applied biosystems™
by life technologies™

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 the sample stream prior to passing through the laser (Figure 1). Cell alignment is maintained in the precise center of the sample stream even as the sample stream widens with increased rate of sample introduction. In addition, cells remain within the optimal focal point of the laser beam regardless of the rate through which they pass (Figure 2A).

- Cells remain in the focal plane regardless of sample rate
- Time cells spend in front of the laser can be optimized
- Cell sample acquisition rates are over 10 times faster than 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 increases, the sample core stream widens and cells become less focused. When sample introduction rates are 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 my results?

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

- Decreasing cell-to-cell variation (reduced CVs)
- Improving rare event detection
- Allowing the collection of more events in a single run
- Increasing sensitivity of 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 randomly through without any acoustic focusing (left). With the application of acoustic focusing, the beads are focused into a single line (right).

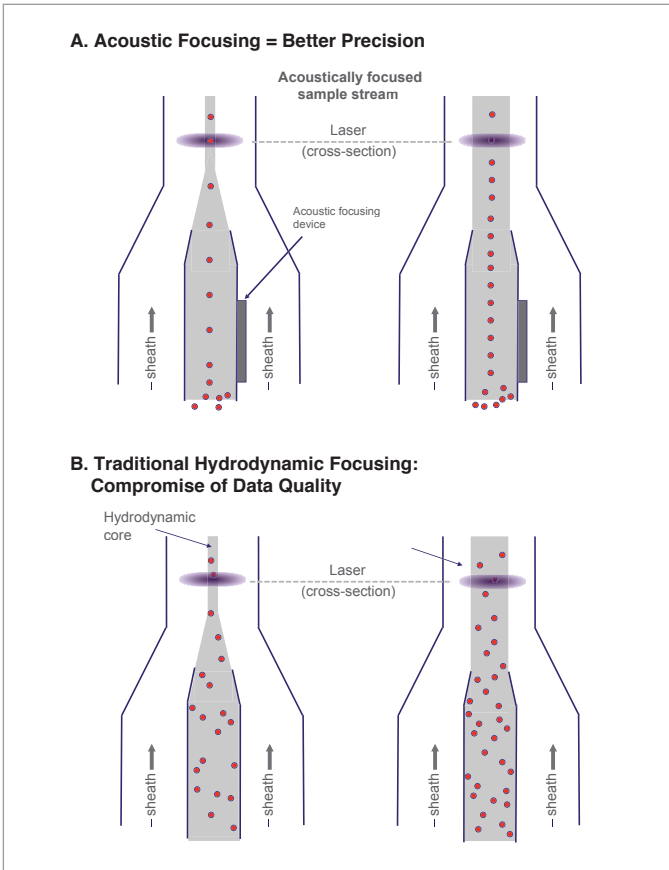


Figure 2. Acoustic focusing vs. traditional hydrodynamic focusing. (**A**) With acoustic focusing, cells remain in tight alignment even at higher sample rates. With this tight alignment, cells pass through the laser beam at its optimal focal point, resulting in less signal variation and improved data quality. (**B**) In traditional hydrodynamic focusing, increasing the number of events collected over a given period of time requires the widening of the sample core stream. The speed at which cells pass through the laser is determined by the speed of the sheath fluid, which is much greater than the sample stream. Because a high sheath flow speed is required for focusing the sample stream, the speed of the cells cannot be slowed. 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 spatial 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

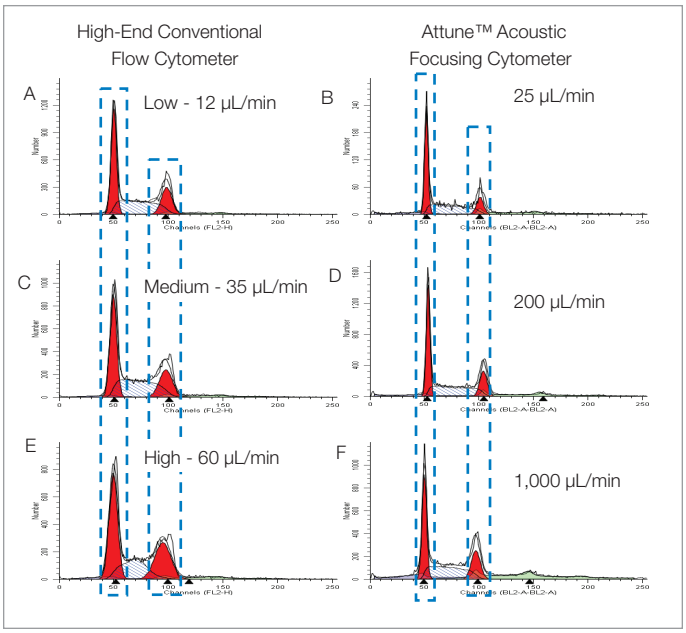


Figure 3. Minimal data variation at high sample rates with the Attune™ Acoustic Focusing Cytometer. Jurkat cells were fixed and stained with propidium iodide, treated with RNase, and analyzed at a concentration of 1 x 10^6 cells/mL on a high-end instrument that uses hydrodynamic focusing, and on the 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 increased on the instrument that uses hydrodynamic focusing, the width of the G_0/G_1 and G_2/M peaks increased, whereas for the Attune™ cytometer the peaks are relatively stable, even at the highest sample rate of 1,000 µL/min. These results are quantitated in Table 1.

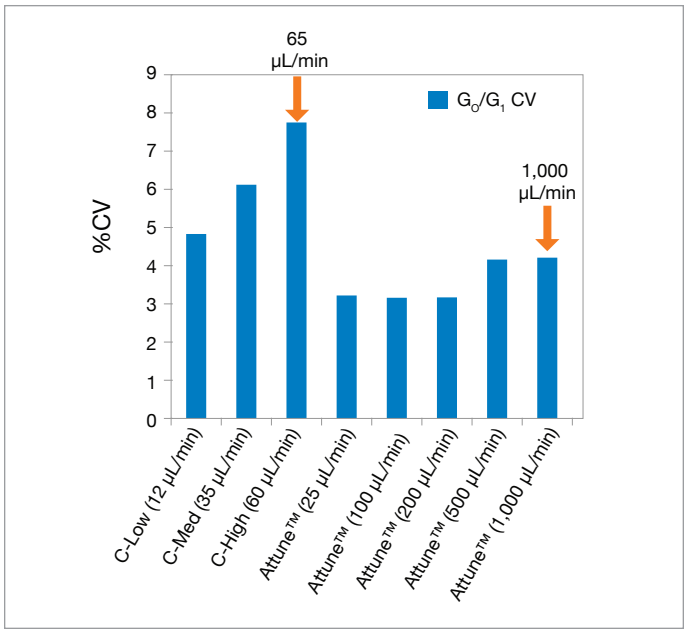
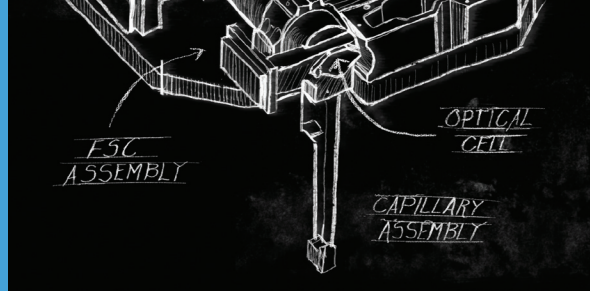


Figure 4. Percent CV of G_0/G_1 at different flow rates on Attune™ cytometer and Competitor (C) at different sample rates. Note the minimal change in variability (%CV) even at high sample rate with the Attune™ Acoustic Focusing Cytometer.

Table 1. Cell Cycle Analysis on the Attune™ Acoustic Focusing Cytometer vs. the 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 µL/min	41.73	38.73	20.44	1.96	4.83
	35 µL/min	40.16	38.95	20.89	1.96	6.12
	60 µL/min	44.60	26.17	29.23	1.90	7.76
Attune™ Acoustic Focusing Cytometer	25 µL/min	45.20	40.29	14.51	1.94	3.22
	100 µL/min	42.81	38.72	18.74	1.94	3.17
	1,000 µL/min	40.25	38.55	21.20	1.94	4.21

**Note the disparity of results in the percentage of cells in S phase of the cell cycle 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 a traditional cytometer (see Table 2 below). 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 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

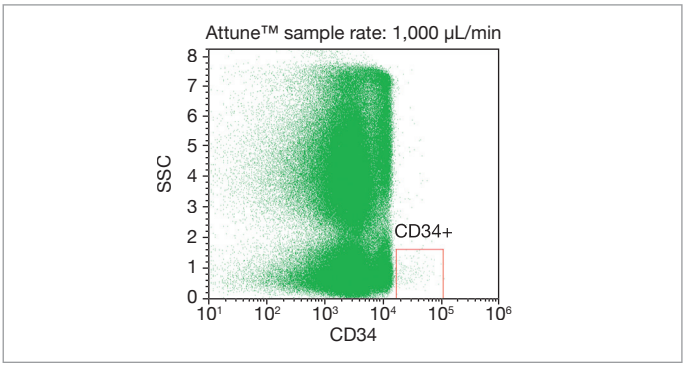


Figure 5. Identification of CD34+ cells from peripheral blood. Peripheral blood from a normal donor was stained and run on the Attune™ Acoustic Focusing Cytometer at a flow rate of 1,000 µL/min with a stop gate set at 500,000 total cells. Two hundred and fifty CD34+ cells (red box) were identified within 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 (µL/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 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)

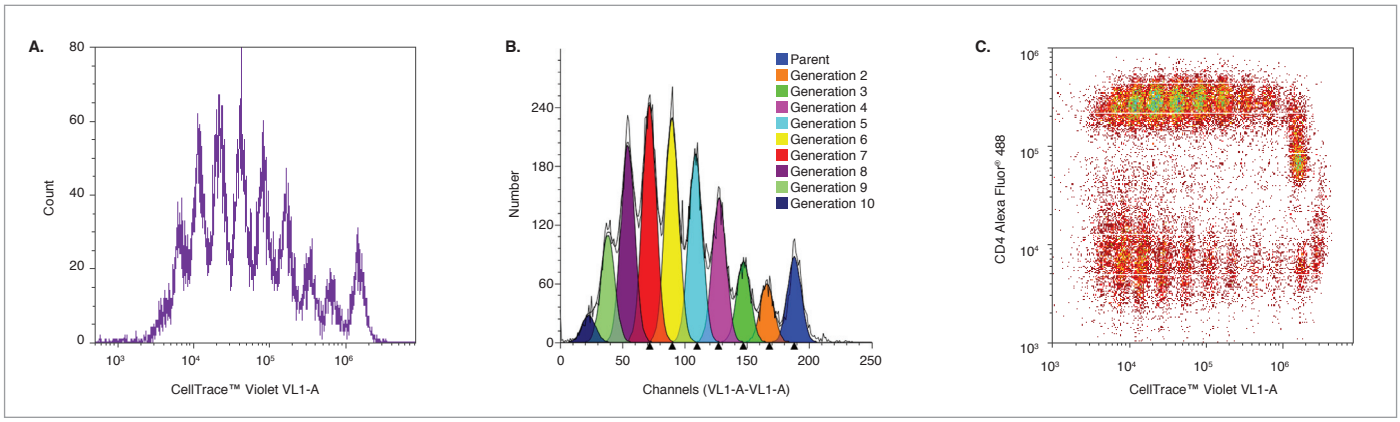
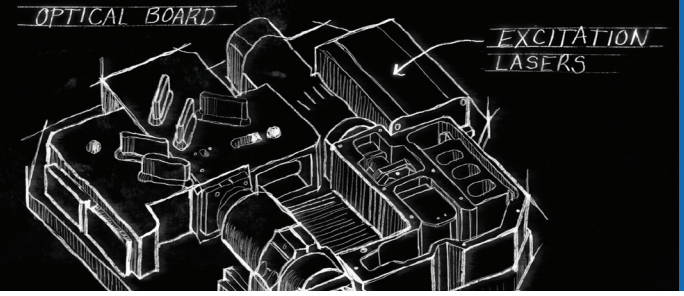


Figure 6. Ten cell divisions identified with the Attune™ Acoustic Focusing Cytometer and the Molecular Probes® CellTrace™ Violet Cell Proliferation Assay. Human peripheral blood mononuclear cells were isolated from whole blood, stained with CellTrace™ Violet, and stimulated to proliferate in culture. 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 µL/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 (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

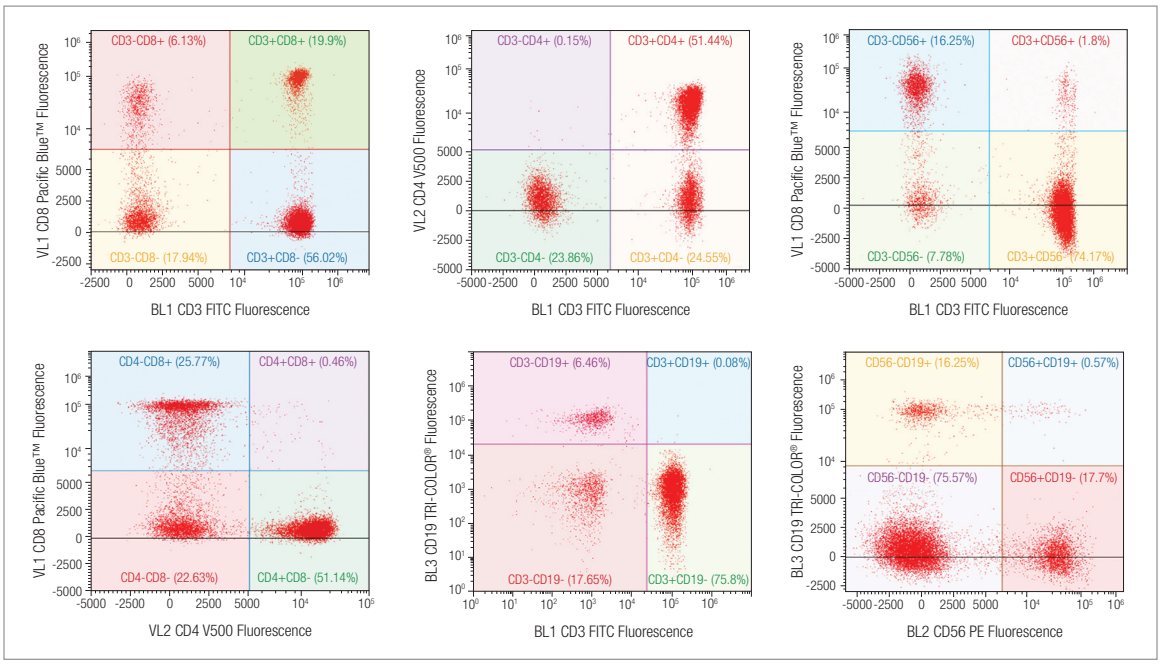


Figure 7. Six-Color Immunophenotyping Analysis on the Attune™ Acoustic Focusing Cytometer. Normal human blood cells were labeled with the following directly labeled mouse anti-human antibody conjugates: CD45-Pacific Orange™, CD3-FITC, CD8-Pacific Blue™, CD56-R-PE, CD19-TRI-COLOR® (all from Invitrogen), and CD4-V500 (BD Biosciences) for six-color immunophenotyping. 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 dim 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

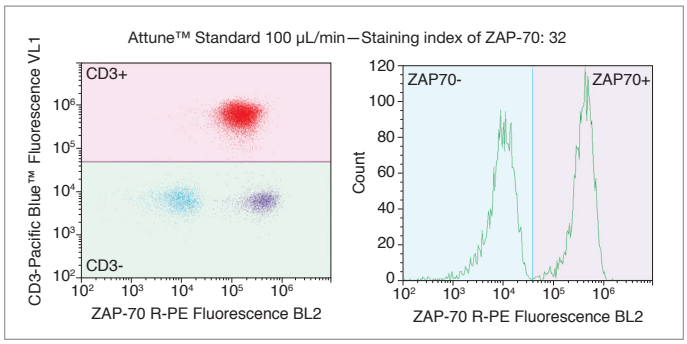
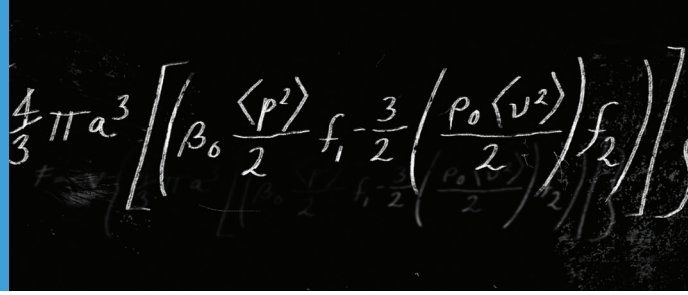


Figure 8. Identification of ZAP-70 positive cells with the Attune™ Acoustic Focusing Cytometer. CD3-ζ associated protein (ZAP-70) is normally expressed primarily in T cells and natural killer (NK) cells. Dual parameter analysis (gated on lymphocytes) was performed for CD3 and ZAP-70 at 100 µL/min collection rate. 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.



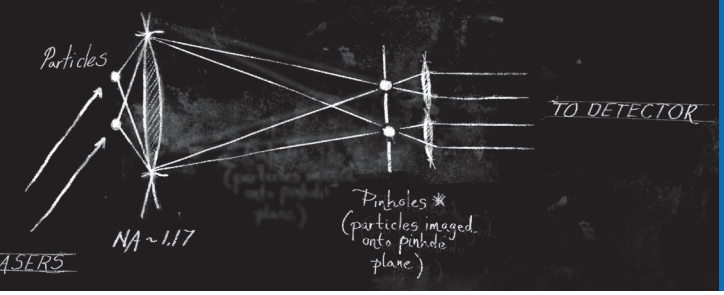
More Choices, More Power to Multiplex

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® fluorescent 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.

Excitation Laser	Fluorescence Channel	Default Filter (nm)	Filter Range (nm)	Fluorophores		Other Fluorescent Dyes
Violet (405 nm)	Blue	450/40	430–470	Pacific Blue™ Alexa Fluor® 405		PO-PRO™-1 DyeCycle™ Violet Fixable Violet Dead Cell Stain CellTrace™ Violet Calcein Violet SYTOX® Blue FxCycle™ Violet
	Green	522/31	507–537	Qdot® 525 Horizon™ V500		Fixable Aqua Dead Cell Stain
	Orange	603/48	579–627	Pacific Orange™ Qdot® 605		Fixable Yellow Dead Cell Stain
Blue (488 nm)	Green	530/30	515–545	Alexa Fluor® 488 Fluorescein		Calcein Fluo-3/Fluo-4 TO-PRO®-1 CFSE GFP JC-1/DiOC$_2$(3) SYTOX® Green DyeCycle™ Green Rhodamine 123 YO-PRO®-1 Fixable Green Dead Cell Stain
	Orange	575/24	563–587	PE		PI Fura Red™ DyeCycle™ Orange JC-1/DiOC$_2$(3) pHrodo™ SNARF® (low pH)
	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	Qdot® 655 Qdot® 705 Qdot® 800	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 the comfort of 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

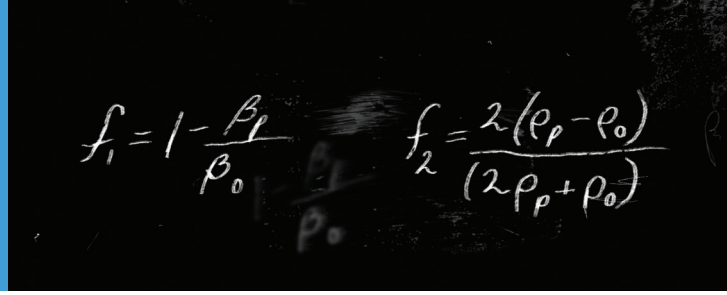
- Plot overlay or side-by-side graph comparison
- Standard or custom gate shapes
- Histogram, dot plot, density plots
- Customize statistics
- Statistics in Microsoft® Excel format
- Copy and paste plots into reports (Microsoft® Word or PowerPoint)

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 to collect and analyze accurate experimental data. The Attune™ Performance beads are designed for use with Attune™ Cytometric Software to automatically characterize, track, and report performance measurements of the Attune™ Acoustic Focusing Cytometer (Figure 10). The beads will be used to define a baseline and conduct daily measurements of the cytometer. Packaged in vials containing enough beads for 50 measurements, specific information for each lot is downloaded into the Attune™ Cytometric Software prior to use.

Performance tracking for 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 the changes in the coefficient of variation and the changes in 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 recommended schedules for maintenance (covered by annual service agreements).

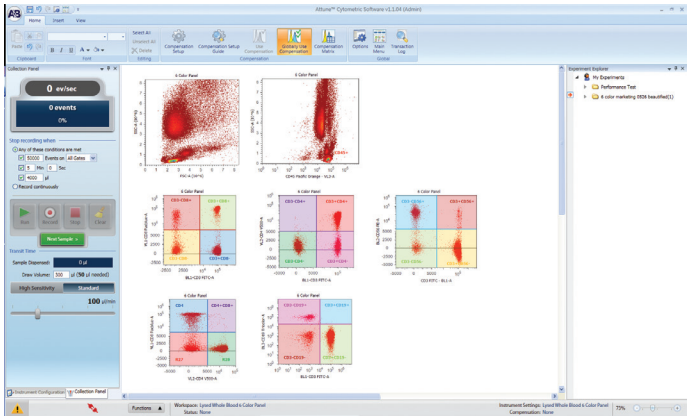


Figure 9. Example of Attune™ Cytometric Software. The instrument control panel on the left side of the screen is used for experiment setup and control of laser selection, optics, and fluidics (flow rates). The main frame of this screen shows data analysis plots, while the right panel shows sub-file organization.

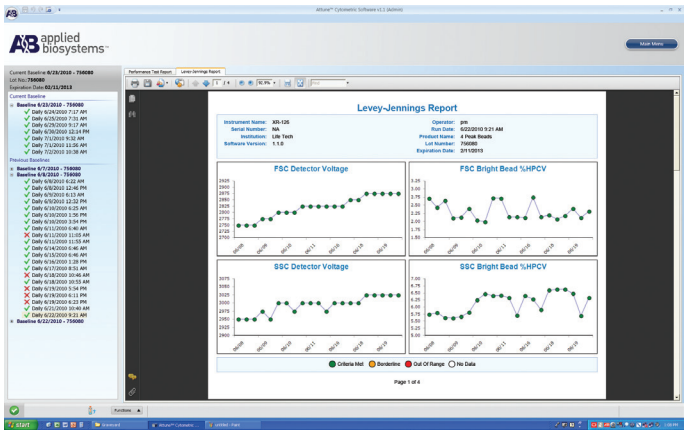
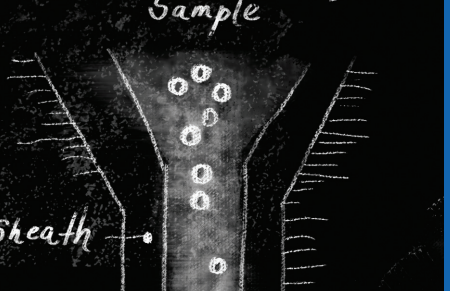


Figure 10. Example of Levey-Jennings Plot Showing Attune Cytometer Performance Over Time. This report is generated using data from performance tracking beads.



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 that the cell transits through 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 assay, 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 of course human whole blood cells. The largest particle size tested was 40 µM, and the smallest particle reliably detected is 1 µM.

What is Attune™ focusing solution?
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 there are no licensing fees, the Attune™ Cytometric Software can be installed on any computer, enabling a broad network for analysis.

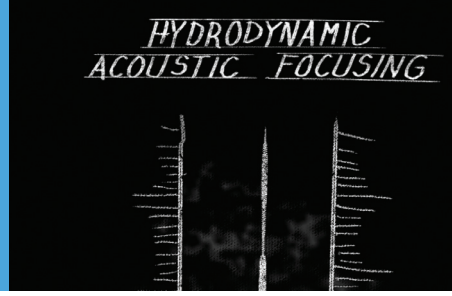
What is the maximum number of events that can be recorded per sample run?
Attune™ Cytometric Software is enabled to record up to 20 million events per sample run, which gives researchers performing rare event analysis a powerful tool to achieve significant numbers and high confidence in the data.

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/attune-software. 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: 575/24 nm
BL3: 640 nm LP
VL1: 450/50 nm
VL2: 522/31 nm
VL3: 603/48 nm

Fluidics
Sample input rates: 1 sample/min
Sample flow 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
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

Service
Supported by Applied Biosystems service with 72-hour response

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
Particle size range: 1–45 µm

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 to add additional users
User and administrator log-in
Capable of collecting 20 x 10^6 events per sample

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—there's 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	6	4449791
Attune™ Wash Solution	1	4449755
Attune™ 10X Shutdown Solution	1	4454955
Attune™ Performance Tracking Beads (5 x 10^6 beads/mL)	1	4449754

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