ThermoFisher SCIENTIFIC

Flow cytometry

Attune flow cytometers, a family built for accelerated discovery

Seamlessly integrating high-speed, acoustic focusing technology for every lab's needs

invitrogen

Core technology—acoustic hydrodynamic focusing

At the heart of all Invitrogen™ Attune™ flow cytometers is our innovative acoustic hydrodynamic focusing technology. This key differentiator allows for:

Higher flow rates and faster time to results—Acoustic focusing enables rapid sample acquisition, significantly reducing the time to results.

Clog-resistant operation—Helps ensure enhanced productivity and uptime by minimizing the risk of clogs, even with complex samples.

Acoustics-assisted hydrodynamic focusing technology

Attune flow cytometers combine ultrasonic waves like those used in medical imaging with hydrodynamic forces to precisely position cells into a single, focused line in the central axis. Enabling cells to be tightly focused at the point of laser interrogation allows the system to collect more photons, helping to ensure data quality regardless of the sample-to-sheath ratio (Figure 1). Increased photon collection allows for a higher degree of data, detail, and throughput to enable processing of a large range of sample types more quickly and accurately than ever before, with no loss in data quality. Sample types that can be processed include large clumpy cells, samples with a low concentration of cells, and precious samples.

Novel optical design

Attune flow cytometers feature a novel optical design that delivers exceptional reliability and excellent performance over time. The flat-top beam profile of the solid-state lasers minimizes the effects of changes in fluidics or optics, which in turn can lead to instability or alignment issues and instrument downtime.

Laser misalignment is a major concern with users of conventional flow cytometers. The flat-top lasers used in Attune flow cytometers have an intensity profile that allows a wider window of alignment over Gaussian lasers used in traditional systems (Figure 2). The flat-top lasers also have a higher tolerance for misalignment that allows them to maintain high sensitivity and low coefficients of variation (CVs).

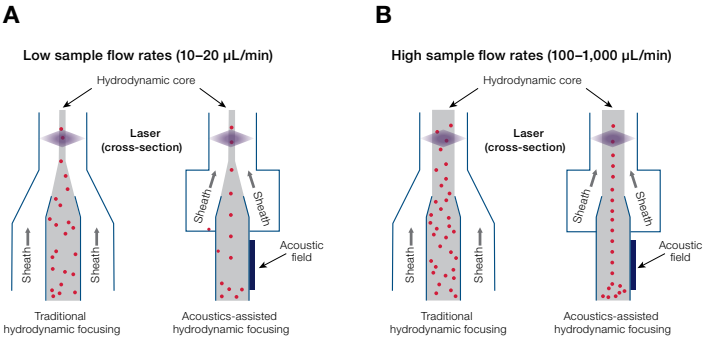

Figure 1. Acoustic focusing vs. traditional hydrodynamic focusing as particles pass through the laser. (A) At low sample rates, cells are tightly aligned with both acoustic-assisted and hydrodynamic focusing. Tight alignment is important for good data quality. **(B)** At high sample rates, traditional hydrodynamic focusing results in widening of the sample core stream, which leads to increased signal variation and compromised data quality relative to the tight alignment of cells seen in the acoustics-assisted hydrodynamic focusing.

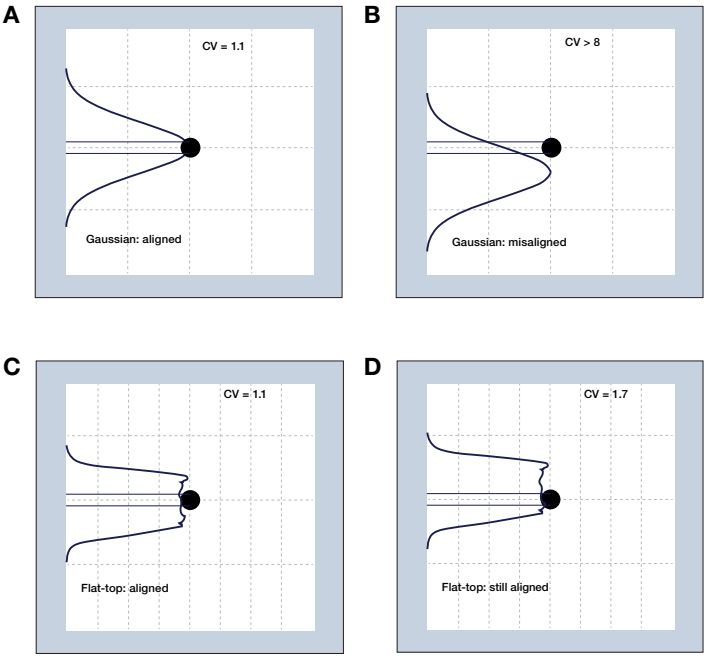

Figure 2. Emission profile of lasers used in flow cytometers. **(A)** Gaussian laser profile with proper alignment, **(B)** Gaussian laser profile with misalignment, **(C)** flat-top laser profile with proper alignment, and **(D)** flat-top laser profile still in proper alignment.

Reduce clogging from difficult samples

Your research samples are precious, as they are often difficult to obtain. Attune flow cytometers are less prone to clogging than traditional flow cytometers, allowing challenging samples such as cardiomyocytes, heterogeneous blood cells, and cancer cells to flow with confidence.

Engineered to actively resist clogging, a syringe-driven system (Figure 3) and larger flow cell help prevent the loss of precious samples such as cancer stem cells from primary pancreatic tumors, and the system is significantly less susceptible to clogs. Attune flow cytometers employ a nonpressurized system that mechanically decreases the occurrence of clogging.

No lyse, no wash, with no compensation

Acoustic focusing allows Attune flow cytometers to deliver a no-wash, no-lyse (NW/NL) protocol to minimize cell loss and significantly shorten and simplify sample preparation (Figure 4). The NW/NL protocol allows for analysis of diluted whole blood to separate out white blood cells (WBCs) from red blood cells (RBCs) and platelets using violet and blue laser side scatter parameters.

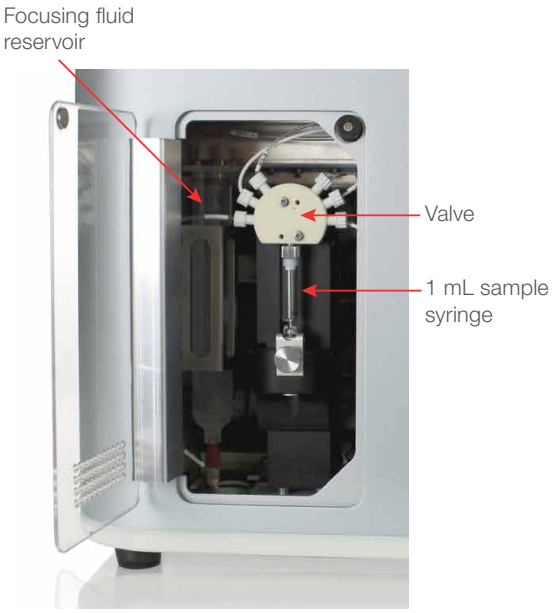

Figure 3. Positive-displacement syringe pump. The syringe is easily removed for cleaning or replacement in this example from the Invitrogen™ Attune™ NxT Flow Cytometer.

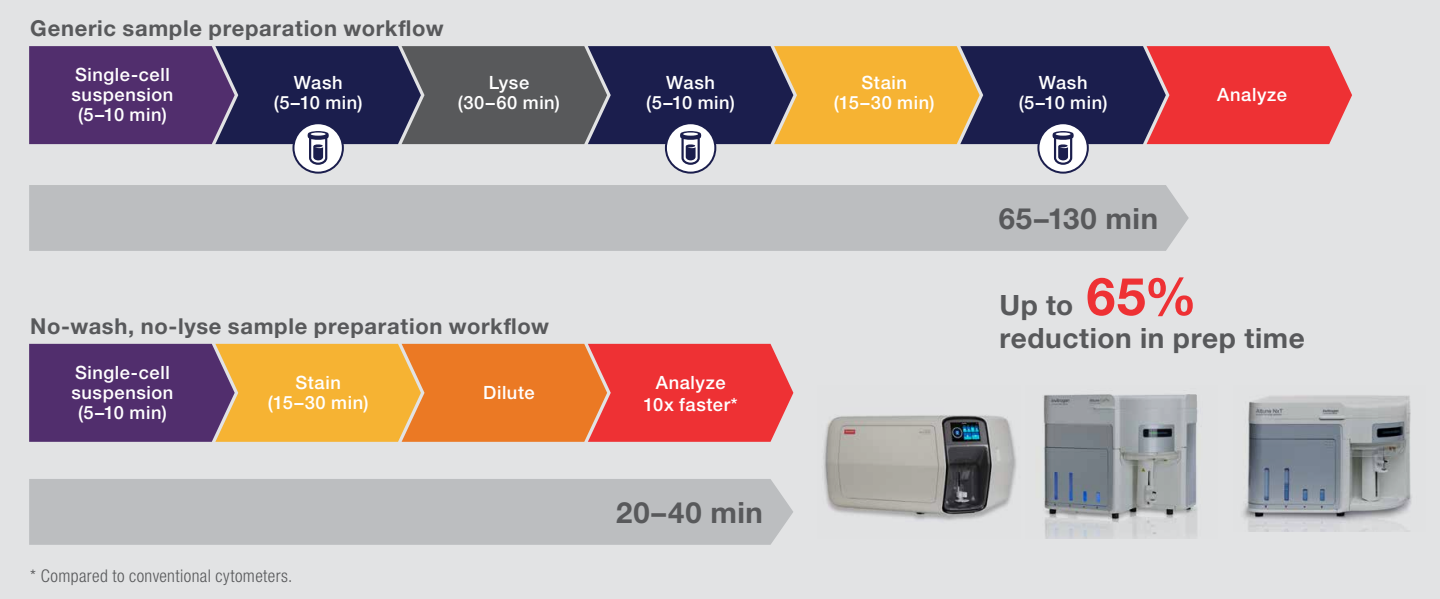

Figure 4. No-wash, no-lyse sample preparation workflow.

Transformative high-speed cell analyzers

In today's fast-paced research environment, speed, precision, and reliability are essential to accelerating discoveries. The Attune flow cytometer family is engineered to set a new standard in flow cytometry, featuring revolutionary acoustic focusing technology that uses sound waves to align cells precisely within the flow cell. This innovative approach surpasses traditional methods, resulting in faster processing speeds, exceptional data quality, and increased throughput.

Whether performing complex immunophenotyping, rare cell analysis, or high-throughput screening, the Attune family delivers excellent performance, intuitive automation, and seamless integration with our flow reagents and software, enabling you to achieve reproducible results quickly.

Explore the next generation of flow cytometry with Attune instruments and transform your cell analysis. Thermo Fisher Scientific offers comprehensive solutions including Invitrogen™ Attune™ flow cytometers, CytKick™ Autosamplers, cell health reagents, and eBioscience™ antibody conjugates to help drive groundbreaking research and new biological insights. These comprehensive solutions are charactrized by:

- **Acoustic focusing technology**—a core feature across all Attune instruments, providing speed, reliability, and clog-resistant operation for enhanced productivity and uptime
- **Reliability and precision**—trusted by researchers worldwide for delivering accurate and reproducible results
- **Ease of use**—designed with user-friendly interfaces and automated features to simplify lab workflows
- **Comprehensive support**—backed by exceptional customer service and technical support to help ensure your success.
- **End-to-end solutions**—from instruments to reagents and automation, Thermo Fisher Scientific offers a comprehensive suite of products to meet all your flow cytometry needs

Table 1. Features of Invitrogen™ Attune™ flow cytometers.

Category	Feature	
Optics	Number of lasers	
	Number of detection channels	
	Imaging illumination	
	Optical alignment	
Fluidics	Acoustic focusing	
	Custom sample flow rates	
Performance (fluorescence detection)	Fluorescence sensitivity	
	Fluorescence resolution	
	Maximum electronic speed	
Performance (imaging)	Image capture rate	
	Objective	
	Pixel resolution	
	Detection limit	
Automated image analysis	Trained models	
	Morphology parameters	
	Sample size range of models	
	Image processing speed	
Quality and regulatory	Instrument tracking	
	Regulatory status	
Physical	Dimensions (H x W x D)	
	Biosafety hood compatibility	
Computer	Memory	
	Hard drives	
	Graphics processor	

* rCV = robust coefficient of variation.

** CV = coefficient of variation.

	Attune™ Xenith™ Flow Cytometer	Attune™ CytPix™ Flow Cytometer	Attune™ NxT Flow Cytometer
	6 **Excitation lasers:** 349 nm, 405 nm, 488 nm, 561 nm, 637 nm, 781 nm	2–4	1–4
	6 scatter channels, 51 fluorescence channels	2 scatter channels, up to 14 fluorescence channels	2 scatter channels, up to 14 fluorescence channels
	NA	405 nm laser with <50 nsec pulse width	NA
	Fixed alignment with pre-aligned welded fiber; no user maintenance required	Fixed alignment with pre-aligned welded fiber; no user maintenance required	Fixed alignment with pre-aligned welded fiber; no user maintenance required
	✓	✓	✓
	✓	✓	✓
	FITC ≤ 30, PE ≤10 APC ≤10	≤80 MESF for FITC, ≤30 MESF for PE, ≤70 MESF for APC	≤80 MESF for FITC, ≤30 MESF for PE, ≤70 MESF for APC
	rCV* <3% for the singlet peak of propidium iodide–stained chicken erythrocyte nuclei (CEN)	CV** <3% for the singlet peak of propidium iodide-stained CEN	CV** <3% for the singlet peak of propidium iodide-stained CEN
	>100,000 events/sec with all parameters	Up to 35,000 events/sec with all parameters	Up to 35,000 events/sec with all parameters
	NA	Up to 6,000 images/sec depending on image size and event rate	NA
	NA	Magnification 20x, numerical aperture 0.45	NA
	NA	0.3 µm/pixel	NA
	NA	Visually detect 800 nm particles	NA
	NA	Leukocytes and beads	NA
	NA	Morphology parameter categories: shape, intensity and texture, pixel, particle interaction, particle count, similarity calculation, moment weighted, co-occurrence, and system features	NA
	NA	5–20 µm	NA
	NA	**Fast:** 1,000 images/second **Standard:** varies by image size and complexity	NA
	NA	Automated daily baseline and performance test with Levey-Jennings plots	Automated daily baseline and performance test with Levey-Jennings plots
	For Research Use Only	For Research Use Only	For Research Use Only
	~50 x 86 x 61 cm (~19.5 x 34 x 24 in.)	~49 x 58 x 43 cm (~19 x 23 x 17 in.)	~40 x 58 x 43 cm (~16 x 23 x 17 in.)
	✓	✓	✓
	64 GB	64 GB (4 x 16 GB) DDR4 2,666 MHz UDIMM non-ECC	32 GB
	2 x 8 TB SSD, 560 MB/sec; controller RAID1, integrated	2 x 8 TB SSD, 2.5-inch Samsung™ 870 QVO, 560 MB/s	2 x 2 TB SATA 3.0 GB/s, 8 MB data burst cache; controller RAID 1, integrated
	NVIDIA™ RTX™ A2000	NVIDIA™ Quadro™ P2200 GPU	NA

Efficient—rapid, accurate acquisition

Acoustic focusing can process samples ~10x faster than conventional cytometers

Fast, accurate acquisition

Acoustic focusing empowers your lab to rapidly acquire high-quality data. You can achieve sample throughput rates of 12.5 µL/min to 1,000 µL/min, up to 10 times faster than traditional hydrodynamic focusing systems, and acquisition speeds of up to 35,000 events per second (Figure 5). This means you can process difficult samples—including low-concentration and precious samples—more quickly and accurately with minimal loss in quality.

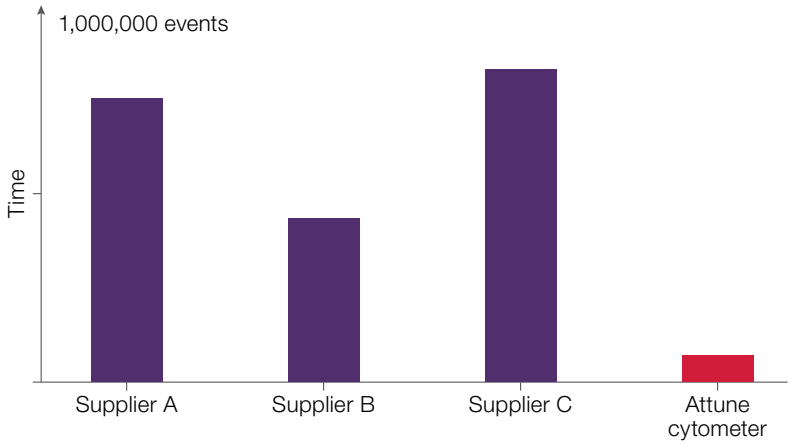

Figure 5. Rapid data acquisition. The time required for an Attune flow cytometer to acquire 1,000,000 events is compared to three instruments from other suppliers, running at maximum sample rates.

Rare-event detection

Detection of rare events requires acquisition of high numbers of cells to attain a reliable measure of accuracy. Attune flow cytometers allow dilute samples to be processed quickly at sample input speeds of up to 1 mL/min, ~10x faster than conventional cytometers that support maximum sample input rates of 60–100 µL/min. Acoustic focusing thus offers an exceptional combination of speed and quality, cutting the time to collect rare events significantly over long acquisition times (Figure 6).

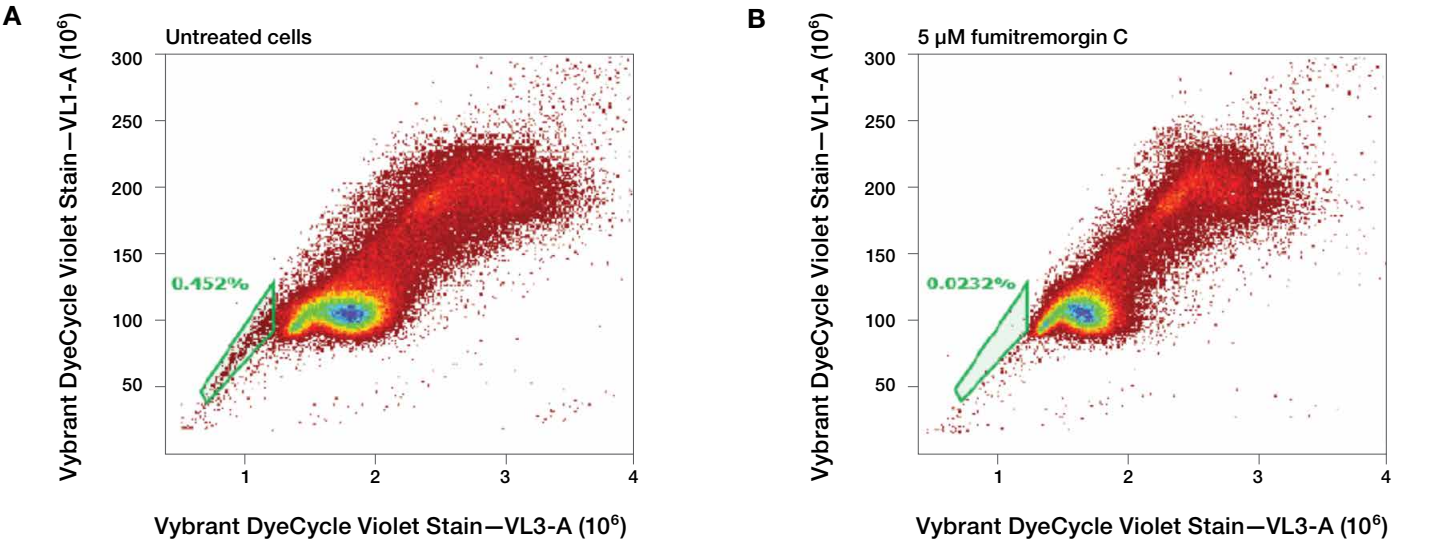

Figure 6. Identifying limbal stem cells (LSC) in a population of differentiated corneal cells. (A) Untreated cells have a side population of limbal stem cells (0.452% of total cells) with decreased Invitrogen™ Vybrant™ DyeCycle™ Violet fluorescence due to ABCG2-mediated dye efflux. **(B)** The percentage of cells in the side population is reduced (0.023% of total cells) when the ABCG2 membrane pump is inhibited with 5 µM fumitremorgin C, preventing efflux of Vybrant DyeCycle Violet dye.

Flexible—multiple configurations and upgradable

Designed for flexibility

Whether you configure your system now or upgrade later, Attune NxT and Attune CytPix Flow Cytometers can grow with you and your research needs. Attune NxT and Attune CytPix Flow Cytometers accommodate up to 14 color panels. The filters and lasers are configurable and field upgradable, giving the freedom to upgrade up to 4 lasers and 16 detection channels (Table 2).

- Modular design for 1–4 laser systems (single laser not available on the Attune CytPix Flow Cytometer)
- Up to 14-color flow cytometry
- Available with violet 6-channel configuration

Table 2. Configurations of lasers and detectors in Attune NxT and Attune CytPix Flow Cytometers.

		Number of detection channels for included lasers							
Lasers	Laser configuration	Violet 405 nm	Blue 488 nm	Green 532 nm	Yellow 561 nm	Red 637 nm	Total detection channels**	Attune CytPix system Cat. No.	Attune NxT system Cat. No.
1	Blue	Available as upgrade	4	Available as upgrade*	Available as upgrade	Available as upgrade	6	NA	A24864
2	Blue/green	Available as upgrade	3	4	–	Available as upgrade	9	NA	A28995
	Blue/yellow	Available as upgrade	3	–	4	Available as upgrade	9	A51842	A24861
	Blue/red	Available as upgrade	4	Available as upgrade*	Available as upgrade	3	9	A51840	A24863
	Blue/violet	4	4	Available as upgrade*	Available as upgrade	Available as upgrade	10	A51841	A24862
	Blue/violet 6	6	3	–	Available as upgrade	Available as upgrade	11	A51843	A29002
3	Blue/green/red	Available as upgrade	3	4	–	3	12	NA	A28997
	Blue/red/yellow	Available as upgrade	3	–	4	3	12	A51845	A28993
	Blue/green/ violet	4	3	4	–	Available as upgrade	13	NA	A28999
	Blue/violet/ yellow	4	3	–	4	Available as upgrade	13	A51846	A24859
	Blue/red/violet	4	4	Available as upgrade*	Available as upgrade	3	13	A51844	A24860
	Blue/red/violet 6	6	3	–	Available as upgrade	3	14	A51847	A29003
4	Blue/red/violet/ green	4	3	4	–	3	16	NA	A29001
	Blue/red/ yellow/violet	4	3	–	4	3	16	A51848	A24858
	Blue/red/ yellow/violet 6	6	2	–	3	3	16	A51849	A29004

* Green laser not available on Attune CytPix system.

** Number of detection channels includes all fluorescence channels as well as a forward scatter and a side scatter channel.

Attune NxT Flow Cytometer

Efficiency, speed, accuracy

The Attune NxT Flow Cytometer combines acoustic focusing technology with traditional hydrodynamic focusing to increase acquisition speeds up to 10x faster than other flow cytometers, helping reduce the time to results. The instrument easily runs most samples, and acoustic focusing allows for minimization of cell loss using a NW/NL protocol (Figure 8). The Attune NxT Flow Cytometer offers a modular design to help provide flexibility for future needs, and its small footprint requires minimal bench space. Additional benefits include:

- **Compact and versatile**—excellent for a wide range of applications with its compact design and customizable configurations
- **High throughput**—enhanced with automated sample loading and processing, improving lab efficiency
- **User-friendly software**—intuitive interface for easy setup and analysis
- **Acoustic focusing**—delivers fast, reliable results and reduces downtime

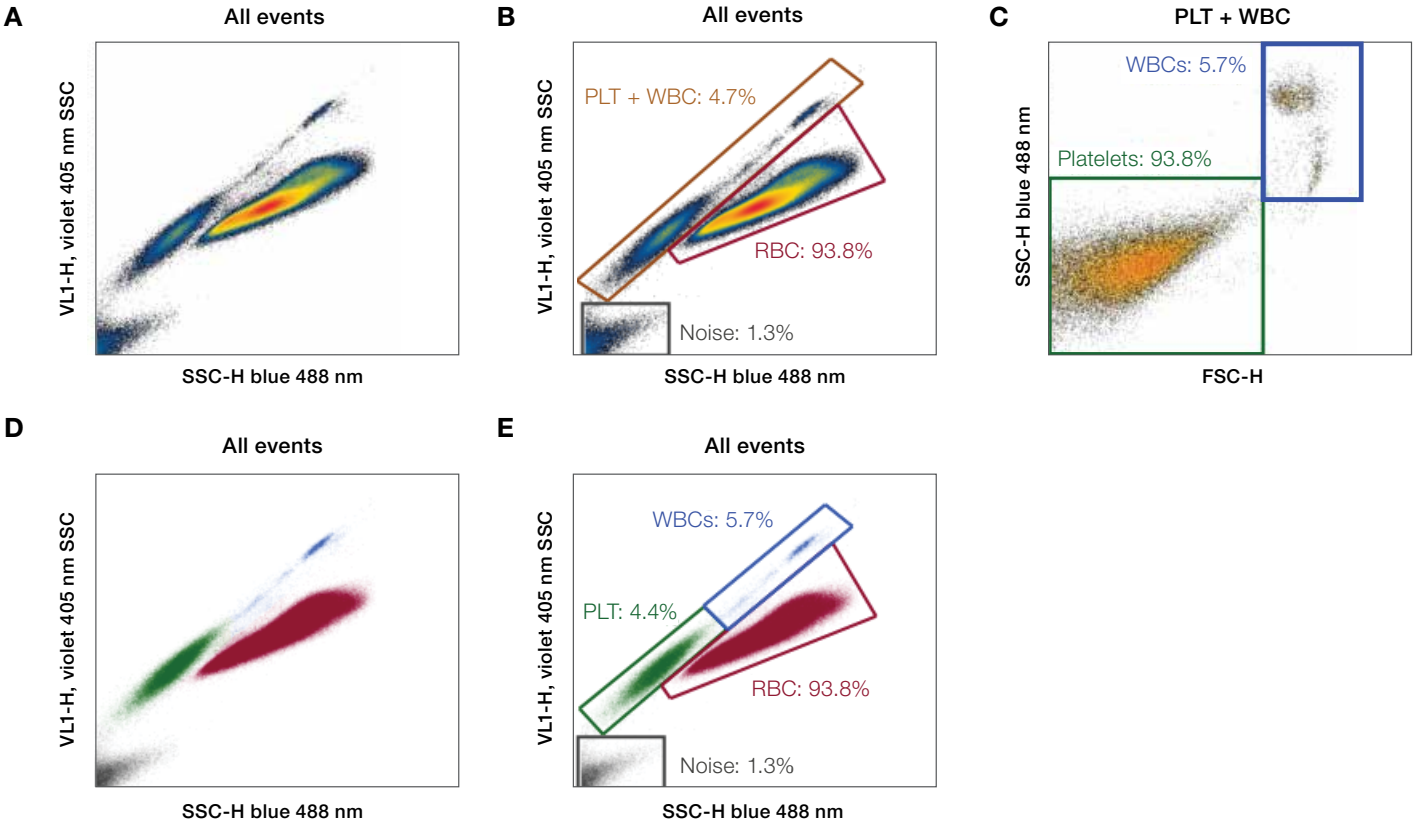

Figure 7. Forward scatter (FSC) and side scatter (SSC) analysis with blue (488 nm) and violet (405 nm) lasers on intact whole blood (no-lyse, no-wash). (A, B) RBCs, white blood cells (WBCs), and platelets (PLTs) are separated on the basis of light scatter only by using a combination of blue and violet laser SSC analysis. Hemoglobin in RBCs readily absorbs light at 405 nm, shifting the RBC population to the right by reducing the SSC for RBCs in the violet laser channel relative to leukocytes and platelets. The dual FSC and SSC threshold is set low enough to show instrument noise, helping ensure the full platelet population is visualized. **(C)** Using the gate that includes WBCs and PLTs, a standard plot of FSC vs. 488 nm SSC can be used to distinguish the PLT population from the WBCs with regions created around the two populations. **(D)** Using color-backgating on plot **(A)**, the RBC population is colored red, the PLT population is colored green, and the WBC population is colored blue, while the noise is black. The three main WBC populations of lymphocytes, monocytes, and granulocytes can be distinguished. **(E)** Placing regions around the RBC, WBC, and PLT populations shows the dominant cell type in whole blood is the RBC, while the WBCs and PLTs are relatively rare events.

Expand the range of performance for your violet laser

Attune flow cytometers are easily upgradable to 6-channel detection for the violet (405 nm) laser (Table 3). Attune flow cytometers with the violet 6-channel configuration are designed to accommodate a wide variety of experimental conditions. Combined with the Invitrogen™ Super Bright™ dyes and other appropriate dyes, the system provides expanded choices for panel design (Table 4). See the available Super Bright dyes at **thermofisher.com/superbright**.

Table 3. The Attune NxT Flow Cytometer filter configurations.

Cat. No.	A24864	A28995	A24861	A24863	A24862	A29002	A28997	A24860	A28999	A28993	A24859	A29003	A29004	A29001	A24858
Detectors	4	7	7	7	8	9	10	11	11	10	11	12	14	14	14
Channel	Emission filter (nm)														
BL1	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30
BL2	574/26	590/40	590/40	574/26	574/26	574/26	590/40	574/26	590/40	574/26	590/40	574/26	695/40	590/40	590/40
BL3	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40		695/40	695/40
BL4	780/60			780/60	780/60			780/60							
GL1		575/36					575/36		575/36					575/36	
GL2		620/15					620/15		620/15					620/15	
GL3		695/40					695/40		695/40					695/40	
GL4		780/60					780/60		780/60					780/60	
YL1			585/16							585/16	585/16		585/16		585/16
YL2			620/15							620/15	620/15		620/15		620/15
YL3			695/40							695/40	695/40		780/60		695/40
YL4			780/60							780/60	780/60				780/60
RL1				670/14			670/14	670/14		670/14		670/14	670/14	670/14	670/14
RL2				720/30			720/30	720/30		720/30		720/30	720/30	720/30	720/30
RL3				780/60			780/60	780/60		780/60		780/60	780/60	780/60	780/60
VL1					440/50	450/40		440/50	440/50		440/50	450/40	450/40	440/50	440/50
VL2					512/25	525/50		512/25	512/25		512/25	525/50	525/50	512/25	512/25
VL3					603/48	610/20		603/48	603/48		603/48	610/20	610/20	603/48	603/48
VL4					710/50	660/20		710/50	710/50		710/50	660/20	660/20	710/50	710/50
VL5						710/50						710/50	710/50		
VL6						780/60						780/60	780/60		

Table 4. Suggested Invitrogen™ fluorophores for the 6 available fluorescence detectors for the violet laser available in the violet-6 configuration of Attune NxT and CytPix flow cytometers.

Detector	Bandpass (nm)	Fluorophores*
VL1	450/40	Super Bright™ 436, eBioscience™ eFluor™ 450, LIVE/DEAD™ Fixable Violet, Vybrant™ DyeCycle™ Violet, SYTOX™ Blue, CellTrace™ Violet, VioBlue™, Brilliant Violet™ 421, Pacific Blue™, BD Horizon™ V450 dyes
VL2	525/50	eBioscience™ eFluor™ 506, LIVE/DEAD™ Fixable Aqua, CFP, VioGreen™, Brilliant Violet™ 510, Pacific Green™, BD Horizon™ V500 dyes
VL3	610/20	Super Bright™ 600, LIVE/DEAD™ Fixable Yellow, Qdot™ 605, Pacific Orange™, Brilliant Violet™ 605 dyes
VL4	660/20	Super Bright™ 645, Brilliant Violet™ 650 dyes
VL5	710/50	Super Bright™ 702, Qdot™ 700, Brilliant Violet™ 711 dyes
VL6	780/60	Super Bright™ 780, Brilliant Violet™ 786 dyes

* List is not inclusive of all available fluorophores.

Attune CytPix Flow Cytometer

Two data sets, one step, zero doubt

With the Attune CytPix Flow Cytometer, you can easily and rapidly highlight structural features of large populations (Figure 8). Using the automated image analysis software, you can rapidly analyze images using trained image processing models to generate morphology parameters that enhance your gating strategy by including cells of interest while excluding aggregates, unwanted cells, and debris (Figure 9). The morphology parameters can also help you gain new insights into sample biology, such as cell–cell interactions (Figure 10).

- **Integrated imaging and flow cytometry**—combines high-speed flow cytometry with high-resolution imaging for comprehensive analysis
- **Real-time image capture**—allows users to capture images of individual cells in real-time, providing additional insights
- **Advanced data analysis**—integrates flow cytometry data with imaging data for a more complete understanding of cell populations
- **User-friendly software**—simplifies the process of correlating flow cytometry data with imaging data
- **Acoustic focusing**—maintains high speed and clog-resistant operation, even with integrated imaging

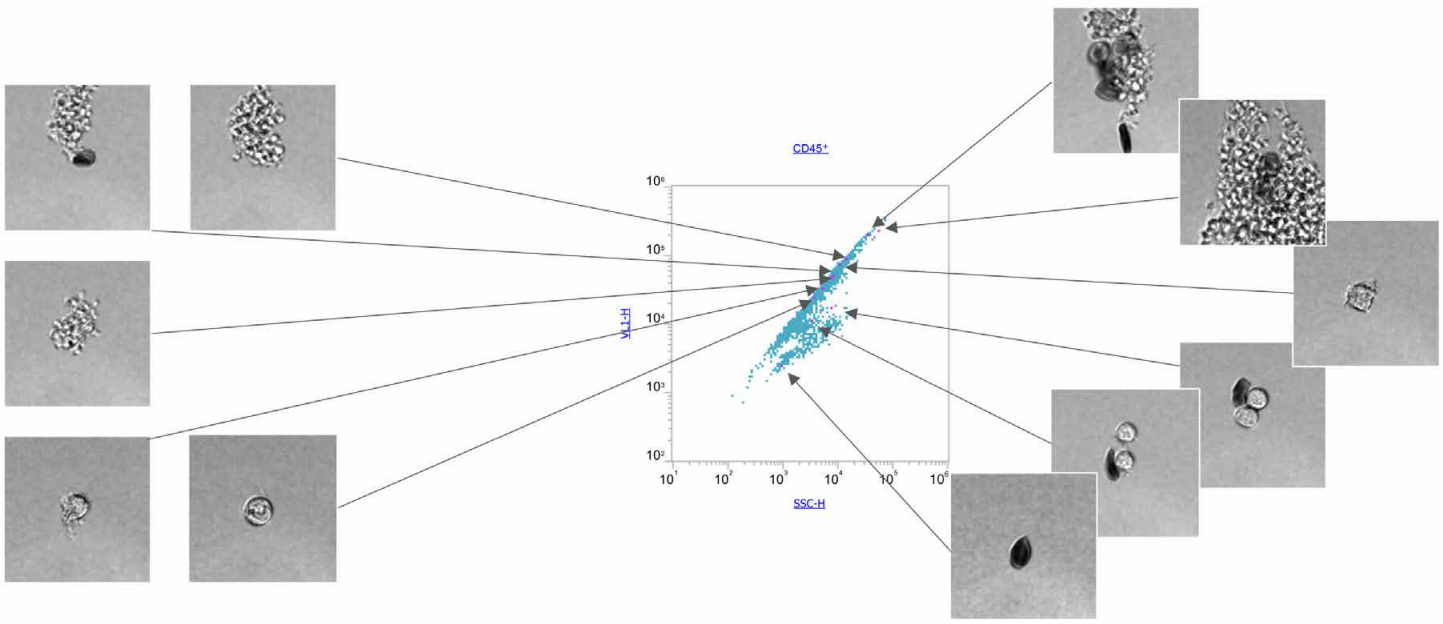

Figure 8. Gain insight with rare-population analysis. Cells were acquired from 24-hour-old blood diluted in 1 mM EDTA (<1:4,000). Samples were acquired at 25 µL/min. The image gate was set to record only CD45⁺ events.

Automated image analysis to optimize single-cell gates

In this example, an experienced user gated singlets confidently using conventional flow parameters. After evaluating (Figure 9A) the manual singlet gate (Figure 9B,C), the Attune CytPix morphology parameter “Particle Count” reveals this gate actually contains more than 4% doublets and aggregates. As shown in Figure 9D, this gating strategy can be visually confirmed with images. Using the “Particle Count” parameter for gating singlets results in a more robust gating strategy and higher data accuracy.

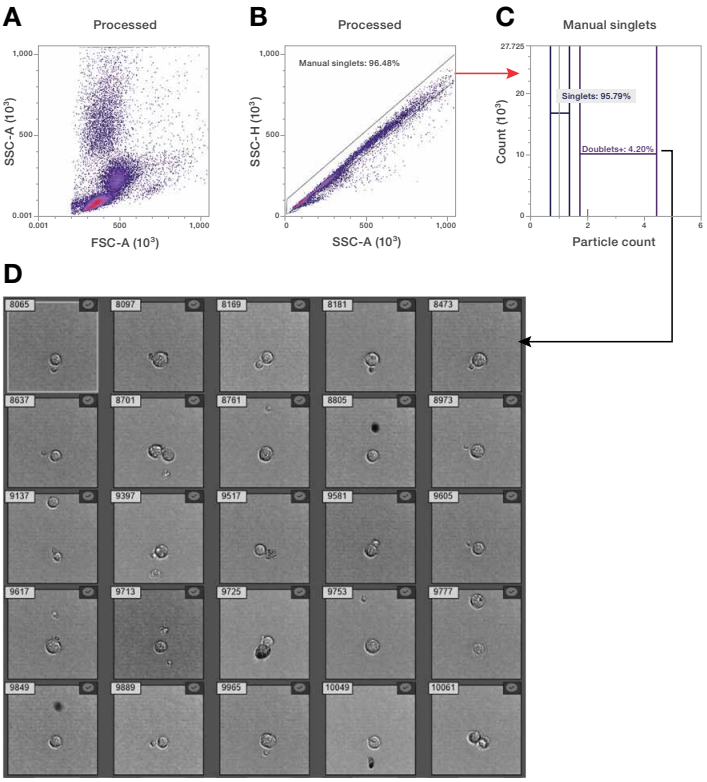

Figure 9. Gating errors in traditional flow assays are highlighted with images. (A) Human blood **(B)** gated for singlets using traditional scatter –A vs. –H approach, which includes **(C)** >4% of events with two or more cells. **(D)** The Image View Gallery is available to rapidly visualize these and other gating inaccuracies.

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Gain new insights into cell biology with morphology parameters

Imaging interactions between CAR T cells and Ramos cells is possible with the Attune CytPix Flow Cytometer. Users can implement morphology parameters (in this example, circularity vs. skewness of intensity) to further examine the features of these populations and refine gating strategy to improve data robustness. Figure 10 shows that by using measured morphology parameters, users can distinguish interacting cells from coincident events.

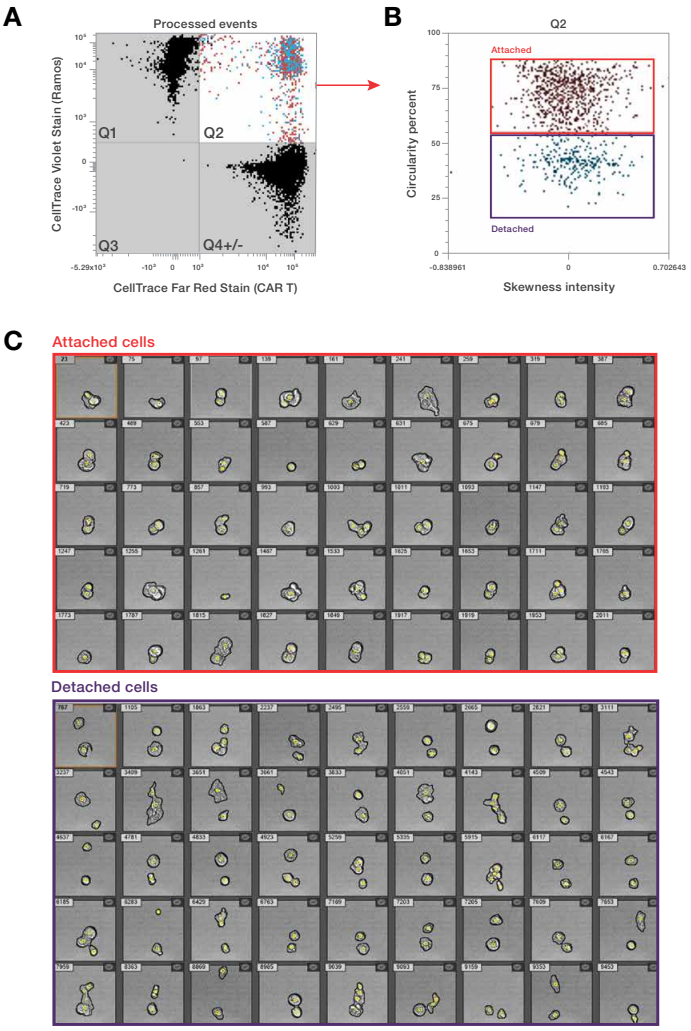


Figure 10. Visualization of CAR T cells targeting lymphoma cells. CAR T and Ramos cells were labeled with Invitrogen™ CellTrace™ Far Red and Violet stains, respectively, and incubated at a 1:1 ratio for 1 hour at 37°C. Unfiltered samples were acquired on the Attune CytPix Flow Cytometer at 200 µL/minute, >8 x 10^5 cells/mL. **(A)** Images of quadrants Q1 (top left), Q4 (bottom right), and Q3 (bottom left) show individual Ramos cells, CAR T cells, and debris, respectively. Images from quadrant Q2 (positive for both stains, top right) reveal both cell types fused together, acquired as a single event as the CAR T cells engulf the Ramos cells. Percentages are % gated. **(B)** Using circularity vs. skewness of intensity, users could differentiate between attached cells (interactions between CAR T and Ramos cells) and detached cells (cells in the same field of view but not showing cell-to-cell interactions). **(C)** In the cell image galleries, annotated events are outlined in black with yellow dots indicating center positioning. Image processing was done using the “Cells Half Resolution” model.

Tailor and train a model to transform your data analysis like never before

We provide the capability to create an unlimited number of retrained models, enabling users to conduct to label-free morphology analysis beyond simple leukocyte and bead samples. This interactive retraining feature allows users to refine the vendor-supplied AI model to reflect their subject matter expertise by improving its accuracy for a variety of sample types and applications (Figure 11A). Here (Figure 11B); both mask fit and singlet counting are improved after model retraining on differentiated macrophages. An unlimited number of training models can be created, exported, imported for later refinement, and shared to support collaboration.

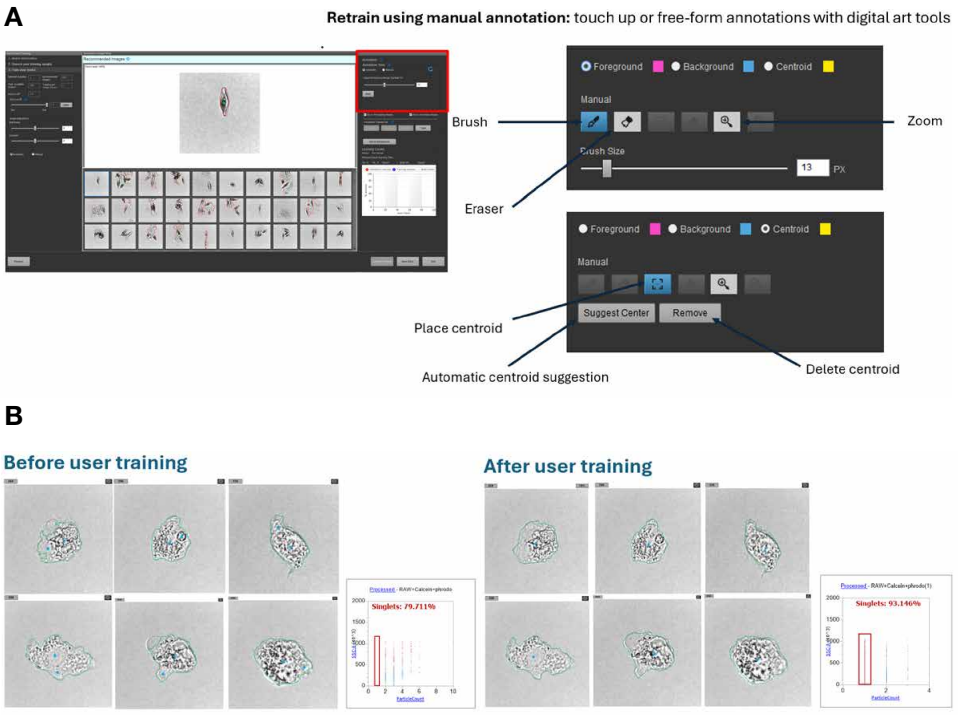


Figure 11. Expert UX/UI interface enables users to train AI models for expanded applications. (A) The "Train Model" feature provides annotation tools and real-time model training accuracy. **(B)** RAW 264.7 macrophages are often annotated inaccurately with respect to particle count due to their complexity and noncircular borders. The number of singlets available for analysis increases once the user trains and employs a more accurate model.

Advanced AI and machine learning integration

The Attune CytPix Flow Cytometer brings the power of advanced AI and machine learning (ML) to your laboratory. The morphology parameters are new markers for identifying target cell populations. Users can correlate phenotype with function, employ label-free profiling for rare populations, and find populations based on morphological similarity. Our image analysis tools perform dimensionality reduction, seamlessly integrating morphology and fluorescence data.

The image similarity feature enables users to choose a reference image and find images with similar morphology throughout a sample (Figure 12). Images can be sorted according to the percent similarity calculation and backgated onto any flow plot. With this calculation, a dynamic similarity percentage parameter for each reference image can be plotted against any parameters for more insight about the population.

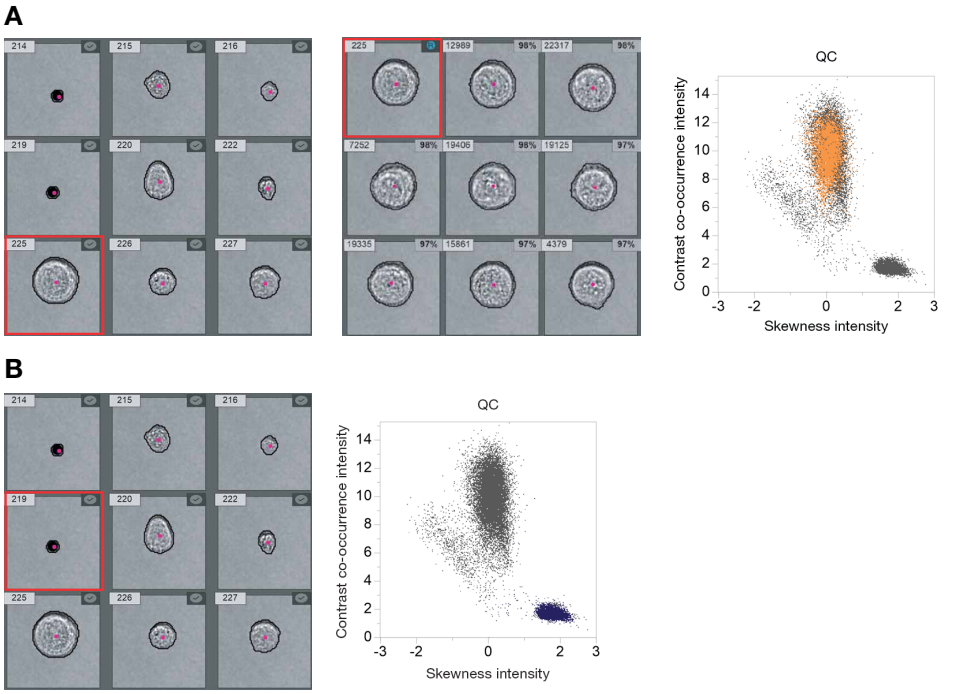


Figure 12. The image similarity feature helps find events of similar morphology image quickly. Human T cells were cultured with IL-2 and Gibco™ Dynabeads™ Human T-Activator CD3/CD28 for T Cell Expansion and Activation, leaving expanded cells and residual detached beads in the final sample. Similarity scores can be calculated for any user image, and the entire image view gallery can be sorted by this score to find morphologically similar events. **(A)** Similarity scores can be used as plot parameters or events can be backgated to workspace plots as shown in the graphs, with orange dots representing images >70% similar to the reference Event 225. **(B)** Multiple similarity scores can be calculated, as shown here with backgated events in blue ranked by reference event 219.

Analyze and identify target cell populations with new layers of data

Gain new insights into your experimental data by visualizing populations using combined fluorescence and morphology data. Built-in software interface wizards enable downsampling, concatentation, and dimensionality reduction for an intuitive, streamlined data analysis experience. Users of any experience level will be able to include all or some (scatter, fluorescence, morphology) parameters as desired and correlate clusters back to flow plots for analysis (Figure 13).

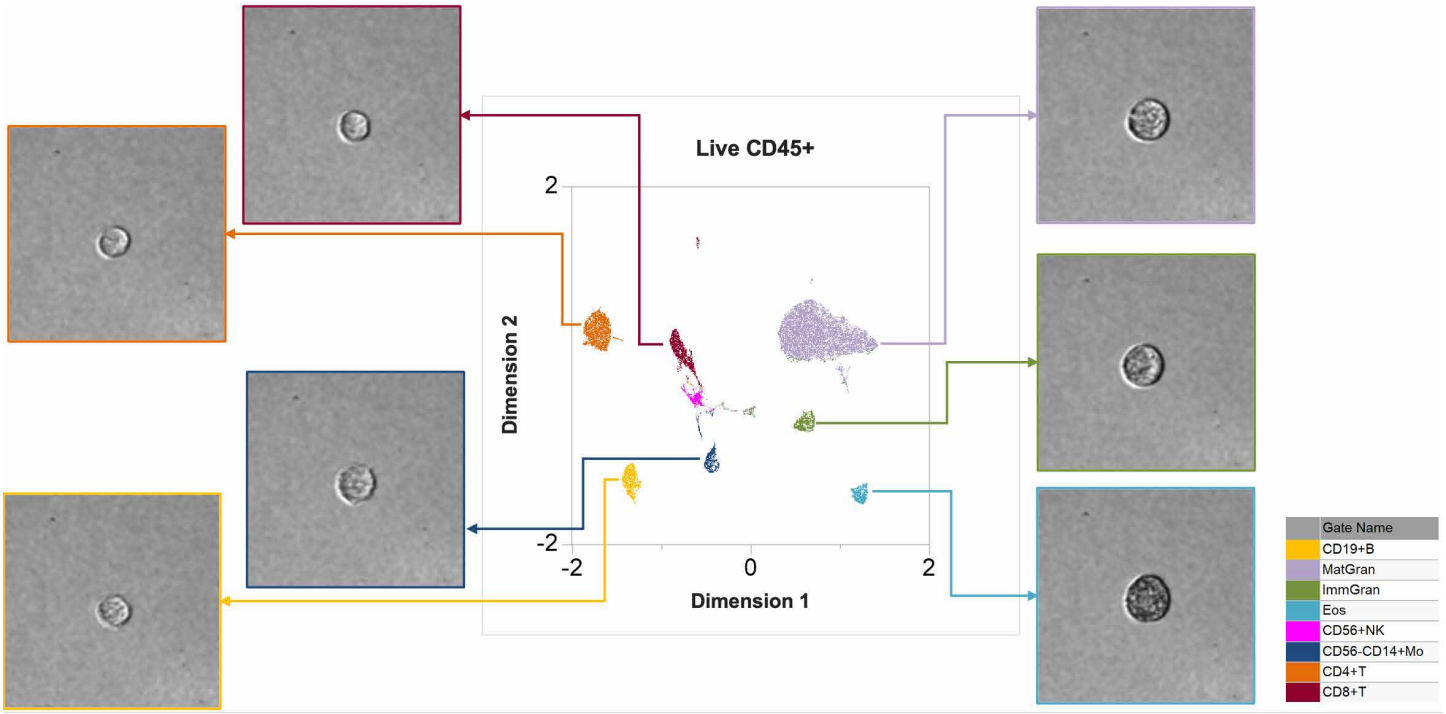


Figure 13. Built-in dimensionality reduction tools increased resolution into populations. Fluorescently gated populations were back-gated onto the UMAP plot to confirm expected population resolution in lysed whole human blood. Users can view events from each cluster for visual confirmation of cell phenotypes.

Attune Xenith Flow Cytometer

Fast, flexible, and spectrally brilliant—simplify high-speed, high-parameter analysis

The Attune Xenith Flow Cytometer is a reliable cell/particle analysis solution designed to help meet the rigorous demands of researchers. Leveraging our legacy core acoustic focusing technology, this innovative instrument combines acoustic-assisted hydrodynamic focusing with advanced spectral capabilities. With expanded optical capabilities, including ultraviolet (UV) and near-infrared (NIR) lasers, the Attune Xenith Flow Cytometer supports both traditional compensation and spectral unmixing analysis. Additional benefits include:

- **Remarkable speed**—operates 5x faster than most flow cytometers
- **Versatility and flexibility**—supports both spectral unmixing and conventional compensation workflows, enabling optimization of experimental design and exploration of new research possibilities
- **Productivity and reliability**—designed with increased uptime and simplified operation in mind, enabled by streamlined maintenance, intuitive touchscreen operation, and advanced remote diagnostics
- **Compatibility with complex samples**—designed to handle challenging sample types, such as tumor and tissue samples, without clogging for smooth sample acquisition and reliable data analysis
- **Extensive detection range**—includes 6 lasers, 51 fluorescence detectors, and 6 scatter channels
- **Exceptional automation options**—compatible with Invitrogen™ CytKick™ Autosamplers for efficient handling of 96- and 384-well plates

Increased uptime and simplified operation

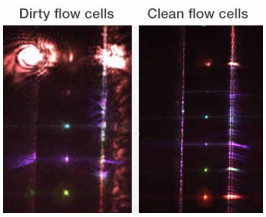
Designed with user convenience in mind, new instrument features include:

Simplified touchscreen maintenance

The built-in touchscreen allows simple one-touch startup and shutdown independent of a PC. Easily view the instrument's continuous fluid-level sensing, acquisition progression, and maintenance instructions.

Increased run time

The fluidics cart design allows for extended runs between fluid changes, helping to enhance productivity. The distinct capability to change fluids without stopping the instrument helps ensure uninterrupted sample runs, minimizing downtime and optimizing experimental efficiency.



Advanced diagnostics and service

Onboard service cameras assess flow cell cleanliness and enable remote support, allowing fast and efficient troubleshooting. Automated maintenance functions and enhanced service logging further streamline operations, helping reduce downtime and maintain optimal speed.

Innovative flexibility

Designed to accommodate various tube sizes, our seal-free, volumetric syringe–based system helps minimize sample loss, enabling fast and efficient analysis. Optional automated sample recovery and rinse further enhance speed and convenience. A sample injection probe (SIP) wash helps reduce carryover and contamination.

A breakthrough in high-speed, multidimensional analysis

Advanced acoustic fluidics technology allows faster acquisition without compromising results

The acoustic focusing technology of the Attune Xenith Flow Cytometer combined with its clog-resistant design help enable the rapid acquisition of data, even from complex sample types like mouse tissue digests. Figure 14 highlights a broad immunophenotyping study that leveraged the high detection sensitivity of the instrument to focus on the development and maturation of B cells. Despite high cell concentrations in the various tissue samples, the Attune Xenith Flow Cytometer enabled rapid data acquisition without compromising the resolution of the analysis.

Figure 15 displays the detection range of the Attune Xenith Flow Cytometer, which results in compatibility with large panels. The instrument showed high sensitivity for an analysis of the expression of NK cell surface markers before and after activation (Figure 16). The data demonstrated the fluidic and optical capabilities of the Attune Xenith Flow Cytometer, which led to a deeper understanding of rare subpopulations at rapid rates of acquisition.

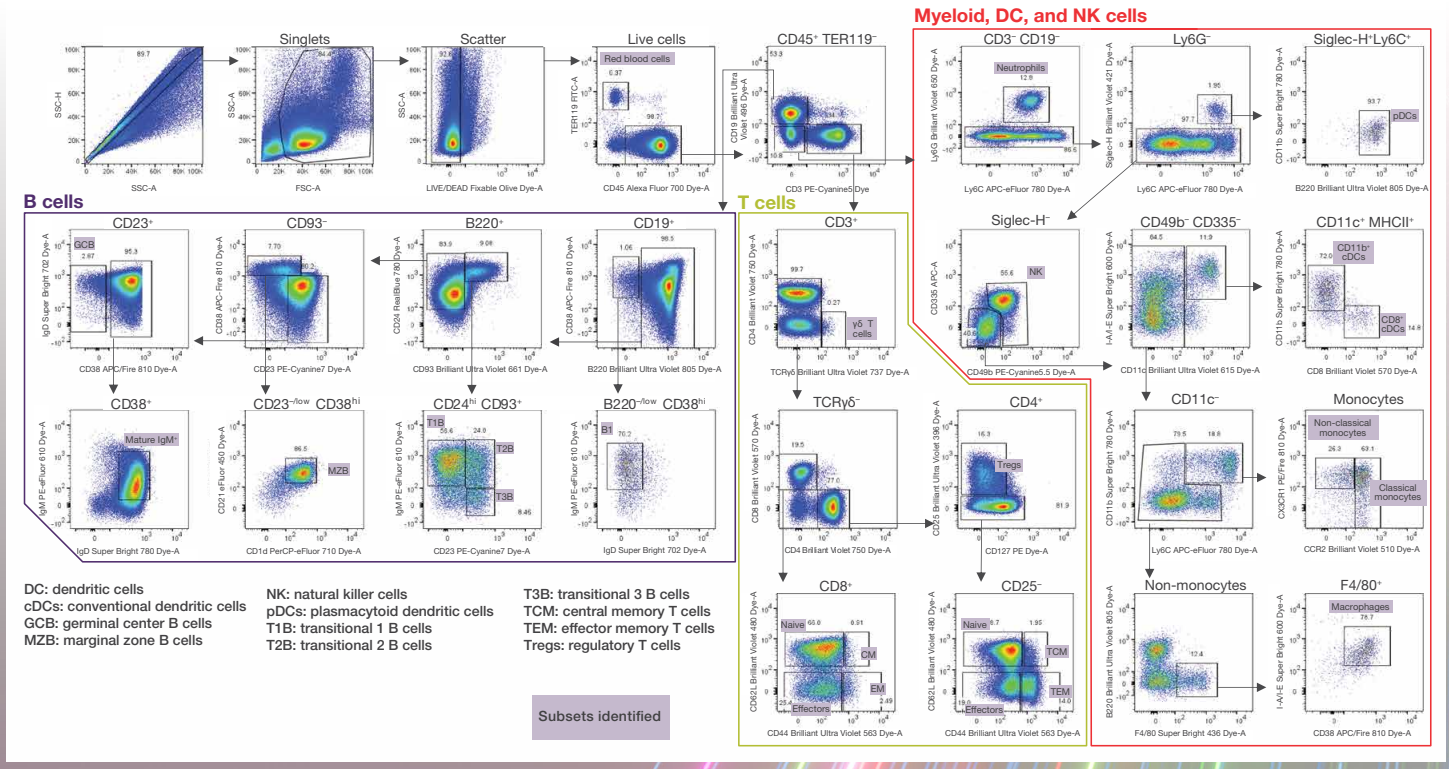


Figure 14. Immunophenotyping of using a 32-color spectral unmixing panel. Even at high cell concentrations of 1 x 10^7 cells/mL in mouse tissue digests, the Attune Xenith Flow Cytometer demonstrated its capability to deliver accurate and reliable results.

Extensive detection range for compatibility with large panels

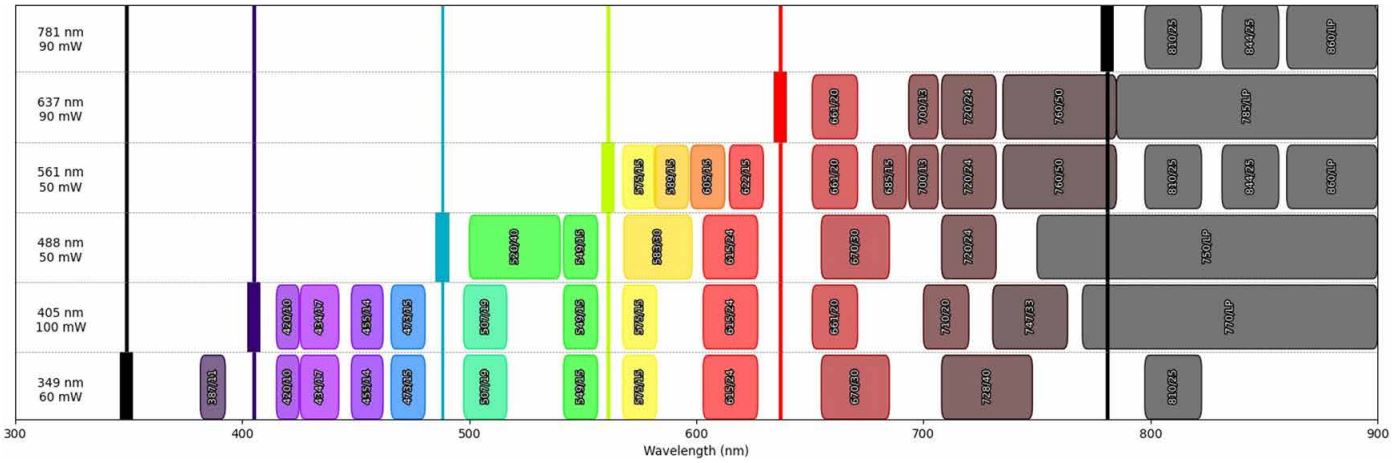


Figure 15. Overview of lasers and fluorescence detectors. The Attune Xenith Flow Cytometer includes 6 lasers (349 nm, 405 nm, 488 nm, 561 nm, 637 nm, and 781 nm) and 51 fluorescence detectors. It also includes 6 scatter detectors for enhanced resolution (488 nm standard FSC and SSC, 405 nm FSC and SSC for small-particle resolution, and 488 nm FSC and SSC for expanded range/polarized detection). The system supports both spectral unmixing and conventional compensation options, making it exceptional for high-parameter workflows.

Sensitivity in detection facilitates robust data resolution to differentiate rare cell populations

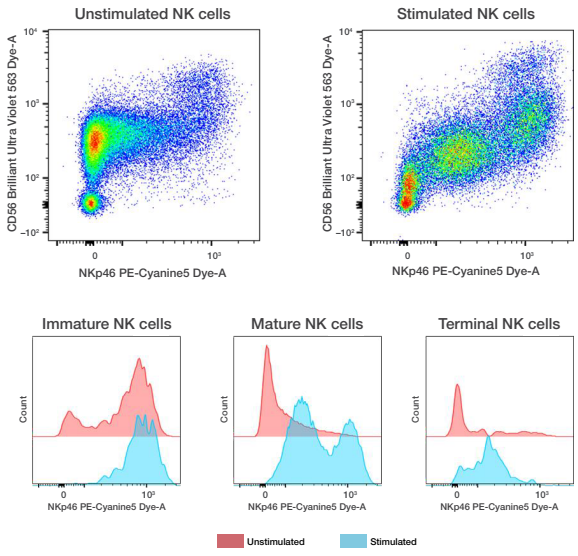


Figure 16. Exploration of expression profiles of NK cells across activation states. Human NK cells were incubated with or without an IL-2, IL-15, and IL-21 cytokine cocktail for a duration of 48 hours. Immature, mature, and terminal NK populations were identified, and surface marker expression of each of these subpopulations was characterized. The Attune Xenith Flow Cytometer demonstrated strong sensitivity in detecting changes in marker expression before and after stimulation using a 25-color spectral panel.

High-performance automation and robotic solutions

Anchor your automation with proven reliability in operation and innovative mechanical integrity

CytKick autosamplers allow walkaway automation

Improve workflow efficiency by choosing the autosampler option that best fits your throughput and experiment requirements (Table 5). Two models of autosamplers are available to deliver walkaway automation seamlessly integrated with your Attune Flow Cytometer for increased productivity.

Table 5. Comparison of Invitrogen™ CytKick™ autosamplers.

Category	CytKick™ Autosampler	CytKick™ Max Autosampler
Throughput	42 min for 96-well plate using high-throughput mode	22 min for 96-well plate (boost mode, using one rinse and one mix and full analysis of a 20 µL sample)
Compatibility	96 deep-well 96-well standard depth 384 deep-well 384-well standard depth	96 deep-well 96-well standard depth 384 deep-well 384-well standard depth 1.5 mL and 2 mL microcentrifuge tube rack (up to 24 per rack) 96-well foil-covered 384-well Customizable to accept additional plate types

Robotic automation

Extend your unmanned runtime settings and scalability with the robotic integration application for Attune flow cytometers. Our range of automation solutions include robotic plate taxiing, extended fluidics, temperature-stable plate storage, and software for operations. An example of robotic plate taxiing is shown in Figure 17. You can leverage both scheduling and integration to get the most from your solution.

Comprehensive specifications are available at **thermofisher.com/flowautomation**.

Figure 17. Optional automation configuration. Maximize operating capacity, mitigate human operator error, and enable the acquisition of rich, reproducible data with the Thermo Scientific™ Orbitor™ RS2 Microplate Mover as part of a comprehensive, multicomponent workcell for robotically automated flow cytometry. The multicomponent workcell, which includes the Attune Flow Cytometer and the Orbitior RS2 Microplate Mover, is configured with 2 hotels and 1 stack.

Momentum Integration Software

Thermo Scientific™ Momentum™ Integration Software includes an easy-to-learn dashboard for operations, scheduling features to handle multiple workflows, and compatibility drivers to speed implementation.

A key factor in choosing your automation solution is helping ensure that the software is both performant and easy to use. The dashboard in Momentum Integration Software is fast to learn, and helps users to actively prioritize and reprioritize runs, visualize progress, and trace plates.

Dynamic scheduling in Thermo Scientific™ Momentum™ Workflow Scheduling Software allows users to successfully create multiple workflows. The software's compatibility drivers support over 200 instruments. They have proven success in numerous implementations and can speed your integration project.

Integrated, powerful, and intuitive software

Attune Cytometric Software

The Invitrogen™ Attune™ Cytometric Software is designed for compatibility with the Attune NxT and Attune CytPix models, as well as the Momentum Workflow Scheduling Software. The buttons on the ribbon in Attune Cytometric Software control the automation settings within the software. When automation is enabled, the Momentum software connects the Orbitor RS2 Microplate Mover to the Attune Flow Cytometer and manages the operations between the instruments. Figure 18 displays a typical Attune NxT software interface.

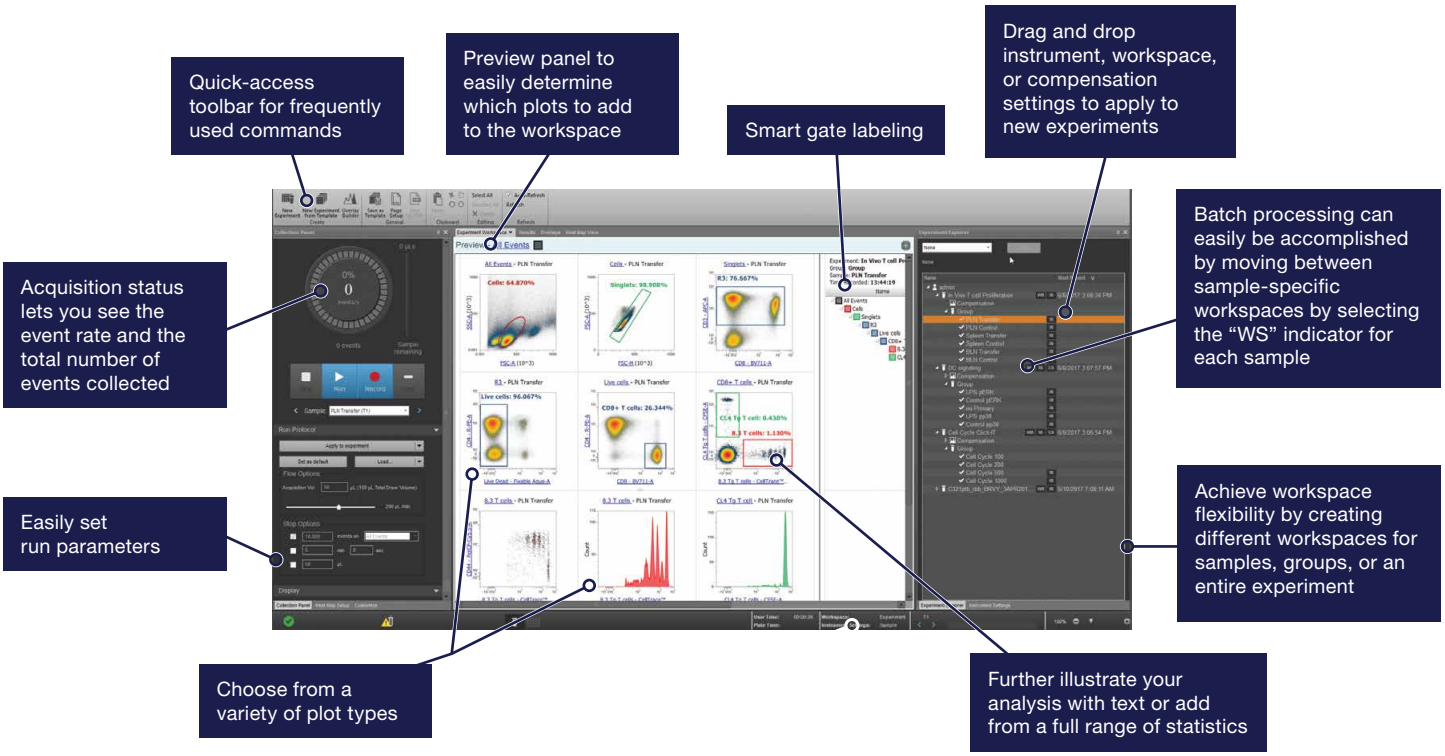


Figure 18. Intuitive, user-friendly software interface with a familiar workflow. A typical Attune NxT workspace interface is shown.

21 CFR Part 11–compliant software module

Regulatory-compliant electronic records and signatures

The Attune Cytometric Software has an optional 21 CFR Part 11–compliant module, which provides users with security features such as user login authentication, logging of any unauthorized attempts to access the system software, and notifications of data tampering. The software also provides the user with a full audit trail—electronic records and electronic signatures that are trustworthy, reliable, and equivalent to paper records.

Learn more about Attune Cytometric Software at **thermofisher.com/attune-cytometer-software**.

Imaging analysis software features

Attune Cytometric Software automates image analysis with a processing rate of up to 1,000 images/second and can be managed by users in an image processing queue. The image analysis software uses models pretrained on leukocytes and beads to provide image-derived measurements. The pre-trained models can be refined by the user on an intuitive software interface. These measurements consist of more than 30 parameters that include quantification of morphology to measure singlets (particle count), roundness (circularity), size (area square), shape (eccentricity), complexity (entropy), and others (Table 6).

Gating on these morphology parameters allows you to analyze populations of interest and confirm or enhance the gating strategy. You can gate your samples using these parameters to verify sample quality, delineate complex samples, and develop new applications. Additionally, you can use back-gating to scan the panel of full-resolution images and correlate what you see with scatter, fluorescence, or image-based parameters to any population on the dot plot. Enhance the quality of your data and feel confident when gating with powerful data-driven cell analysis.

Table 6. Morphology parameters available for image analysis.

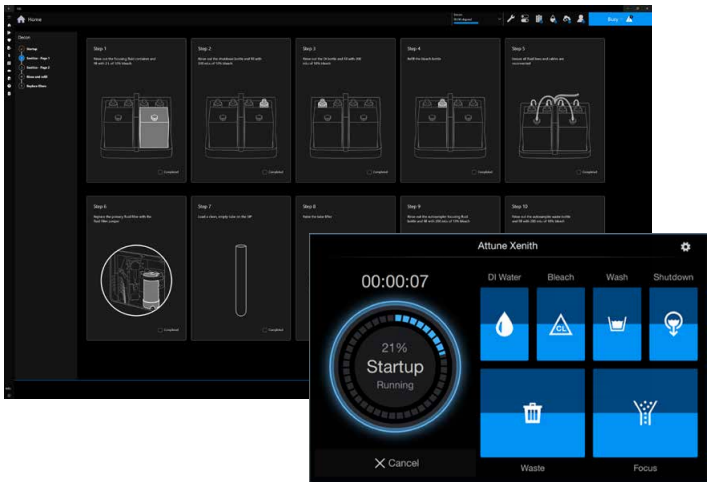
Image-derived parameters	
Co-occurrence features	
AngularSecondMomentCoOccurrence	EntropyCoOccurrenceIntensity
ContrastCoOccurrenceIntensity	MaximumCoOccurrenceIntensity
Intensity and texture features	
AverageIntensity	MaxIntensity
AverageNormIntensity	MinIntensity
CVIntensity	SkewnessIntensity
CVNormIntensity	StandardDeviationIntensity
EntropyIntensity	StandardDeviationNormIntensity
KurtosisIntensity	TotalIntensity
Moment weighted features	
CoherencyWeighted	MajorRadiusMicrons
GyrationRadiusWeightedMicrons	MinorRadiusMicrons
Object features	
ParticleCount	
Particle interaction features	
ClumpIndexMax	ObjectCount
Pixel features	
NumPixels	
Shape features	
AreaSquareMicrons	MinorDiameterMicrons
CircularityPercent	MinorMajorRatioPercent
EccentricityPercent	PerimeterMicrons
MajorDiameterMicrons	PseudoDiameterMicrons
System features	
ConfidenceScore	IsProcessable
IsOnBorder	IsProcessed

Invitrogen™ Sasquatch Software—designed for the Attune Xenith Flow Cytometer

- Multiple autofluorescence subtraction support for users to more accurately analyze highly autofluorescent samples
- Keyword management to assist users in customizing information in the database for each sample and experiment
- Addition of instrument-specific fluorophores to significantly aid users in building a panel that is specific to the Attune Xenith Flow Cytometer
- User-specific experiment management to support multiple users in shared labs
- Experiment acquisition settings management for improving experiment setup time
- N x N plots to enable a single view of quality control unmixing results and comparison of results across different settings

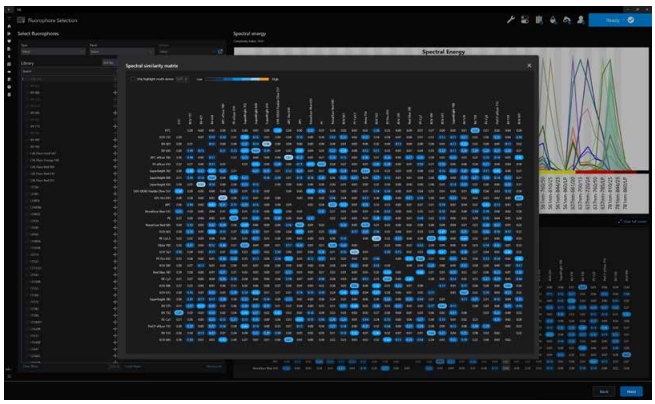
Automated maintenance made easy

Sasquatch Software provides automated maintenance with on-screen instructions that can be completed in both the desktop application and on the touch screen.



Advanced panel design tools

Sasquatch Software includes built-in panel design tools to evaluate fluorophore compatibility that show both the complexity score and fluorophore similarity matrix.



Flow cytometry reagents

Accelerate your science with a comprehensive suite of solutions for the analysis of cells and their functions with Invitrogen™ reagents.

Antibodies—Build and expand your panels using our extensive portfolio of antibodies conjugated to over 30 types of fluorophores, including traditional, Invitrogen™ eFluor™, Alexa Fluor™, and Super Bright™ violet-excitable polymer dyes.

Reagents—Incorporate a comprehensive variety of cell function assays for studying viability, apoptosis, cell cycle, metabolism, and cell proliferation.

Fixable viability dyes—Invitrogen™ LIVE/DEAD™ fixable dead cell stains are fixable viability dyes that help ensure accurate assessment of cell viability in samples, and are compatible with fixation and/or permeabilization.

Instrument and compensation—Select from our wide range of easy-to-use beads for your experimental needs.

Buffers—Choose from a wide variety of buffers to suit your research needs.

Compensation and instrument beads—Build flow cytometry panels with accurate compensation using Invitrogen™ UltraComp eBeads™ Plus Compensation Beads.

Flow Cytometry Panel Builder

Design your panel for your Attune Flow Cytometer using the Invitrogen™ Flow Cytometry Panel Builder or our free panel design service to streamline your experimental design.

Get more information at **thermofisher.com/flow-cytometry**.

Free flow cytometry panel design service

Panel Builder tool—for self-designing panels

The Invitrogen™ Flow Cytometry Panel Builder is a free online tool to help select antibody conjugates and reagents for a multicolor flow cytometry panel. This allows for improved panel design with greater separation and detection of individual cell populations of interest.

With the Flow Cytometry Panel Builder tool, you can:

- Create a new immunophenotyping experiment or add antibodies and reagents to an existing panel
- Check fluorophore emission spectra with the built-in Fluorescence SpectraViewer
- Export a Microsoft™ Excel™ document with your antibody choices, or order directly

Key features of our free panel design service include:

- **Free and fast**—no purchase necessary; typical response time within one business day
- **Personalized**—one-on-one, customized assistance with a live specialist
- **Flexible**—accommodates antibodies that you already have, and those that you need

For panel design service and panel builder tool visit **thermofisher.com/flowpanel**

Services and support

Partner with a flow cytometry company invested in supporting you through a lifetime of research

Choose a service plan that is right for you—beyond repair to proactive care

Our technical services, field engineering, and training teams are fully committed to your success using Attune flow cytometers for your research. Instrument service plans, consulting, and training programs are designed to help ensure instrument performance, team readiness, and overall optimal research outcomes using the system (Table 7). In the field or on the phone, our team has the professional know-how to support your research, and the personal dedication to help ensure your satisfaction with our instruments.

- **Peace of mind**—during every stage of ownership: instrument install, repair, and maintenance
- **Flexible service options**—over 1,000 technical specialists delivering 30 years of experience servicing life sciences instrumentation
- **Applied Biosystems™ AB Assurance™ Plan and extended warranty**—covers all costs associated with instrument repairs

Table 7. Comparison of Applied Biosystems™ service plans.

	AB Maintenance Plus	AB Assurance	AB Complete
Response time	3 business days*	2 business days**	Next business day
Planned maintenance	✓	✓	✓
Access to technical support (Monday–Friday, standard business hours)	✓	✓	✓
Parts, labor, and travel	10% discount	✓	✓
Qualification service	Available as add-on	Available as add-on	Available as add-on
Field application scientist (FAS) consultation	Available as add-on	Available as add-on	✓

* After receipt of purchase order.

** Availability limited in some geographic areas.

Technical resources

Videos and webinars

Check out how-to videos and virtual demos at thermofisher.com/attunevideos.

Customer stories

See what researchers around the world have to say and how they are advancing research using Attune flow cytometers at thermofisher.com/attune.

Protocols and application notes

View protocols and learn more about how to use the Attune flow cytometers in different applications at thermofisher.com/attune.

Ordering information

Product	Cat. No.
Attune flow cytometers	
Attune Xenith Flow Cytometer	A59358
Attune CytPix Flow Cytometer	Various
Attune NxT Flow Cytometer	Various
Automation options	
CytKick Autosampler	A42901
CytKick Max Autosampler	A42973
Attune NxT External Fluid Supply	A28006
Orbitor RS2 Microplate Mover	ZG30SCORBROBNXT
Orbitor RS2 Microplate Mover, Stacks	A33007
Orbitor RS2 Microplate Mover, Hotels	A33008
Orbitor RS2 Microplate Mover, Stacks/Hotels	A35220
Upgrade options	
Attune NxT Yellow Laser Upgrade Kit	100022779
Attune NxT Red Laser Upgrade Kit	100022778
Attune NxT Green Laser Upgrade Kit	A32701
Attune NxT Violet 6 Conversion Kit, Blue Laser	A35428
Attune NxT Violet 6 Conversion Kit, Violet Laser	A36569
Attune NxT Violet 6 Conversion Kit, Red Laser	A36571
Attune NxT Violet 6 Conversion Kit, Yellow Laser	A36572
Attune NxT Fluorescent Protein Filter Kit—GFP, YFP, mCherry	100022775
Attune NxT Custom Filter Holder Kit	A27784
Attune NxT Small Particle Side-Scatter Filter	100083194

Product	Cat. No.
Reagents and consumables	
Attune Debubble Solution (1X), 50 mL	A10496
Attune Focusing Fluid (1X), 1 L	4488621
Attune Focusing Fluid (1X), 10 L	A24904
Attune Focusing Fluid (1X), 20 L	J106627
Attune Wash Solution, 250 mL	A24974
Attune Wash Solution, 1 L	J24974
Attune Shutdown Solution (1X), 250 mL	A24975
Attune Shutdown solution (1X), 1 L	J106628
Attune NxT No-Wash No-Lyse Filter Kit	100022776
Attune Performance Tracking Beads	4449754
Attune Flow Cell Cleaning Solution, 30 mL	A43635
Software	
Attune NxT Software, single license	A25554
Attune NxT Software, 5 licenses	A24856
Attune NxT Software, 10 licenses	A24855
Attune Software, multiple user server license, 5 users	A25555
Attune NxT Software, multiple user server license, 10 users	A25556
Attune NxT Software 21 CFR Part 11, single license	A47288
Attune NxT Software 21 CFR Part 11, server license, 5 users	A47289
Attune NxT Software 21 CFR Part 11, server license, 10 users	A47290
Attune NxT IQ/IPV, Attune NxT Operation Qualification and Instrument Performance Qualification (IQ/IPV)	4465413
Attune NxT IQ/OQ, Attune NxT Installation Qualification and Operation Qualification (IQ/OQ)	4465445
Attune CytPix IQ/OQ, Attune CytPix Installation Qualification and Operation Qualification (IQ/OQ)	A51888
Attune CytPix OQ, Attune CytPix Operation Qualification (OQ)	A51889
Attune Xenith IQ/OQ, Attune Xenith Installation Qualification and Operation Qualification (IQ/OQ)	A66978
Attune Xenith OQ, Attune Xenith Operation Qualification (OQ)	A66979

Learn more at **thermofisher.com/attune**

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