Attune® instrument a “greener” solution
- Fluidics onboard—no need for a separate fluidics cart

Optics

- Change-it-yourself optical filters
- Filter holder is conveniently located under the lid
- Violet and blue laser combination, giving you a choice of six colors for analysis

Best of all, the Attune® cytometer is affordable enough for most labs to own.

Ordering Information

Description	Quantity	Part Number
Attune® Acoustic Focusing Cytometer (includes computer, monitor, startup solutions, installation and warranty)	1	4445315
Attune® Focusing Fluids, 1X solution, 1 L	1	4449790
Attune® Focusing Fluids, 10X solution, 1 L	1	4449792
Attune® Focusing Fluids, 1X solution, 6 x 1 L	1	4449791
Attune® Wash Solution	1	4449755
Attune® 10X Shutdown Solution	1	4454955
Attune® Performance Tracking Beads (5 x 10 ⁶ beads/mL)	1	4449754

Life Technologies offers a breadth of products DNA | RNA | protein | cell culture | instruments

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