invitrogen

The next generation in flow cytometry

Attune NxT Flow Cytometer—
expand your research capabilities
with acoustic focusing technology

ДИА•М
современная лаборатория

30 лет

ThermoFisher
SCIENTIFIC

Small in size but big in performance

Attune NxT Flow Cytometer and Autosampler

Combining precision with performance, the Invitrogen™ Attune™ NxT Flow Cytometer is a benchtop analyzer configurable with up to 4 lasers and 16 parameters while offering superior speed (up to 10x faster than traditional flow cytometers) and clog-resistant engineering (Figure 1). Convert between tubes and plates in seconds and leverage complete walkaway automation of 96- or 384-well plates with the robotic automation–capable Invitrogen™ Attune™ NxT Autosampler. Together, these systems were designed to help researchers gather data like never before and achieve superior results.

Figure 1. Front view featuring the light panel and tube loader of the Attune NxT Flow Cytometer.

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

Weight:
- 64 lb. (29 kg)

Electrical requirements:
- 100–240 VAC, 50/60 Hz, <150 W

All fluids are stored within the instrument (Figure 2) and include active level sensors. Flashing lights are displayed when fluid levels are low (or high, for the waste tank), allowing users to identify fluid level issues from across the lab.

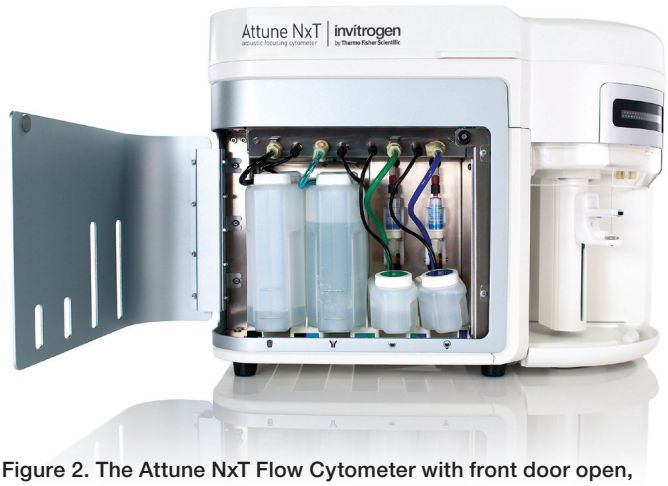


Figure 2. The Attune NxT Flow Cytometer with front door open, revealing the fluid storage containers.

Standard fluid tanks:
- 1.8 L focusing fluid tank
- 1.8 L waste tank
- 175 mL shutdown solution tank
- 175 mL wash solution tank

External fluid tank option:
- Configuration for 10 L of fluid (Figure 3)
- Nominal fluid consumption of 1.8 L/day

Figure 3. Optional external fluid tank with 10 L fluid capacity.

High sensitivity at all sample rates

The Attune NxT Flow Cytometer enables higher sensitivity when you need it most. The system maintains precise alignment, thanks to the acoustic focusing, even at sample rates of up to 1,000 µL/min.

Data acquisition rate

The Attune NxT Flow Cytometer is designed to acquire data at up to 35,000 events/second.

Particle size range

The Attune NxT Flow Cytometer is designed to detect particles with diameters from 0.5 µm to 50 µm. Figure 4 demonstrates its ability to distinguish small particles, sometimes less than 0.5 µm.

Fluorescence resolution

The Attune NxT Flow Cytometer is designed to have a coefficient of variation (CV) of less than 3% for the singlet peak of chicken erythrocyte nuclei stained with propidium iodide (Figure 5).

Forward and side scatter resolution

The Attune NxT Flow Cytometer is designed to discriminate platelets from noise and is optimized to resolve lymphocytes, monocytes, and granulocytes in lysed whole blood (Figure 6).

Multi-peak fluorescent microspheres are often used in flow cytometry to determine the sensitivity of an instrument. Figure 7 demonstrates the higher sensitivity of the Attune NxT Flow Cytometer compared to flow cytometers that use conventional hydrodynamic focusing at both low (12.5 µL/min) and high (500 µL/min) sample input rates.

Predicted molecules of equivalent soluble fluorochrome (MESF):

- ≤80 MESF (FITC)
- ≤30 MESF (PE)
- ≤70 MESF (APC)

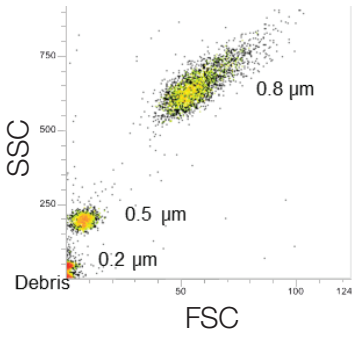


Figure 4. Forward scatter (FSC) and side scatter (SSC) discrimination of 0.2 µm, 0.5 µm, and 0.8 µm particles.

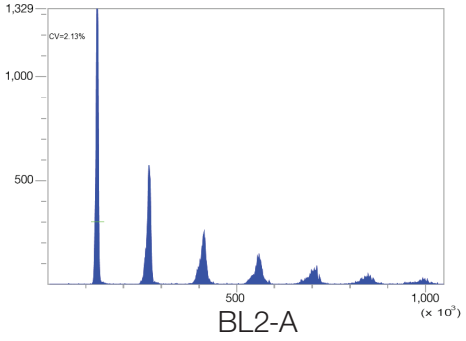


Figure 5. Chicken erythrocyte nuclei stained with propidium iodide and analyzed at a sample rate of 100 µL/min.

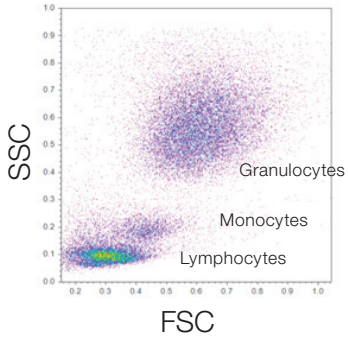
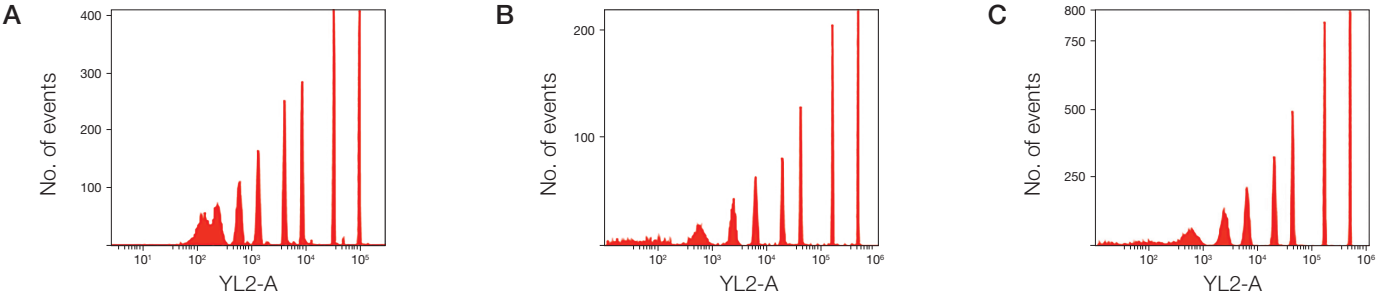


Figure 6. FSC and SSC parameters can be used to identify lymphocyte, monocyte, and granulocyte populations in ammonium chloride–lysed whole blood.



Conventional flow cytometer
(highest sensitivity = ~12.5 µL/min)

Attune NxT Flow Cytometer
(12.5 µL/min)

Attune NxT Flow Cytometer
(500 µL/min)

Figure 7. 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 Flow 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 Flow Cytometer was run at **(B)** 12.5 µL/min, which is equivalent to the traditional flow cytometer and at **(C)** 500 µL/min, a 40x increase in sample flow rate. The Attune NxT Flow Cytometer showed equal or better results even at the highest flow rates.

What is acoustic focusing?

The Attune NxT Flow Cytometer uses ultrasonic waves (over 2 MHz, similar to those used in ultrasound medical imaging), in combination with hydrodynamic forces, to position cells into a single, focused line along the central axis of a capillary (Figure 8). 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. This, in turn, allows the collection of more photons for high-precision analysis at superior volumetric sample throughput, as demonstrated by the minimal variation demonstrated in cell cycle analysis (Figure 9).

Sample input rates:

- 12.5 µL/min to 1 mL/min, up to 10 times faster than traditional hydrodynamic focusing systems

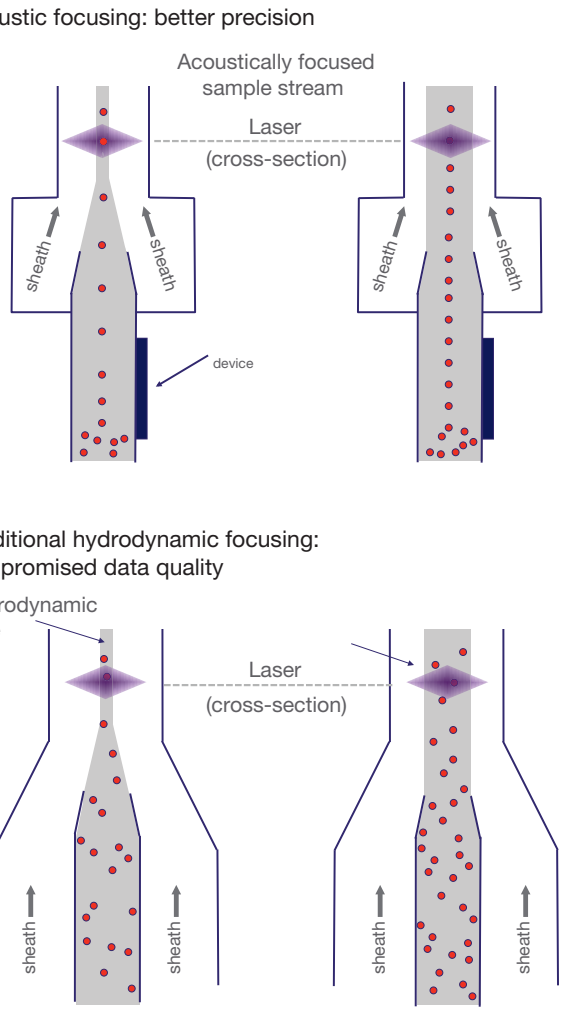
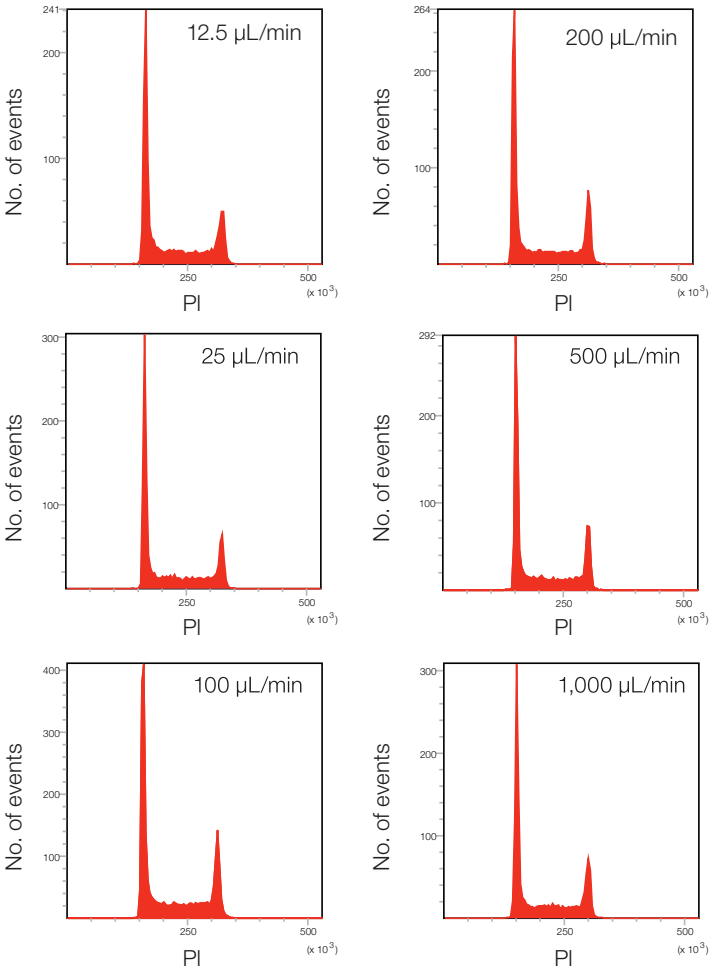


Figure 8. Acoustic focusing vs. traditional hydrodynamic focusing as particles pass through the laser. (A) In acoustic focusing, cells remain in tight alignment even at higher sample rates, resulting in less signal variation and improved data quality. **(B)** In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream, resulting in increased signal variation and compromised data quality.



Sample rate (µL/min)	12.5	25	100	200	500	1,000
G_0/G_1 CV (%)	2.99	3.03	2.76	2.94	2.70	2.96
G_2/M CV (%)	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 Flow 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 the Attune NxT Flow 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. Regardless of sample rate, the width of the G_0/G_1 and G_2/M peaks and coefficient of variation (CV) remains consistent for the Attune NxT Flow Cytometer, even at the highest sample rate of 1,000 µL/min.

Dilute your samples, not your data quality

When working with whole blood, washing of the sample and lysis of red blood cells (RBCs) can cause significant cell loss and damage. Significantly higher sample collection rates, due to acoustic focusing, allow the Attune NxT Flow Cytometer to deliver a no-wash/no-lyse protocol to minimize cell loss and simplify sample preparation. Figure 10 demonstrates three strategies for identifying leukocytes from whole blood: (1) utilizing violet side scatter to discriminate leukocytes from RBCs, (2) utilizing CD45 antibodies to "trigger" and gate on leukocytes and (3) utilizing Invitrogen™ Vybrant™ DyeCycle™ dyes to identify nucleated cells (therefore discriminating the nucleus-free RBCs).

This feature is also useful for samples that are inherently low in concentration, dilute samples such as cerebrospinal fluid (CSF), and stem cell samples with low cell numbers, all of which can take a long time to acquire due to their high volumes and need to acquire the entire sample. High sample collection rates of up to 1 mL/min make acquisition of these samples possible—you can analyze up to 4 mL in just 4 minutes. In addition, no sample loss occurs due to washing and centrifugation of the samples, and full panel testing with up to 14 colors is possible for all precious samples.

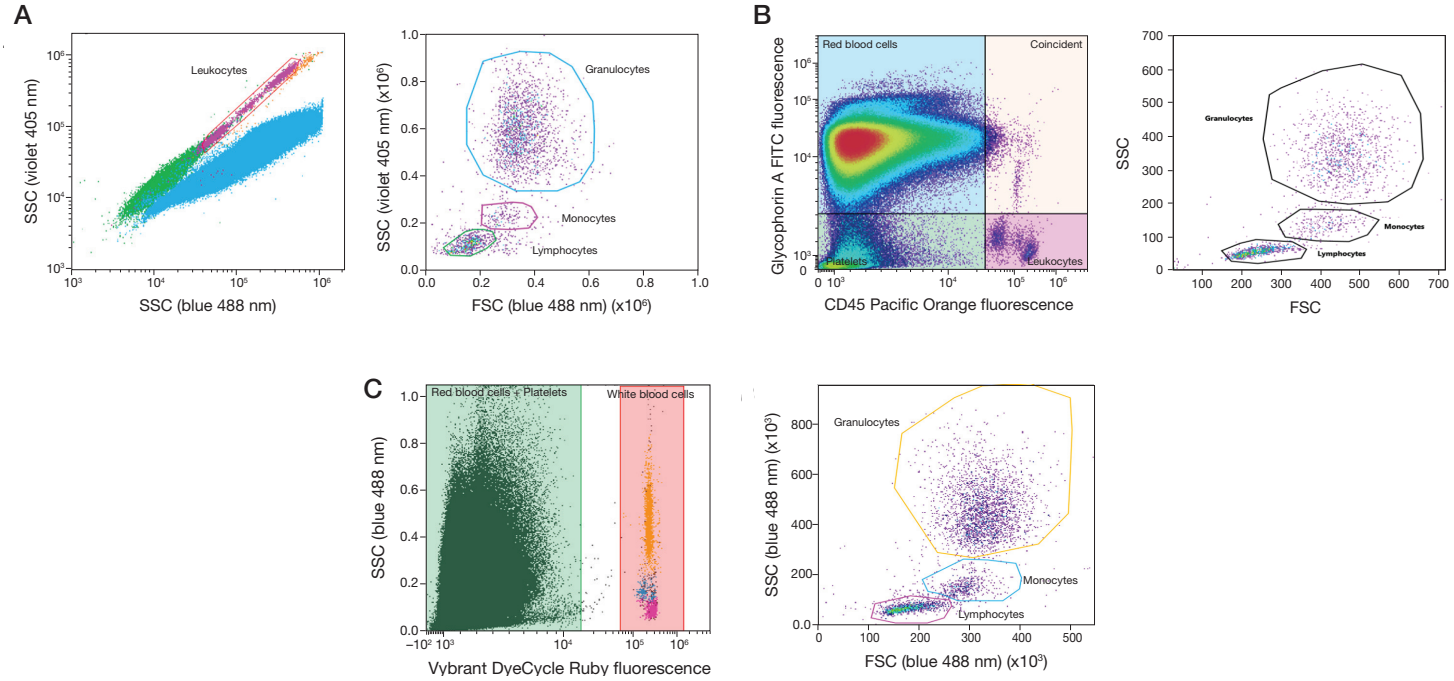


Figure 10. Problematic samples easily analyzed with the Attune NxT Flow Cytometer. (A) Identification of leukocytes in human whole blood using violet side scatter is illustrated. Leukocytes are outnumbered ~1,000 to 1 by red blood cells in whole blood and generally require enrichment by red blood cell lysis or gradient centrifugation prior to analysis. The rapid sample collection rates and inclusion made possible by the Invitrogen™ Attune™ NxT No-Wash No-Lyse Filter Kit (Cat. No. 100022776) on the Attune NxT Flow Cytometer allow the identification of leukocytes by scatter properties alone. Using both violet and blue side scatter permits leukocytes to be distinguished from RBCs (which contain hemoglobin and absorb light at 405 nm) in whole blood. When leukocytes are gated based on violet light scatter properties, the 3 main leukocyte cell populations in human blood (lymphocytes, monocytes, and granulocytes) are detected. **(B)** A fluorochrome-conjugated antibody approach to identifying leukocytes in whole human blood is illustrated. CD45-expressing leukocytes can be targeted with fluorochrome-conjugated anti-CD45 antibodies, in this case Invitrogen™ CD45 Antibody, Pacific Orange™ Conjugate (Cat. No. MHCD4530TR). Invitrogen™ Glycophorin A Antibody, FITC Conjugate (Cat. No. MHGLA01-4) is used to eliminate red blood cell–coincident events. Gating on CD45-positive glycophorin A–negative cells allows the identification of the 3 primary leukocyte populations (lymphocytes, monocytes, and granulocytes), while avoiding glycophorin A–CD45 double-positive coincident events. **(C)** Identification of leukocytes in whole human blood using Vybrant DyeCycle dyes is illustrated. Vybrant DyeCycle dyes label live nucleated cells, thus allowing for the identification of leukocytes in whole human blood. Human whole blood labeled with the cell membrane–permeant DNA dye Vybrant DyeCycle Ruby (Cat. No. V10309) allows the identification of the 3 main leukocyte populations in human blood: lymphocytes, monocytes, and granulocytes.

Rapid detection of rare cells with acoustic focusing

Detection of rare events includes populations of cells comprising less than 1% of total cells, which includes the detection of stem cells, minimal residual disease cells, natural killer cells, and fetomaternal hemorrhage cells. 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 Flow Cytometer allows dilute samples to be processed quickly at sample input speeds of up to 1 mL/min.

Conventional cytometers utilize traditional hydrodynamic focusing technology that allows for maximum sample input rates of 60–100 µL/min, limiting detection of rare cell populations due to the incredibly long acquisition times required to collect enough cells for analysis. By combining acoustic and hydrodynamic focusing, the Attune NxT Flow Cytometer can analyze cells at sample input rates of 500 µL/min or 1 mL/min, allowing for quick collection time in experiments involving detection of rare events (Figures 11 and 12).

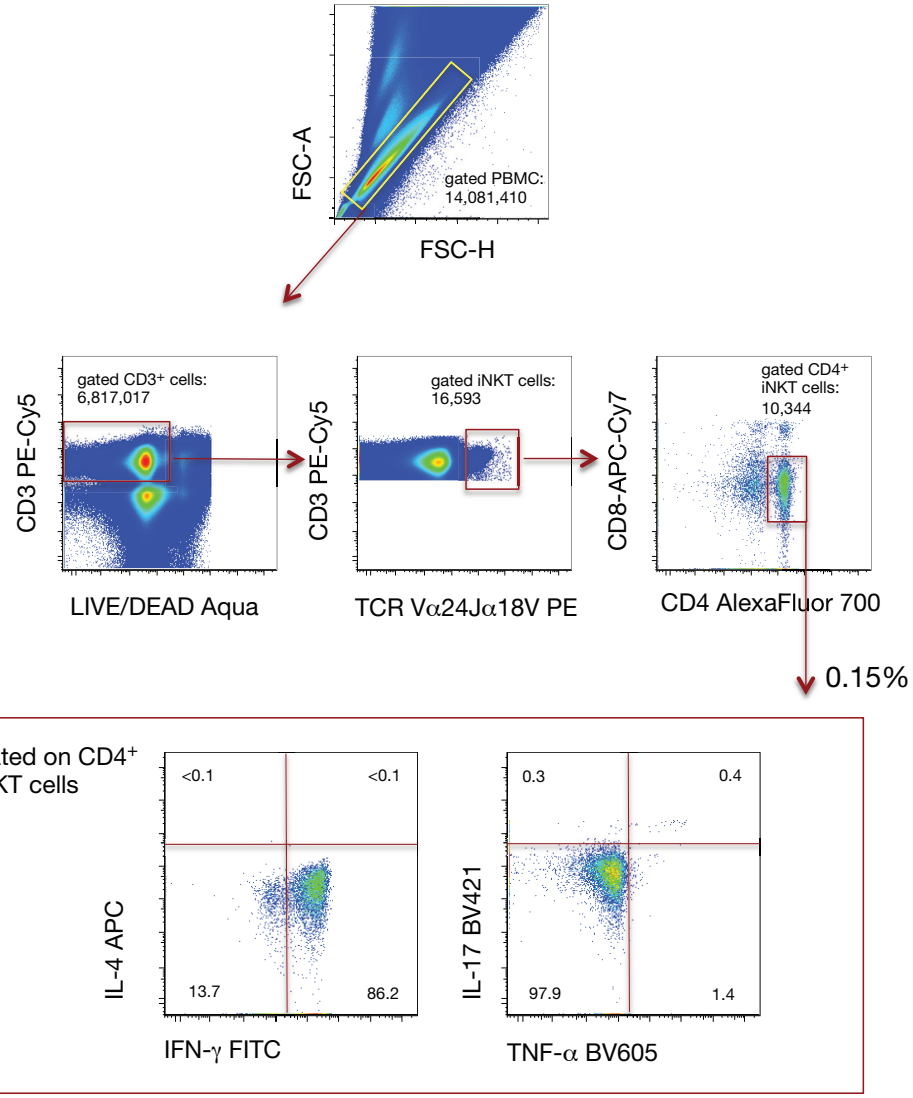


Figure 11. Peripheral blood mononuclear cells (PBMC) were stimulated for 4 hours with 200 ng/mL PMA plus 1 µg/mL ionomycin at 37°C. Rare cell analysis gating strategy is demonstrated. Production of 4 different cytokines (IL-4, IL-17, IFN-γ, and TNF-γ) by human peripheral blood iNKT cells that express CD4 was analyzed. Data courtesy of Dr. Andrea Cossarizza (University of Modena and Reggio Emilia School of Medicine).

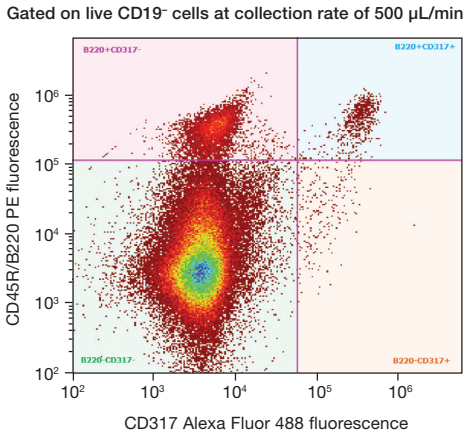


Figure 12. Collection of more than 1 million live cells and detection of a rare population (0.2%) of dendritic cells. Plasmacytoid dendritic cells (pDCs) are a specialized cell population that produces large amounts of type I interferons in response to viruses and are identified using the immunophenotype CD19⁻/B220^{high}/CD317⁺. Four-color staining of mouse splenocytes included CD19 Pacific Blue™, CD317 Alexa Fluor™ 488, and CD45R/B220 PE antibodies and SYTOX™ AADvanced™ Dead Cell Stain. A gate was made on live cells using SYTOX AADvanced Dead Cell Stain, followed by gating on CD19⁻ cells. A two-parameter plot of CD45R/B220 vs. CD317 was used to identify pDCs. A collection rate of 500 µL/min was used to acquire 1.3 x 10^6 total cells with a cell concentration of 7.5 x 10^7 cells/mL. pDCs were identified as dual B220⁺/CD317⁺ (upper right quadrant) and constitute 0.851% of live CD19⁻ cells, which is 0.194% of total splenocytes.

Volumetric measurements and cell counts

Smooth sample delivery

- Sample delivered by positive displacement syringe pump (Figure 13) for volumetric analysis

Volumetric cell counts for all samples

- Count cells in a known volume (gated or total events)
- Live/dead analysis—easily gate out dead cells to count live cells

Sample analysis volume

- Don't lose precious sample—choose from 20 µL to 4 mL sample volumes

Sample flow rates

- Maximize your sample flow rate for specific applications
- Choose from flow rates of 12.5, 25, 100, 200, 500, and 1,000 µL/min

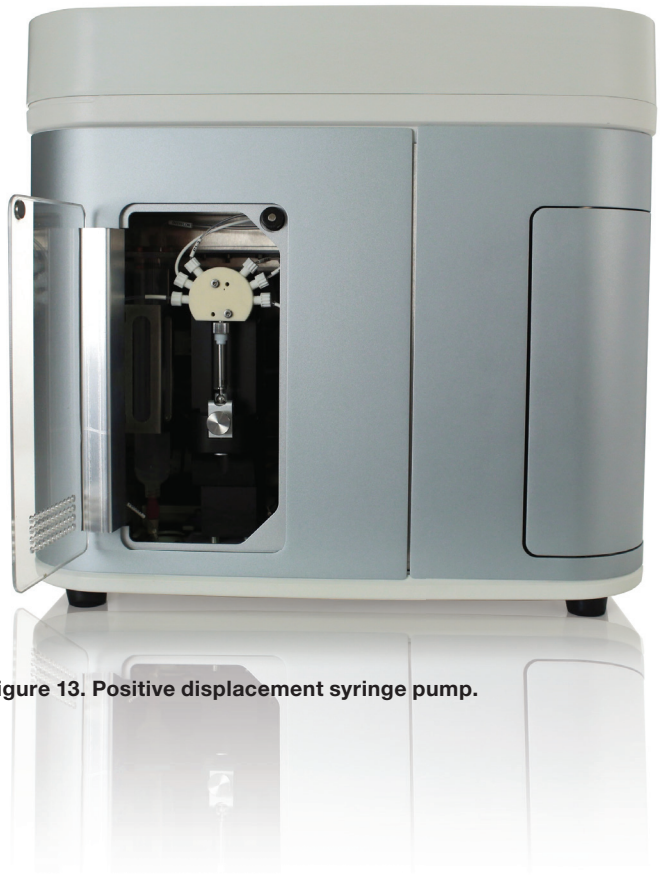


Figure 13. Positive displacement syringe pump.

Flow that fits: modular design configurable to your needs and budget

Attune NxT Flow Cytometer and Autosampler

Designed to grow in lockstep with the research needs and budgetary confinements of labs, the Attune NxT Flow Cytometer features modularity and uncomplicated, rapid upgrade options.

Lasers:

- Choose from 1 to 4 lasers
  - Violet (405 nm)
  - Blue (488 nm)
  - Yellow (561 nm)
  - Red (637 nm)

Up to 16 detection channels:

- Forward scatter (FSC)
- Side scatter (SSC)
- Up to 14 colors

User-exchangeable bandpass filters

- Easy access to filters (Figure 14)

Figure 14. Filters are easy to access on the Attune NxT Flow Cytometer.

Spatially separated lasers

- All lasers spatially separated (Figure 15)
- Improved compensation for multicolor panels
  - More choices for color combinations
  - 6-color experiments with no compensation required with 4-laser instrument (Figure 17)

Minimal compensation for popular dyes

- FITC (from blue laser) vs. PE (from yellow lasers) (Figure 16)

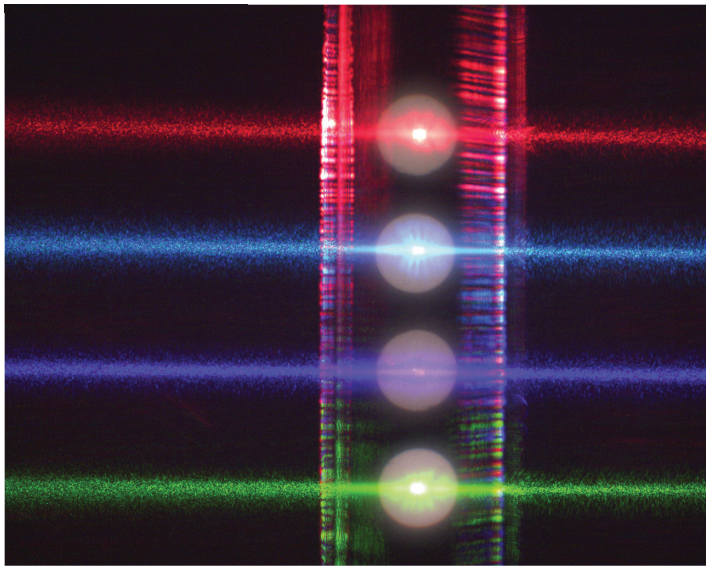


Figure 15. The Attune NxT Flow Cytometer can be configured with up to 4 spatially separated lasers.

Multicolor analysis in a modular design

With the option to be configured with up to 4 lasers and 14 colors for multiparameter analysis, the Attune NxT Flow Cytometer was envisioned as a modular system to fit most experimental design needs and lab budgets. The novel design of the optical path helps ensure precise fixed alignment of 4 spatially separated lasers onto the sample stream, leading to consistency in data over time, superior performance, and excellent reliability. Minimal compensation is required for popular dye combinations (Figures 16 and 17).

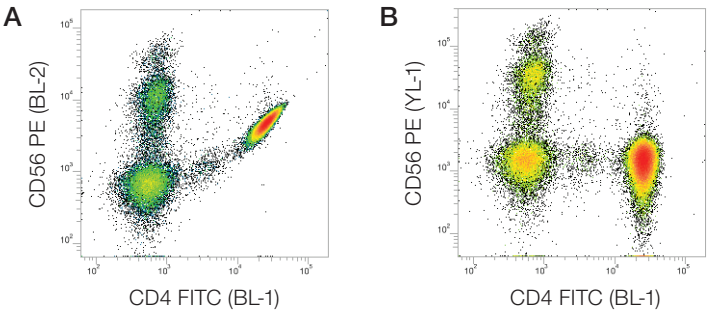
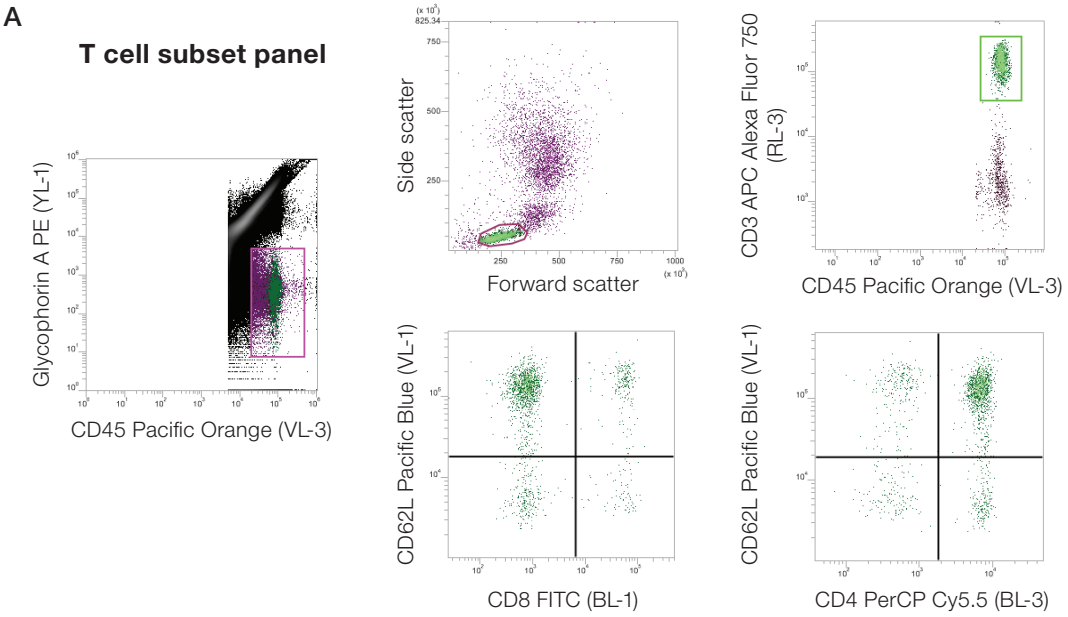


Figure 16. With the Attune NxT Flow Cytometer, minimal compensation is required for popular dyes such as FITC and PE. **(A)** When FITC and PE use the same excitation and optical detection pathways after excitation by the 488 nm blue laser, there is a significant amount of spillover of FITC signal into the PE detector, requiring compensation. **(B)** When excitation and detection of FITC and PE are uncoupled, using the 561 nm laser to excite PE and the 488 nm laser to excite FITC, there is little or no spillover of FITC signal into the PE signal, thus no compensation is required.



B

Compensation matrix						
	Pacific Blue (VL-1)	Pacific Orange (VL-3)	FITC (BL-1)	PerCP-Cy5.5 (BL-3)	PE (YL-1)	APC-Alexa Fluor 750 (RL-3)
Pacific Blue (VL-1)		0.0	0.0	0.0	0.0	0.0
Pacific Orange (VL-3)	0.0		0.0	0.0	0.0	0.0
FITC (BL-1)	0.0	0.0		0.0	0.0	0.0
PerCP-Cy5.5 (BL-3)	0.0	0.0	0.0		0.0	0.0
PE (YL-1)	0.0	0.0	0.0	0.0		0.0
APC-Alexa Fluor 750 (RL-3)	0.0	0.0	0.0	0.0	0.0	

Figure 17. Minimize compensation for multicolor panels. (A) Optimal design of a no-lyse, no-wash 6-color immunophenotyping panel for human T cell subsets acquired on the Attune NxT Flow Cytometer requires no compensation. Whole human blood was stained with Invitrogen™ anti-CD45 Pacific Orange™ (Cat. No. MHCD4530TR), anti–glycophorin A PE (Cat. No. MHGLA04), anti-CD3 APC-Alexa Fluor™ 750 (Cat. No. MHCD0327), anti-CD62L Pacific Blue™ (Cat. No. MHCD62L28), anti-CD8 FITC (Cat. No. MHCD0801), and anti-CD4 PerCP-Cy®5.5 (Cat. No. A15858) antibodies. Samples were acquired on the Attune NxT Flow Cytometer using 405 nm excitation to detect Pacific Blue and Pacific Orange dyes, 488 nm excitation to detect FITC and PerCP-Cy5.5 dyes, 561 nm excitation to detect PE, and 637 nm excitation to detect APC–Alexa Fluor 750 dye. A fluorescence threshold was set on the CD45–Pacific Orange conjugate and red blood cell–coincident events were excluded based on glycophorin A–PE positivity. Lymphocytes were gated based on scatter properties, from which T cells were identified by CD3 expression. T cells were then analyzed for their expression of the lineage markers CD4 and CD8 and the activation marker CD62L to identify naive/central memory T cells ($CD62L^+$) and effector memory T cells ($CD62L^-$). **(B)** Compensation matrix from the Attune NxT Flow Cytometer for the 6-color immunophenotyping panel. Zero compensation is needed.

Novel optical design to minimize instrument downtime

The innovative design of the optical path helps ensure precise fixed alignment of four spatially separated lasers onto the sample stream, leading to consistency in data over time, superior performance, and first-class reliability. The instrument can be configured with up to 4 solid-state lasers with flat-top beam profiles (Figure 18) to minimize the effects of changes in fluidics or optics, which may cause instability and alignment issues potentially 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 signal.

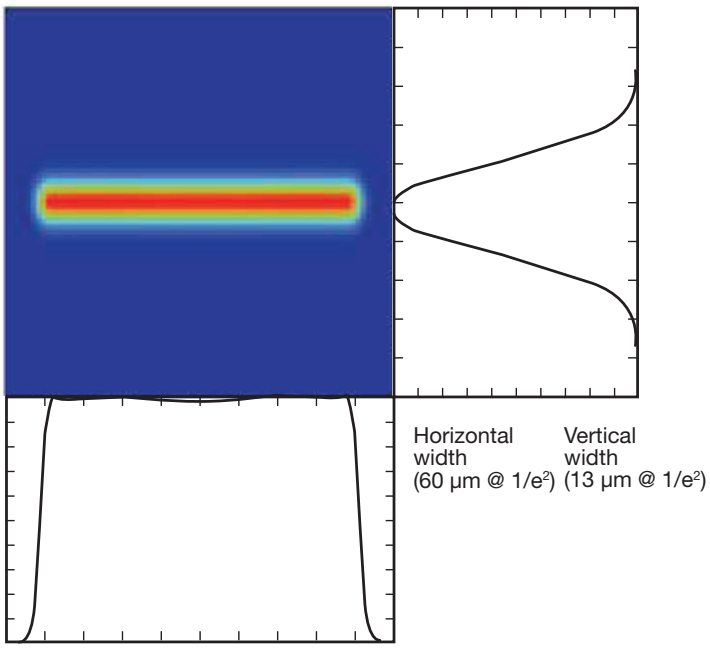


Figure 18. Example of flat-top beam profiles.

Benefits of the state-of-the-art optical design

- Onboard thermal-electric cooler
  - No warm-up delay: fiber isn't affected by instrument warm-up
- Simmer mode: automatic shutoff prolongs laser usage lifetime by 10x
  - Only on when acquiring samples
  - Reports hours of usage
- Pre-aligned and welded fiber to laser interface (permanent)
- Pre-aligned fiber to beam-shaping optics (BSO) interface (alignment-free)
- Focus lens per laser: power where you want it
- Flat-top laser specified at the flow cell
- Field upgradable

Benefits of flat-top lasers

- ~50% reduction in scattered light
- No problems with lateral shift in alignment

Superior reliability and maximum signal efficiency

Instrument downtime due to laser misalignment is a major concern with users of conventional flow cytometers. Most flow cytometers use lasers that have a Gaussian emission profile (Figure 19A), where the intensity increases until it hits a maximum. The maximum window for alignment is very small, so alignment needs to be very precise. Gaussian lasers have very little flexibility and may yield poor results due to slight shifts in the laser alignment (Figure 19B), leading to loss of sensitivity and high CVs. The Attune NxT Flow Cytometer uses flat-top lasers, with emission profiles as seen in Figure 19C, which have an intensity profile that allows a much wider window of alignment. With these lasers, slight shifts in the alignment do not affect sensitivity and CVs, because they have a higher tolerance for misalignment, allowing them to maintain high sensitivity and low CVs (Figure 19D).

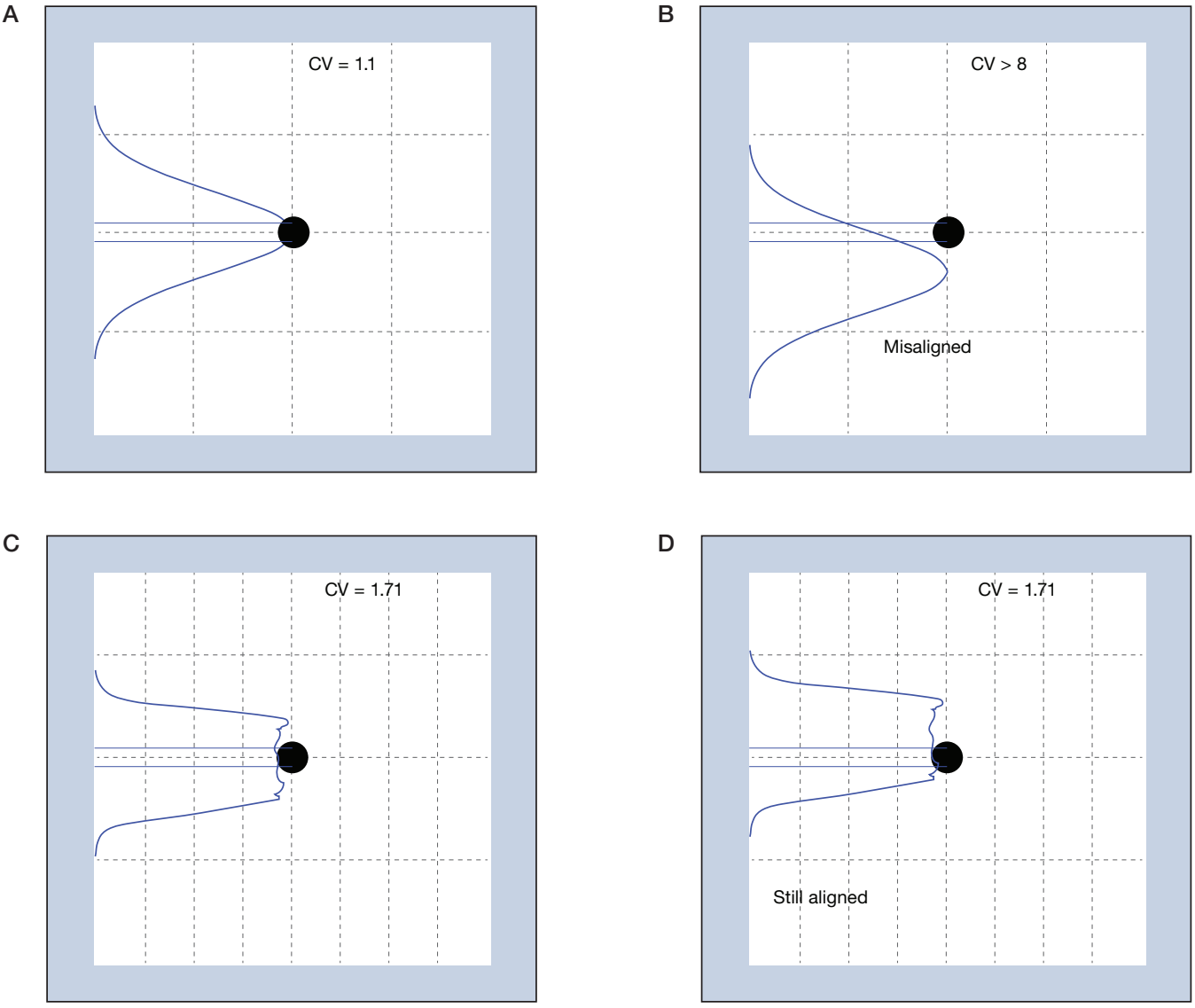


Figure 19. Emission profiles of lasers used in flow cytometers. (A) Gaussian laser profile with proper alignment, **(B)** Gaussian laser profile with misalignment, **(C)** flat-top laser profile with proper alignment and **(D)** flat-top laser profile, still in proper alignment.

Software that performs to your specifications

Invitrogen™ Attune™ NxT Software is designed to provide powerful data acquisition and analysis using an intuitive, user-friendly interface (Figure 20). 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.

- **Compensation**—fully automated and manual compensation
- **Instrument tracking**—automated baseline and performance test for all channels and linearity
- **Automated maintenance**—startup (<15 min), shutdown, rinse, wash, debubble, deep clean, monthly decontamination
- **User account maintenance**—administrative and individual accounts with user log
- **Gates**—standard and customizable gates
- **Plot previews**—easy workspace setup
- **Zoom filmstrip viewer**—easy visualization and navigation of plots to set gates and plot attributes
- **File formats**—FCS 3.0 and FCS 3.1
- **Graphics resolution**—publication-quality images (customizable)

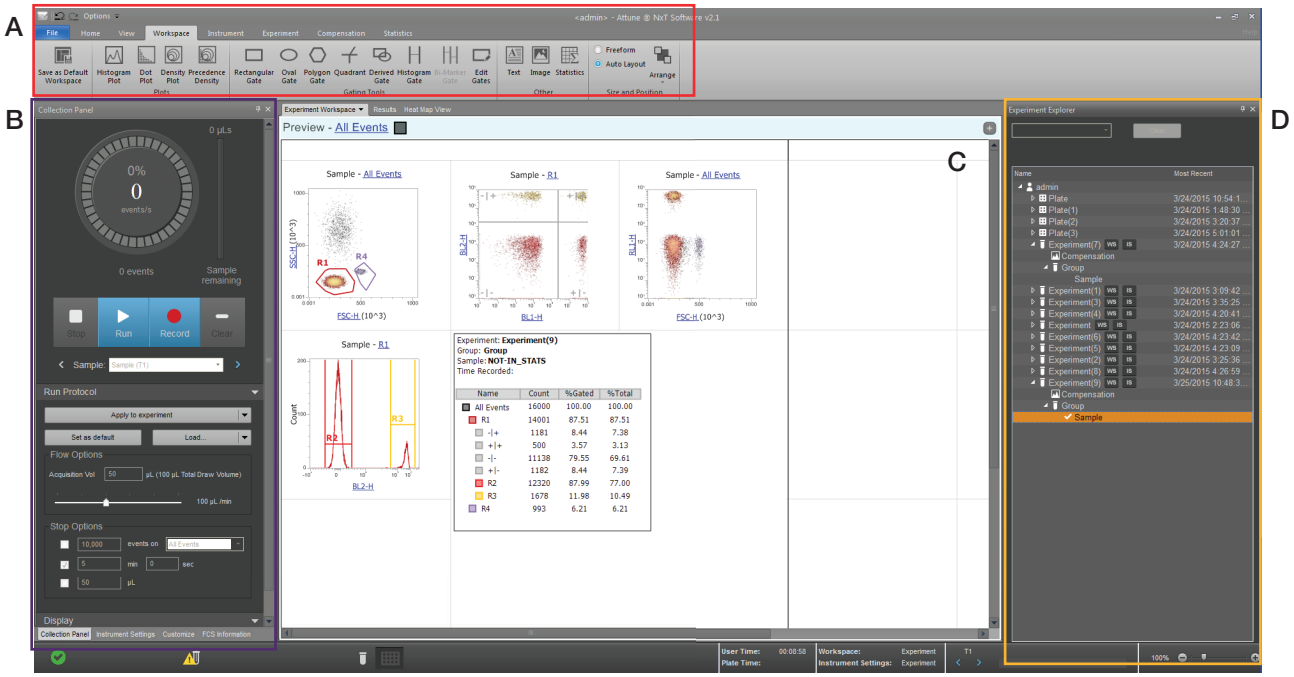


Figure 20. Intuitive software interface with familiar workflow. The user interface is divided into four panels for simple use: **(A)** the Top panel uses ribbons and tabs for most functions (similar to Microsoft™ Office™ products); **(B)** the Collection panel presents an easy setup window to acquire data; **(C)** the Experiment Workspace panel offers display of plots and statistics; and **(D)** the Experiment Explorer panel makes it easy to manage samples and data files.

Improved ease of operation and maintenance

Automated processes make startup, cleaning between samples, and shutdown as easy as a push of a button (Figure 21).

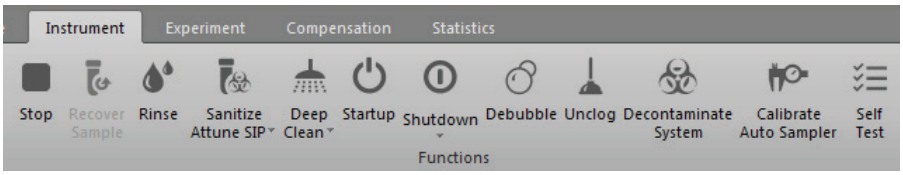


Figure 21. Instrument control tab on Attune NxT Software.

Versatility to meet your analysis needs

Powerful Attune NxT Software allows efficient data acquisition and analysis. Attune NxT Software provides flexible analysis, offering a variety of plotting tools (Figure 22) and plot types (Figure 23).

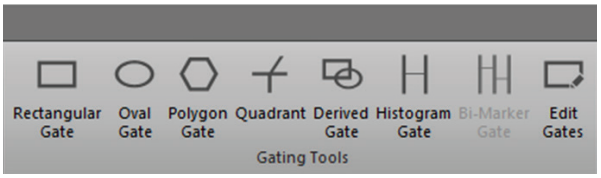


Figure 22. Plotting tools available on Attune NxT Software.

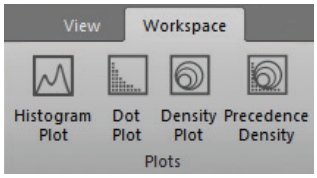


Figure 23. Customizable plot types on Attune NxT Software.

Pre- and post-compensation—automated or manual

Compensation results can be easily achieved by utilizing the automated compensation setup (Figure 24), allowing rapid and accurate compensation, thus eliminating tedious trial-and-error adjustments of compensation matrix coefficients.

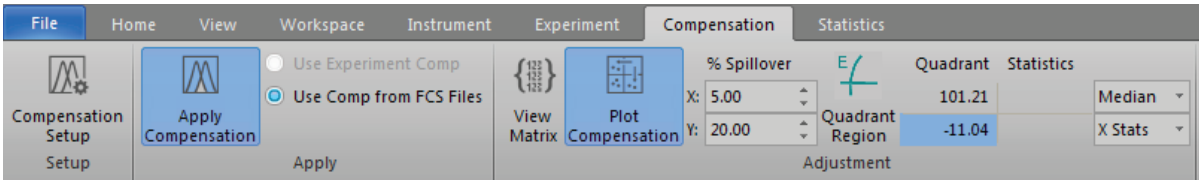


Figure 24. Compensation tab on Attune NxT Software.

Quality control

Performance history and Levey-Jennings plots for all channels are available to track instrument performance over time (Figure 25).

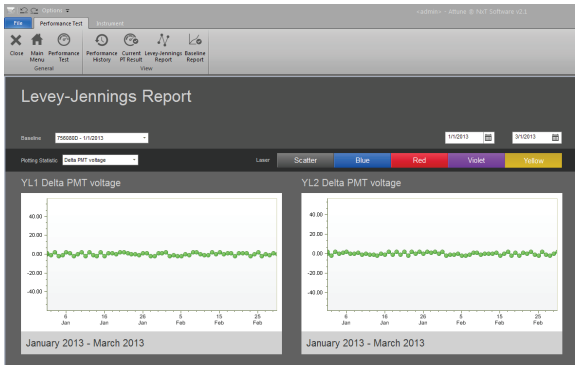


Figure 25. Levey-Jennings tracking plots.

Rapidly process multiple samples

An optional accessory for the Attune NxT Flow Cytometer, the Attune NxT Autosampler enables the rapid processing of multiple samples.

Key features

- Compatible with many different plate formats, including 96-well, 384-well, and deep-well plates (Figure 26)
- Intelligent probe design minimizes clogging and carryover (<1%) (Table 1) and helps prevent damage to the instrument
- Mixes sample by aspiration to maintain sample homogeneity and cell viability (Table 2)

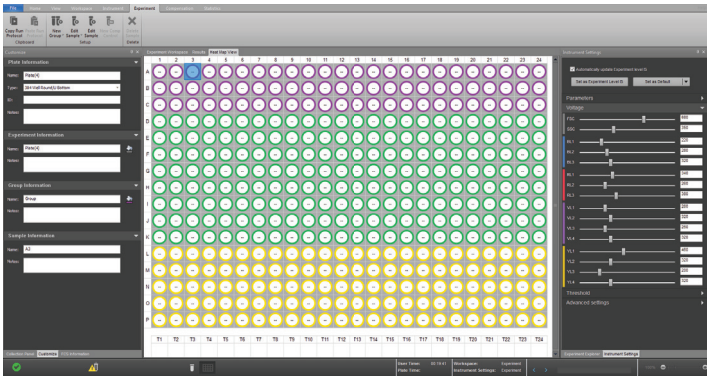


Figure 26. Example of a 384-well heat map.

- Performs automated cleaning when the instrument is shut down
- Helps maintain consistent data while easily switching between use of the autosampler and individual tubes
- Consistent data across different collection rates

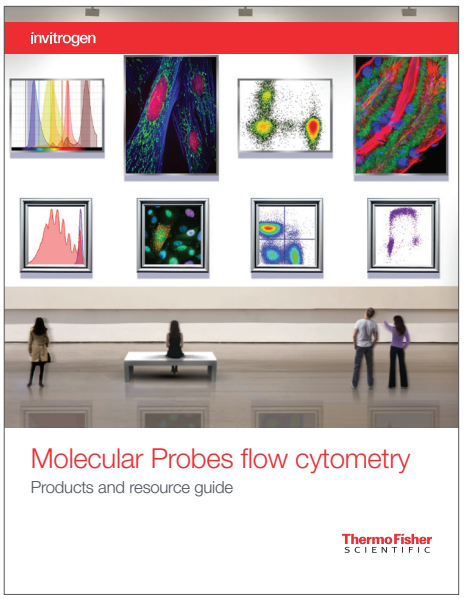
Table 1. Minimal carryover using the Attune NxT Autosampler. Jurkat cells at a concentration of 1 x 10^6 cells/mL were dispensed into a 96-well V-bottom plate and sampled using the Attune NxT Autosampler. Samples were analyzed on the Attune NxT FLow Cytometer using collection rates in Standard mode (200 µL/min) and High Throughput mode (500 µL/min). Each sample was mixed once and the Attune NxT Autosampler was washed 1–3 times prior to sampling the next well. Percent sample carryover was calculated.

	Number of washes and % carryover		
Mode	**1**	**2**	**3**
Standard	0.01	0.01	0.01
High Throughput	0.02	0.02	0.02

Table 2. Gentle sample mixing using the Attune NxT Autosampler: increasing the number of mixing cycles does not adversely affect cell viability. Ammonium chloride–lysed whole blood (LWB) and NIH/3T3 (live/heat-treated) cells were stained with 2 µg/mL propidium iodide and loaded in triplicate into a 96-well V-bottom plate. Prior to acquisition, samples were mixed 0–5 times by the Attune NxT Autosampler and then samples were analyzed using Standard mode collection rates (100 µL/min for NIH/3T3, 200 µL/min for LWB) on the Attune NxT Flow Cytometer. Propidium iodide was excited using a 488 nm laser and fluorescence emission was collected using a 640 nm longpass filter. Minimal variation was observed within each cell type, regardless of the number of mix cycles used prior to acquisition.

	Percentage of dead cells	
Number of mix cycles	**LWB**	**NIH/3T3**
0	0.75	34.10
1	0.78	32.83
2	0.74	33.52
3	0.74	32.75
4	0.74	33.26
5	0.75	31.58

Invitrogen flow cytometry reagents



We have been at the forefront of expanding cellular analysis with the continued invention and development of fluorescent detection molecules and probes for nearly 40 years. This enabled a new, expanded world of flow cytometry. From conjugated antibodies through functional dyes to cell function assays, look to Invitrogen™ flow cytometry products to elucidate your research.

Go to **thermofisher.com/flow-cytometry** for more information on Invitrogen flow cytometry products and resources.

Sample preparation

Quality data require quality starting material. Invitrogen™ sample preparation reagents, which include reagents for blood cell preservation, red blood cell lysis, and sample fixation and permeabilization, are designed to help you achieve the best possible results. Learn more about these products and get protocols at **thermofisher.com/flow-sample**

Instrument setup and calibration

Flow cytometers are designed to perform quantitative measurements on individual cells and other particles with high precision, speed, and accuracy. As with all high-performance instrumentation, flow cytometers must be calibrated regularly to ensure accuracy and reliability. The stability, uniformity, and reproducibility of Invitrogen™ microsphere products make them excellent tools for flow cytometer instrument setup and calibration. Learn more about all of these products at **thermofisher.com/flow-standards**

Antigen detection—primary antibodies

We offer a diverse array of highly specific RUO, ASR, and IVD primary antibodies. From immunophenotyping to rare-event detection, Invitrogen™ multicolor fluorescently conjugated primary antibodies for flow cytometry can help answer complex cell biology questions. Search for primary antibody conjugates at **thermofisher.com/flow-searchantibodies**

Cell analysis—functional dyes

An extensive array of Invitrogen™ stains and kits have been developed to assess cell function, health, and viability (Figure 27). Whether the health of cells is your primary question or simply a critical factor in getting the right answers to other questions, we have a solution for you. For more information and more products, go to **thermofisher.com/flow-cellhealth**

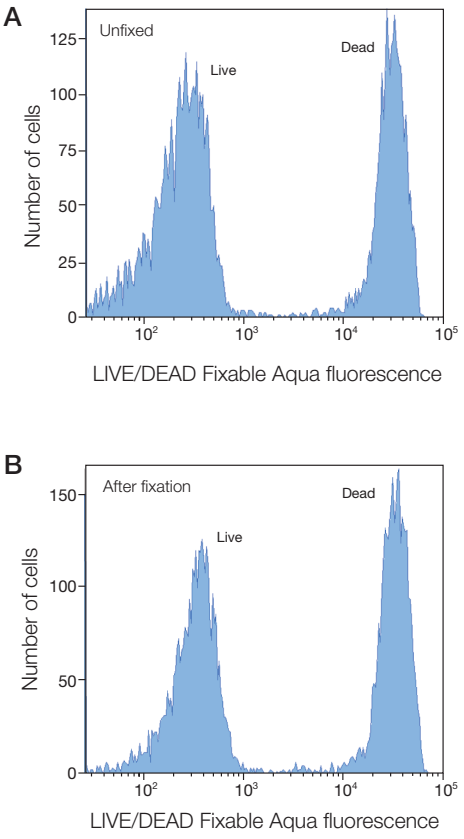


Figure 27. Retention of Invitrogen™ LIVE/DEAD™ Fixable Dead Cell Stains after formaldehyde fixation. The LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Cat. No. L34957) was used to differentially stain a mixture of live (left peak) and heat-treated (right peak) Jurkat cells. **(A)** Unfixed cells. **(B)** Cells fixed in 3.7% formaldehyde following staining. Samples were analyzed by flow cytometry using 405 nm excitation and ~525 nm emission.

Fluorophore selection guide for the Attune NxT Flow Cytometer

The Attune NxT Flow Cytometer is designed to accommodate the most common fluorophores and fluorescent proteins used in flow cytometry, to match the panels you are currently running. Multiple fluorescent proteins can be interrogated with the 4-laser version of the Attune NxT Flow Cytometer.

Table 3. Fluorophore selection guide for the Attune NxT Flow Cytometer.

Excitation laser	Emission filter (nm)	Channel	Recommended labeling dyes	Viability dyes (compatible with fixation)	Viability dyes (nonfixed cells)	DNA content/cell cycle dyes (live cells)	DNA content/cell cycle dyes (fixed cells)	
Violet (405 nm)	440/50	VL1	Alexa Fluor™ 405 Pacific Blue™	LIVE/DEAD™ Fixable Violet	DAPI SYTOX™ Blue	Vybrant™ DyeCycle™ Violet	FxCycle™ Violet	
	512/25	VL2	Pacific Green™	LIVE/DEAD™ Fixable Aqua				
	603/48	VL3	Pacific Orange™ Qdot™ 605	LIVE/DEAD™ Fixable Yellow				
	710/50	VL4	Qdot™ 705					
Blue (488 nm)	530/30	BL1	Alexa Fluor™ 488 FITC	LIVE/DEAD™ Fixable Green	SYTOX™ Green	Vybrant™ DyeCycle™ Green		
	574/26	BL2 (without yellow laser present)	PE		PI SYTOX™ Orange	Vybrant™ DyeCycle™ Orange		
	590/40	BL2 (with yellow laser present)	PE PE–Alexa Fluor™ 610 PE–Texas Red™	LIVE/DEAD™ Fixable Red	7-AAD PI SYTOX™ AADvanced™ SYTOX™ Orange		FxCycle™ PI/RNase	
	695/40	BL3	PE–Alexa Fluor™ 700 PE-Cy®5.5 PerCP PerCP-Cy®5.5 Qdot™ 705 TRI-COLOR™		7-AAD PI SYTOX™ AADvanced™			
	780/60	BL4	PE-Cy®7 Qdot™ 800			Vybrant™ DyeCycle™ Ruby		

	Apoptosis dyes	Cell proliferation dyes	ROS detection dyes	Phagocytosis dyes	Fluorescent proteins	Other dyes
	Annexin V Pacific Blue™ PO-PRO™-1	CellTrace™ Violet Click-iT™ Plus EdU Pacific Blue™			Azurite Cerulean eBFP eCFP mTurquoise Sirius	BV421 eFluor™ 450 Horizon™ V450 VioBlue™
	Violet Ratiometric Probe (F2N12S)				vGFP	AmCyan BV510 Horizon™ V500 VioGreen™
	Violet Ratiometric Probe (F2N12S)					BV570 BV605 BV650 eFluor™ 605NC
						BV650 BV711
	Annexin V–Alexa Fluor™ 488 Annexin V–FITC Anti-PARP–FITC APO-BrdU TUNEL–Alexa Fluor™ 488 CellEvent™ Caspase-3/7 Green MitoProbe™ $DiOC_2(3)$ MitoProbe™ JC-1 YO-PRO™-1 Iodide	CellTrace™ CFSE Click-iT™ Plus EdU Alexa Fluor™ 488	CellROX™ Green	pHrodo™ Green *E. coli* BioParticles™ conjugate pHrodo™ Green *S. aureus* BioParticles™ conjugate	eGFP Emerald eYFP	BB515
	Annexin V PE MitoProbe™ JC-1 TMRE TMRM			pHrodo™ Red *E. coli* BioParticles™ conjugate pHrodo™ Red Phagocytosis Kit	eYFP mCitrine Venus	
	Annexin V PE TMRE TMRM					PE-eFluor™ 610
						PE-CF594 PE/Dazzle™ 594 PerCP-eFluor™ 710 PerCP-Vio700™ VioGreen™ Vio770™
						PE-Vio770™ VioGreen™

Table 3. Fluorophore selection guide for the Attune NxT Flow Cytometer. (continued)

Excitation laser	Emission filter (nm)	Channel	Recommended labeling dyes	Viability dyes (compatible with fixation)	Viability dyes (nonfixed cells)	DNA content/cell cycle dyes (live cells)	DNA content/cell cycle dyes (fixed cells)	
Yellow (561 nm)	585/16	YL1	PE		PI SYTOX™ Orange	Vybrant™ DyeCycle™ Orange		
	620/15	YL2	PE–Alexa Fluor™ 610 PE–Texas Red™		7-AAD PI SYTOX™ AADvanced™ SYTOX™ Orange		FxCycle™ PI/RNase	
	695/40	YL3	PE–Alexa Fluor™ 700 PE-Cy®5.5 Qdot™ 705 TRI-COLOR™		7-AAD PI SYTOX™ AADvanced™			
	780/60	YL4	PE-Cy®7 Qdot™ 800			Vybrant™ DyeCycle™ Ruby		
Red (638 nm)	670/14	RL1	Alexa Fluor™ 647 APC Qdot™ 655	LIVE/DEAD™ Fixable Far Red	SYTOX™ Red		FxCycle™ Far Red	
	720/30	RL2	Alexa Fluor™ 680 Alexa Fluor™ 700 APC–Alexa Fluor™ 700 Qdot™ 705					
	780/60	RL3	APC-Alexa Fluor™ 750 APC-Cy®7	LIVE/DEAD™ Fixable Near-IR		Vybrant™ DyeCycle™ Ruby		

	Apoptosis dyes	Cell proliferation dyes	ROS detection dyes	Phagocytosis dyes	Fluorescent proteins	Other dyes
	Annexin V Alexa Fluor™ 568 Annexin V Alexa Fluor™ 594 MitoTracker™ Orange CMTMRos MitoTracker™ Red CMXRos TMRE TMRM		CellROX™ Orange		dTomato mOrange RFP	
					DsRed mCherry mKate mStrawberry	ECD PE-CF594 PE-Dazzle™ 594 PE-eFluor™ 610 PE-Vio™ 610 Texas Red™
						PE-Vio770™
	Annexin V Alexa Fluor™ 647 Annexin V Alexa Fluor™ 680 Annexin V APC MitoProbe™ $DiIC_1(5)$ TO-PRO™-3	CellTrace™ Far Red Click-iT™ Plus EdU Alexa Fluor™ 647	CellROX™ Deep Red			eFluor™ 660
						APC-H7 APC-eFluor™ 750 APC-eFluor™ 780 APC-Vio770™

Choose a service plan that is right for you—whatever your priorities

Beyond repair to proactive care

- Gain peace of mind during every stage of ownership: instrument install, repair, and maintenance
- Over 1,000 technical specialists delivering on flexible and reliable service options
- 30 years of servicing life sciences instrumentation
- Concierge and excellent escalation process to help get you up and running faster
- Wide range of flexible service plans to help realize savings in instrument care

AB Assurance Plan*

This is our premium repair plan designed to help maximize instrument performance and help ensure availability of critical systems. The plan can help you keep your lab running smoothly with preventive maintenance, proactive instrument monitoring, and fast response should an instrument require repair. Our Uptime option provides guaranteed next–business day, on-site response.

The AB Assurance Plan features:

- Guaranteed 2-business day response time*
- All-inclusive contract price—all labor, parts, and engineer travel included: no caps or hidden fees
- Complete computer coverage
- Consumables used for troubleshooting included
- Remote monitoring and diagnostics
- Phone and email access to technical support
- Proactive planned maintenance and Pure Dye Calibration Service scheduling
- Experienced, certified engineers
- Certified parts and consumables
- Proactive software updates

AB Maintenance Plan*

Ideal for labs that don't have critical availability needs, this plan is designed to help you maximize instrument performance, even on a limited budget. This plan is also an ideal fit for those with internal resources and expertise to maintain instrumentation on an ongoing basis.

Features:

- Planned maintenance visit(s)—includes engineer labor and travel
- Phone and email access to application technical support
- Discount on labor and parts for each service call (Plus option)

A la carte services

Many of the value-added services are available on an a la carte basis, allowing you to supplement your in-house abilities or mix and match services based on your needs and budget.

These services include:

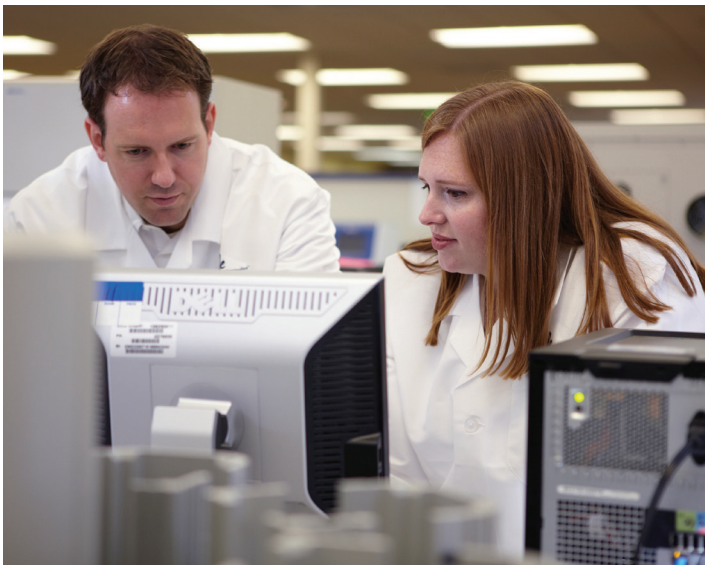
- Qualification service
- Smart services
- On-site application consulting
- Professional services
- Time and materials service

*Availability limited in some geographic areas.

Smart Monitor Service: remote monitor and diagnostics

Smart Monitor Service is a remote monitoring and diagnostics service used to help improve instrument uptime by enabling service personnel to proactively respond to and remotely diagnose instrument issues. We use a proprietary software application that provides instrument and instrument computer performance data on a periodic basis to a secure, centrally located server and database. Smart Monitor Service monitors the instrument's critical operating parameters and provides peace of mind, during unattended operation, that your unit is functioning as designed.*

- Helps improve uptime
- Remote monitoring and diagnostics from anywhere in the world where instrument is able to be networked
- Helps decrease total cost of product ownership
- Telephone access to Remote Service Engineers
- Historic performance data and reporting



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If your laboratory operates in a regulated environment, you may be required to qualify and validate your systems according to vendor specifications and your internal requirements. Global standards and country-specific regulations require documented verification that your system is doing the job correctly as intended. Complex, time-consuming, and costly, this process can be overwhelming. We can help.

Solutions

Installation Qualification (IQ) verifies that, at the time of testing, your system was received as ordered and installed according to the manufacturer's specifications. It also establishes that your laboratory environment is suitable for operation of the system.

Operational Qualification (OQ) verifies that, at the time of testing, your system functions according to operational specifications per the manufacturer. It also verifies that the equipment operates consistently within established limits and tolerances over the defined operating ranges per the manufacturer's specifications.

IQ/OQ is an integral part of a validation process for compliance with Good Laboratory Practices (GLP), ISO 9000, and other standards.

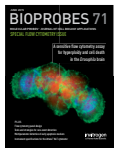
* Instrument needs to be networked.

Resources

Reference guides

***The Molecular Probes™ Handbook*, 11th Edition**
The most complete reference on fluorescent labeling and detection available, this resource features extensive references and technical notes and contains over 3,000 technology solutions representing a wide range of biomolecular labeling and detection reagents. See the online version of The *Molecular Probes Handbook* and request your free copy* at **thermofisher.com/handbook**

Molecular Probes™ Flow Cytometry Resource Guide
Our flow cytometry products and resource guide presents an overview of primary antibody conjugates, cell analysis reagents, and other tools optimized for flow cytometry. Access your free copy* at **thermofisher.com/flowguide**



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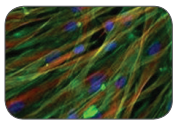

Antibody selection tool
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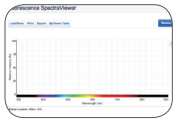

Fluorescence SpectraViewer
Plot up to 14 fluorophores on a single graph that you can then print or save for later.
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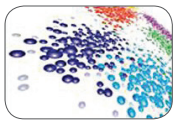
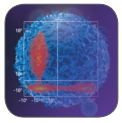
Molecular Probes™ Flow Cytometry Panel Design Tool
Choose fluorescent antibody conjugates: pick the antibody species reactivity, select up to 14 targets of interest (choices include viability dyes), and choose the lasers or fluorophores you want to view. Print or email your list.



Flow cytometry resources center
Search for protocols, tutorials, application notes, fluorophore and product selection guides, literature, and many other technical resources in a single place.
thermofisher.com/flowresources

Interactive instrument 3D explorer demo
Explore the features of the Attune NxT Flow Cytometer with the virtual demo. Spin the unit around and interact with key features. **thermofisher.com/attune**

Mobile apps for research on the go



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*Not available in all countries.

Ordering information

Lasers	Laser colors	Parameters	Cat. No.
Attune NxT Flow Cytometer			
4	Blue, red, yellow, violet	16	A24858
3	Blue, violet, yellow	13	A24859
3	Blue, red, violet	13	A24860
3	Blue, red, yellow	12	A28993
2	Blue, yellow	9	A24861
2	Blue, violet	10	A24862
2	Blue, red	9	A24863
1	Blue	6	A24864

Product		Cat. No.
Attune NxT upgrades		
Attune NxT Yellow Laser Upgrade Kit		100022779
Attune NxT Violet Laser Upgrade Kit		100022777
Attune NxT Red Laser Upgrade Kit		100022778
Attune NxT Fluorescent Protein Filter Kit—GFP, YFP, mCherry		100022775
Attune NxT No-Wash No-Lyse Filter Kit		100022776
Attune NxT Custom Filter Holder Kit		A27784
Attune NxT accessories	**Quantity**	
Attune NxT Autosampler		4473928
Attune NxT Software Dongle	1	A25554
Attune NxT Software Dongle	5	A24856
Attune NxT Software Dongle	10	A24855
Attune NxT reagents and consumables		
Attune Focusing Fluid (1X), 1 L		4488621
Attune Focusing Fluid (1X), 10 L		A24904
Attune Wash Solution		A24974
Attune Shutdown Solution		A24975
Attune Performance Tracking Beads		4449754
Attune NxT Service Contracts		
Attune NxT 1-Laser System—AB Maintenance including 1 planned maintenance (PM)		ZG51SCATTUNEB
Attune NxT 1-Laser System—AB Assurance including 1 PM		ZG11SCATTUNEB
Attune NxT 2-Laser System—AB Maintenance including 1 PM		ZG51SCATTUNEBRBVBY
Attune NxT 2-Laser System—AB Assurance including 1 PM		ZG11SCATTUNEBRBVBY
Attune NxT 3-Laser System—AB Maintenance including 1 PM		ZG51SCATTUNEBRVBVY
Attune NxT 3-Laser System—AB Assurance including 1 PM		ZG11SCATTUNEBRVBVY
Attune NxT 4-Laser System—AB Maintenance including 1 PM		ZG51SCATTUNEBVRY
Attune NxT 4-Laser System—AB Assurance including 1 PM		ZG11SCATTUNEBVRY

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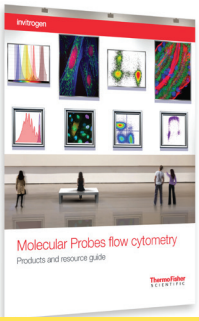

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