ThermoFisher SCIENTIFIC

# Attune Flow Cytometers

## Transformative. Efficient. Flexible.

invitrogen

# Building a legacy of advancing flow cytometry technology

Technological advancements in flow cytometry are creating an entirely new lineage of reagents and instruments. Flow cytometry innovations enable researchers to create more transformative, efficient, and flexible research solutions. Thermo Fisher Scientific is committed to developing products that fuel groundbreaking research. Our suite of comprehensive flow cytometry solutions, including Invitrogen™ Attune™ Flow Cytometers and the Invitrogen™ CytKick™ family of autosamplers and automation options, together with the Invitrogen™ cell health reagent portfolio and Invitrogen™ eBioscience™ antibody conjugates, helps drive discovery of new biological insights for many applications.

Find out more at **thermofisher.com/attune**

# Transformative 14-color cell analyzers with high-speed camera option

Both Attune Flow Cytometer models are efficient, flexible, and transformative cell analyzers with acoustic focusing and up to 14 colors. Choose between the Invitrogen™ Attune™ CytPix Flow Cytometer with high-speed camera and the Invitrogen™ Attune™ NxT Flow Cytometer without it.

**Table 1. Features of Attune Flow Cytometers.**

| Category | Feature | Attune CytPix Flow Cytometer | Attune NxT Flow Cytometer |
|---|---|---|---|
| **Optics** | Number of lasers | 2–4 | 1–4 |
| | Number of detection channels | 2 scatter channels, up to 14 fluorescence channels | |
| | Imaging illumination | 405 nm laser with <50 nsec pulse width | N/A |
| | Optical alignment | Fixed alignment with pre-aligned welded fiber; no user maintenance required | |
| **Fluidics** | Acoustic focusing | ✓ | ✓ |
| | Custom sample flow rates | ✓ | ✓ |
| **Performance (fluorescence detection)** | Fluorescence sensitivity | ≤80 MESF for FITC, ≤30 MESF for PE, ≤70 MESF for APC | |
| | Fluorescence resolution | CV <3% for the singlet peak of propidium iodide–stained chicken erythrocyte nuclei (CEN) | |
| | Maximum electronic speed | Up to 35,000 events/sec with all parameters | |
| **Performance (imaging)** | Image capture rate | Up to 6,000 images/sec depending on image size and event rate | N/A |
| | Objective | Magnification 20x, numerical aperture 0.45 | N/A |
| | Pixel resolution | 0.3 µm/pixel | N/A |
| | Detection limit | Visually detect 800 nm particles | N/A |
| **Automated image analysis** | Trained models | Leukocytes and beads | N/A |
| | Image-derived parameters | More than 25 image-derived parameters in 5 categories: shape, intensity, object, pixel, and system | N/A |
| | Sample size range of models | 5–20 µm | N/A |
| | Image processing speed | Up to 1,000 images/second depending on image size and complexity | N/A |
| **Quality and regulatory** | Instrument tracking | Automated daily baseline and performance test with Levey-Jennings plots | |
| | Regulatory status | For Research Use Only | |
| **Physical** | Dimensions (H x W x D) including fluid bottles | ~49 x 58 x 43 cm (~19 x 23 x 17 inch) | ~40 x 58 x 43 cm (~16 x 23 x 17 inch) |
| | Biosafety hood compatibility | ✓ | ✓ |
| **Computer** | Memory | 64 GB (4 x 16 GB) DDR4 2666 MHz UDIMM non-ECC | 32 GB |
| | Hard drives | 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 |
| | Graphics processor | Nvidia™ Quadro™ P2200 GPU | N/A |

# Innovative flow technology—acoustic focusing and high-speed camera

Acoustic focusing fluidics is the key to the high sensitivity of Attune Flow Cytometers, even at high throughput. The Attune CytPix model adds a high-speed camera that captures brightfield images of cells passing through the flow cell to verify that cell populations consist of single cells and verify morphology.

## 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). This allows for a higher degree of data, detail, and throughput that enables processing of a large range of sample types, including large clumpy cells, samples with low concentration of cells, and precious samples, more quickly and accurately than ever before with no loss in data quality.

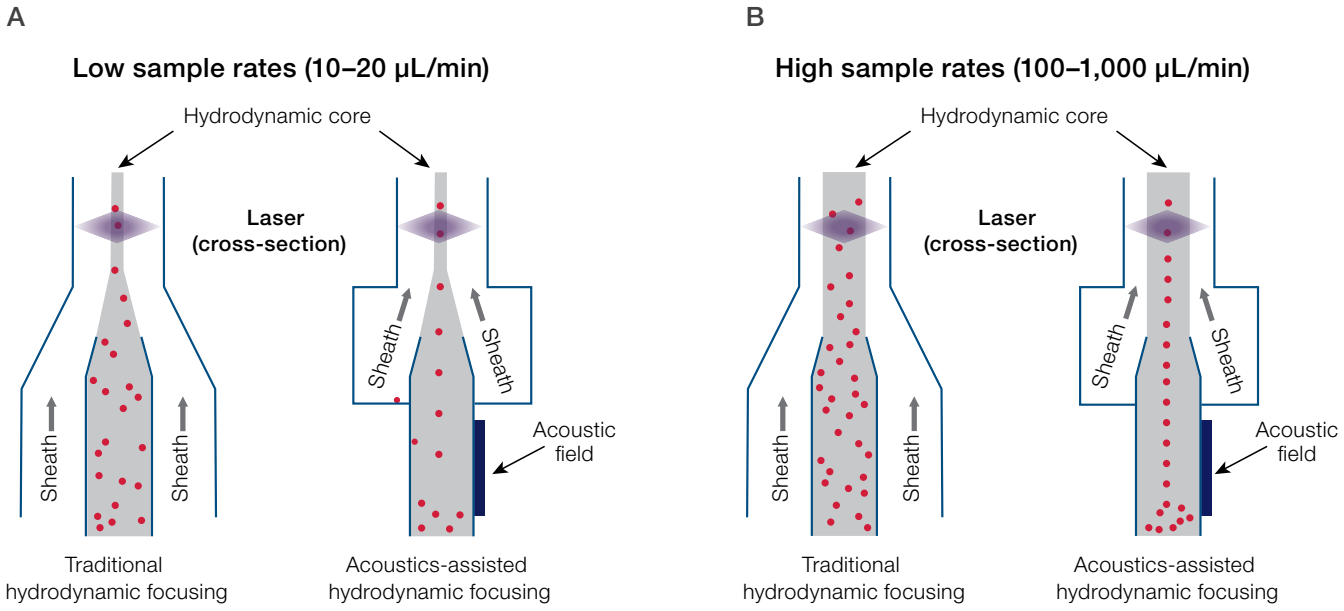



**Figure 1. Acoustic focusing vs. traditional hydrodynamic focusing as particles pass through the laser. (A)** In acoustic focusing, cells remain in tight alignment even at higher sample rates, resulting in less signal variation and improved data quality. **(B)** In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream, resulting in increased signal variation and compromised data quality.

## High-speed camera

The distinguishing feature of the Attune CytPix Flow Cytometer is a high-speed brightfield camera that records images of individual events coming through the flow cell (Figure 2). The camera and Invitrogen™ Attune™ Cytometric Software help ensure that events recorded by the detector are single cells as opposed to doublets, clumps, or debris. This is crucial in cell and gene therapy research applications, but is useful in almost any flow cytometry experiment to help researchers understand and record the morphology of each cell population identified for analysis. The images can also aid in identifying debris and optimizing protocols.

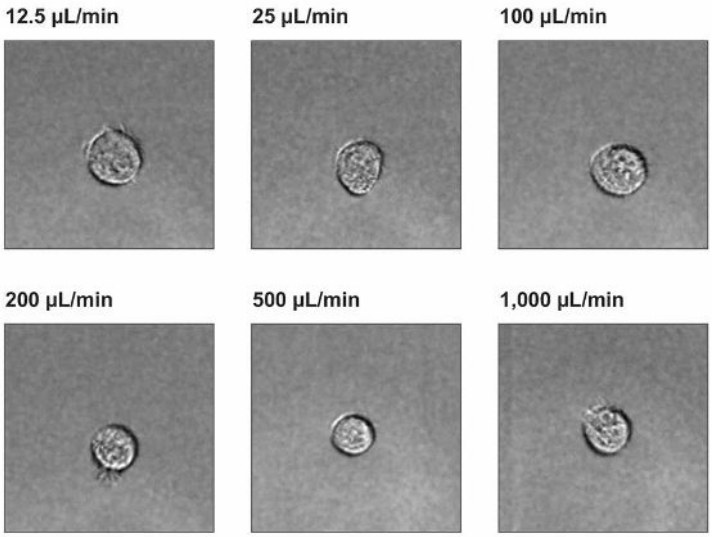



**Figure 2. Consistent image quality even at high flow rates.** Acoustic focusing and a high-speed camera combine to image these CAR T cells consistently at low or high flow rates. No changes to sample rate are required for imaging capabilities. Easily adjust focus and camera settings to meet experimental requirements.

## Novel optical design

Attune Flow Cytometers feature a novel optical design that delivers first-class reliability and superior 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 3). The flat-top lasers also have a higher tolerance for misalignment that allows them to maintain high sensitivity and low CVs.

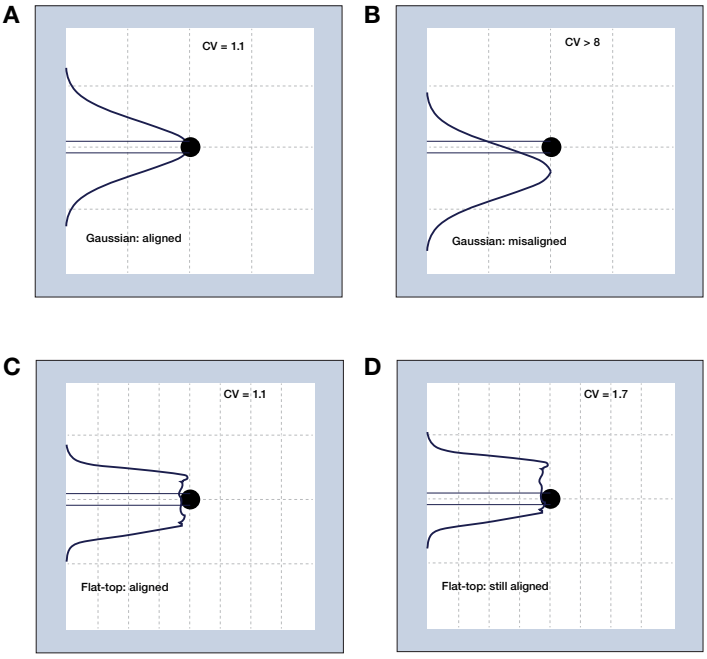



**Figure 3. Emission profile of lasers used in flow cytometers.** **(A)** Gaussian laser profile with proper alignment, **(B)** Gaussian laser profile with misalignment, **(C)** flat-top laser profile with proper alignment, and **(D)** flat-top laser profile still in proper alignment.

# Flexible—multiple configurations and upgradable

## Designed for flexibility

Whether you configure your system now or upgrade later, Attune Flow Cytometers can grow with you and your research needs. Attune 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. Attune Flow Cytometer system laser and detector configurations.**

| | | Number of detection channels for included lasers | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Lasers | Laser configuration | Violet 405 nm | Blue 488 nm | Yellow 561 nm | Green 532 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 | N/A | A24864 |
| 2 | Blue/green | Available as upgrade** | 3 | – | 4 | Available as upgrade** | 9 | N/A | 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 | N/A | A28997 |
| | Blue/red/yellow | Available as upgrade** | 3 | 4 | – | 3 | 12 | A51845 | A28993 |
| | Blue/green/violet | 4 | 3 | – | 4 | Available as upgrade** | 13 | N/A | 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 | N/A | A29001 |
| | Blue/red/yellow/violet | 4 | 3 | 4 | – | 3 | 16 | A51848 | A24858 |
| | Blue/red/yellow/ violet 6 | 6 | 2 | 3 | – | 3 | 16 | A51849 | A29004 |

* Number of detection channels includes all fluorescence channels as well as a forward scatter and a side scatter channel.
** Green laser not available on Attune CytPix system.

## 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 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 | 10 | 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™ fluorophore for the 6 available fluorescence detectors for the violet laser available in the violet-6 configuration of Attune Flow Cytometers.

| Detector | Bandpass (nm) | Fluorophores* |
|---|---|---|
| VL1 | 450/40 | Super Bright 436, 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 | 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.

# Transformative—break the mold of traditional flow

## Reduce clogging from difficult samples

Your research samples are precious, as they are often difficult to produce. 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 4) and larger flow cell help prevent the loss of precious sample such as cancer stem cells from primary pancreatic tumors, and is significantly less susceptible to clogs. Attune Flow Cytometers employ a non-pressurized 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 5). 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 6).

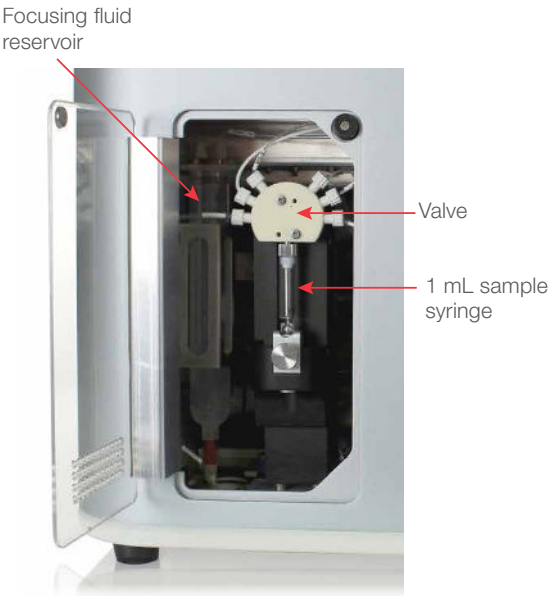



**Figure 4. Positive-displacement syringe pump.** The syringe is easily removed for cleaning or replacement.

## Protocol

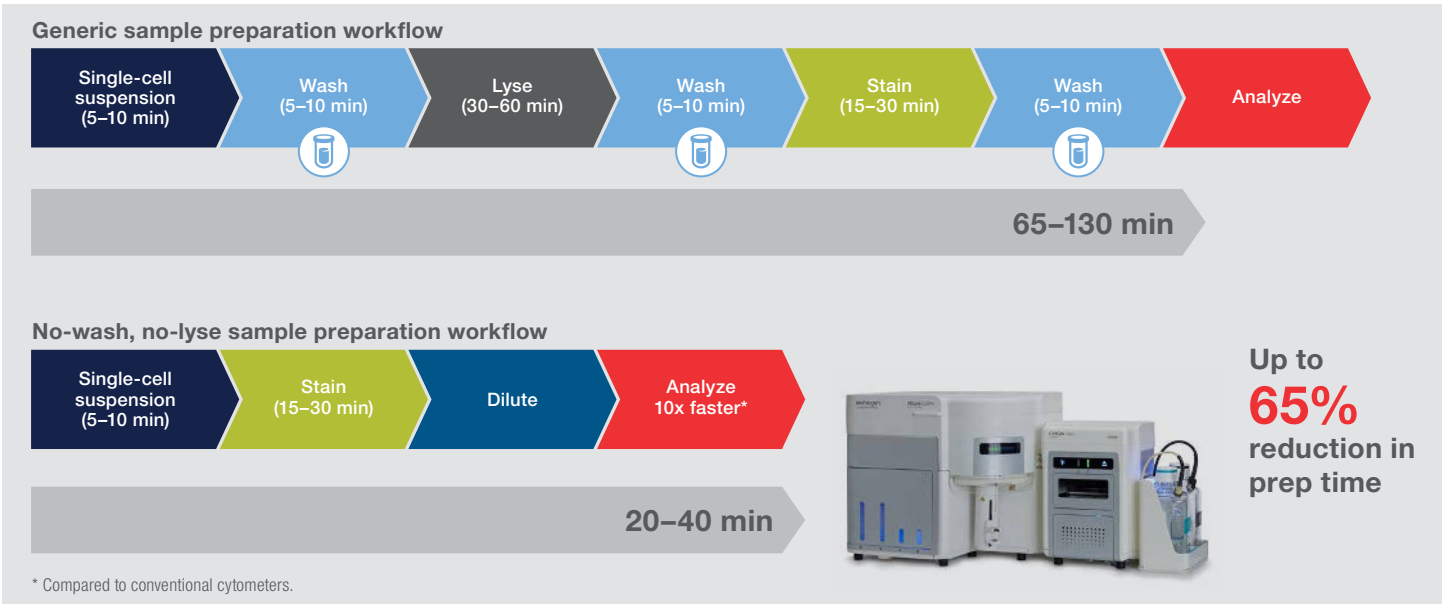



**Figure 5. No-wash, no-lyse sample preparation workflow.**

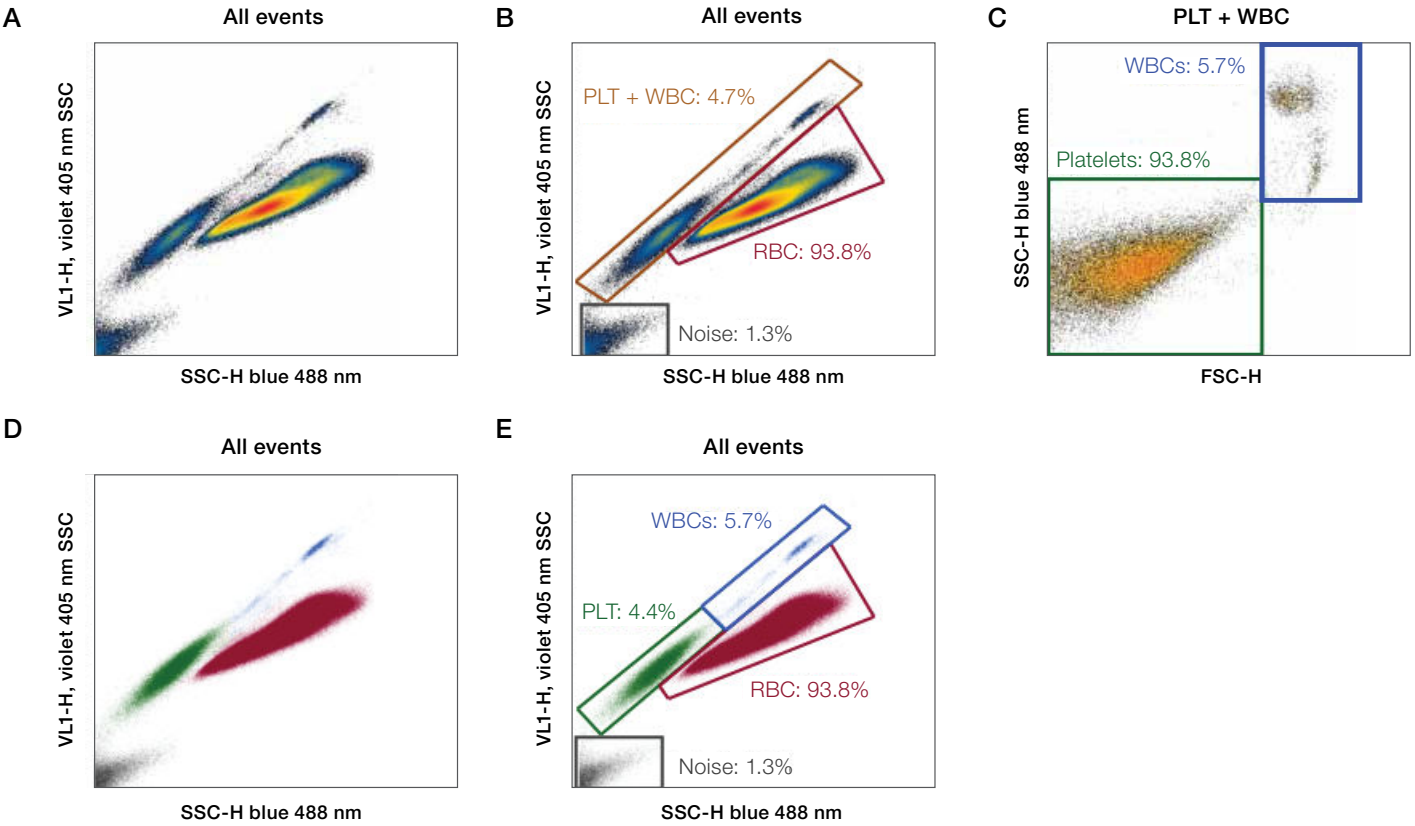



**Figure 6. Forward scatter (FSC) and side scatter (SSC) analysis with blue (488 nm) and violet (405 nm) lasers on intact whole blood (no-lyse, no-wash). (A, B)** RBCs, white blood cells (WBCs), and platelets are separated on the basis of light scatter only by using a combination of blue and violet laser 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. Dual FSC and SSC threshold is set low enough to show instrument noise, ensuring the full platelet population is visualized. **(C)** Using the gate that includes WBCs and platelets, a standard plot of FSC vs. 488 nm SSC can be used to distinguish the platelet 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 platelet 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 platelet populations shows the dominant cell type in whole blood is the RBC, while the WBCs and platelets are relatively rare events.

# Revealing—brightfield imaging and automated image analysis

With the Attune CytPix Flow Cytometer, you can easily and rapidly highlight structural features of large populations (Figures 7 and 8). Using the automated image analysis software feature, you can rapidly process images to measure image-derived parameters that enhance your gating strategy to include cells of interest while excluding aggregates, unwanted cells, and debris (Figure 9). Image-derived parameters can also help you gain new insights into sample biology, such as cell–cell interactions (Figure 10).

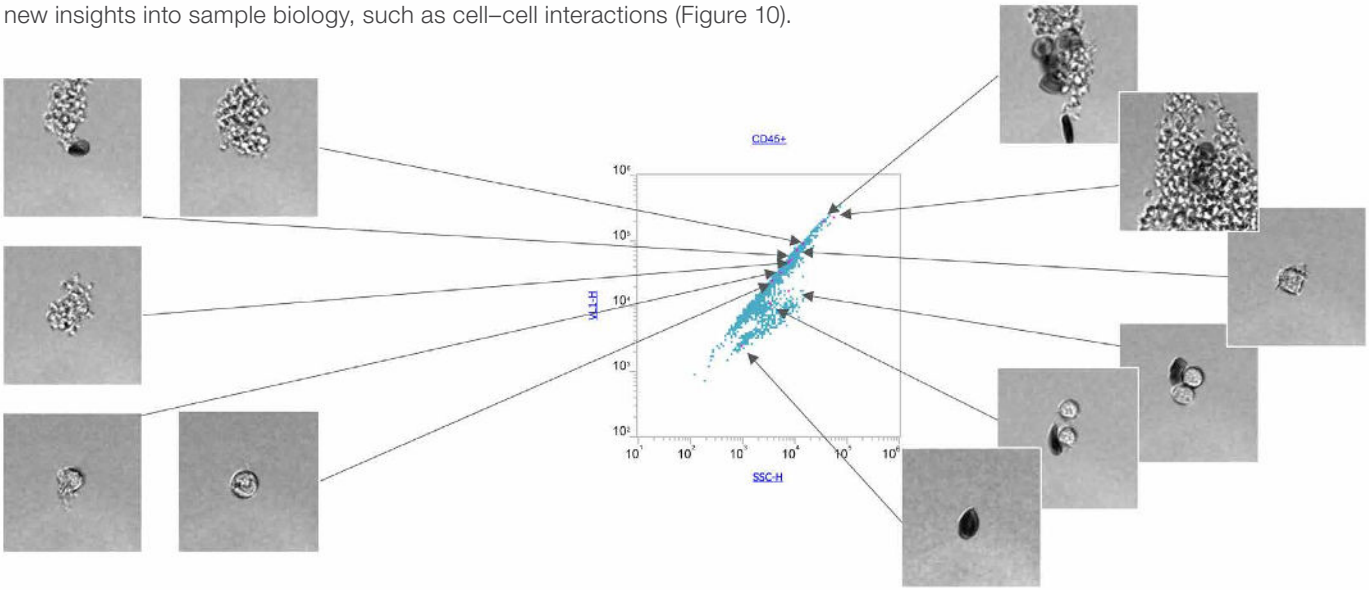



**Figure 7. Gain insight with rare-population analysis.** Cells were acquired from 24-hour-old blood diluted in 1 mM EDTA (<1:4,000). Samples were acquired at 25 µL/min. The image gate was set to record only CD45⁺ events.

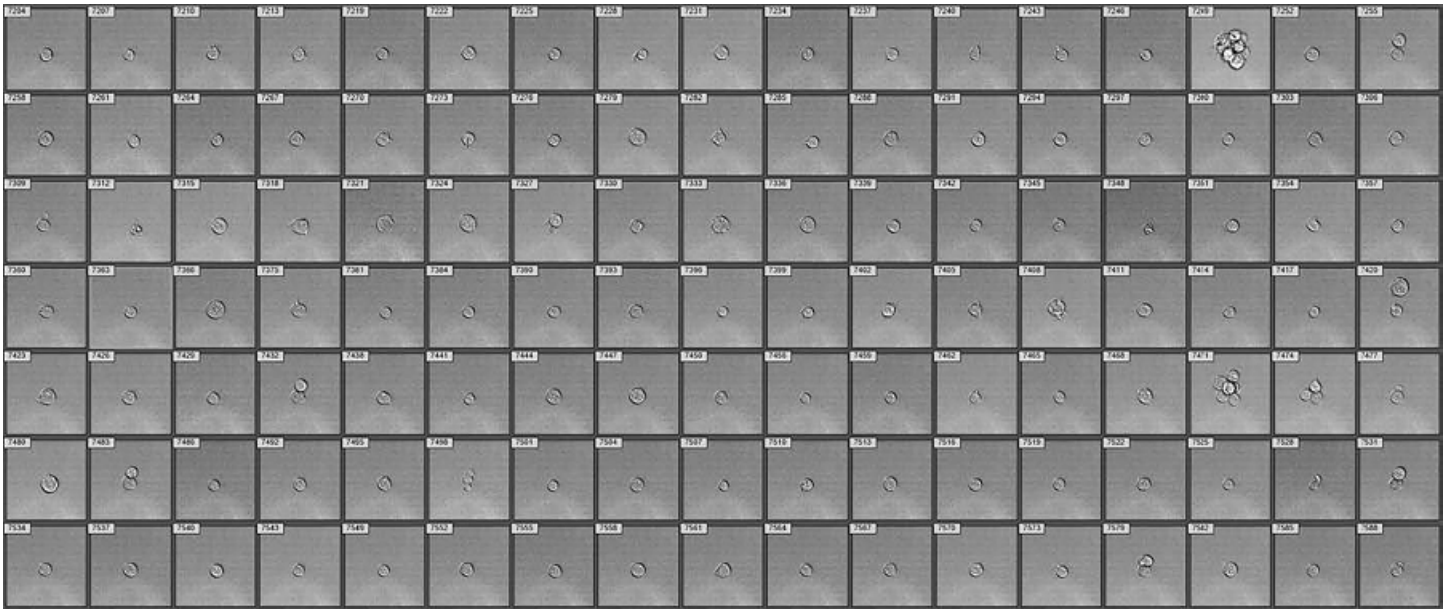


**Figure 8. High-level image gallery view.** Early log phase Jurkat cells were acquired unfiltered on the Attune CytPix Flow Cytometer at 200 µL/min, >$10^5$ cells/mL. Image gallery view was used to rapidly scan cellular events.

# Automated image analysis to optimize single-cell gates

In this example, an experienced user gated singlets confidently. After evaluating the manual singlet gate, the Attune CytPix image-derived parameter “Particle Count” reveals this gate actually contains more than 4% doublets and aggregates. As shown in Figure 9, this result can be confirmed with images. Using the “Particle Count” parameter for gating singlets results in a more robust gating strategy.

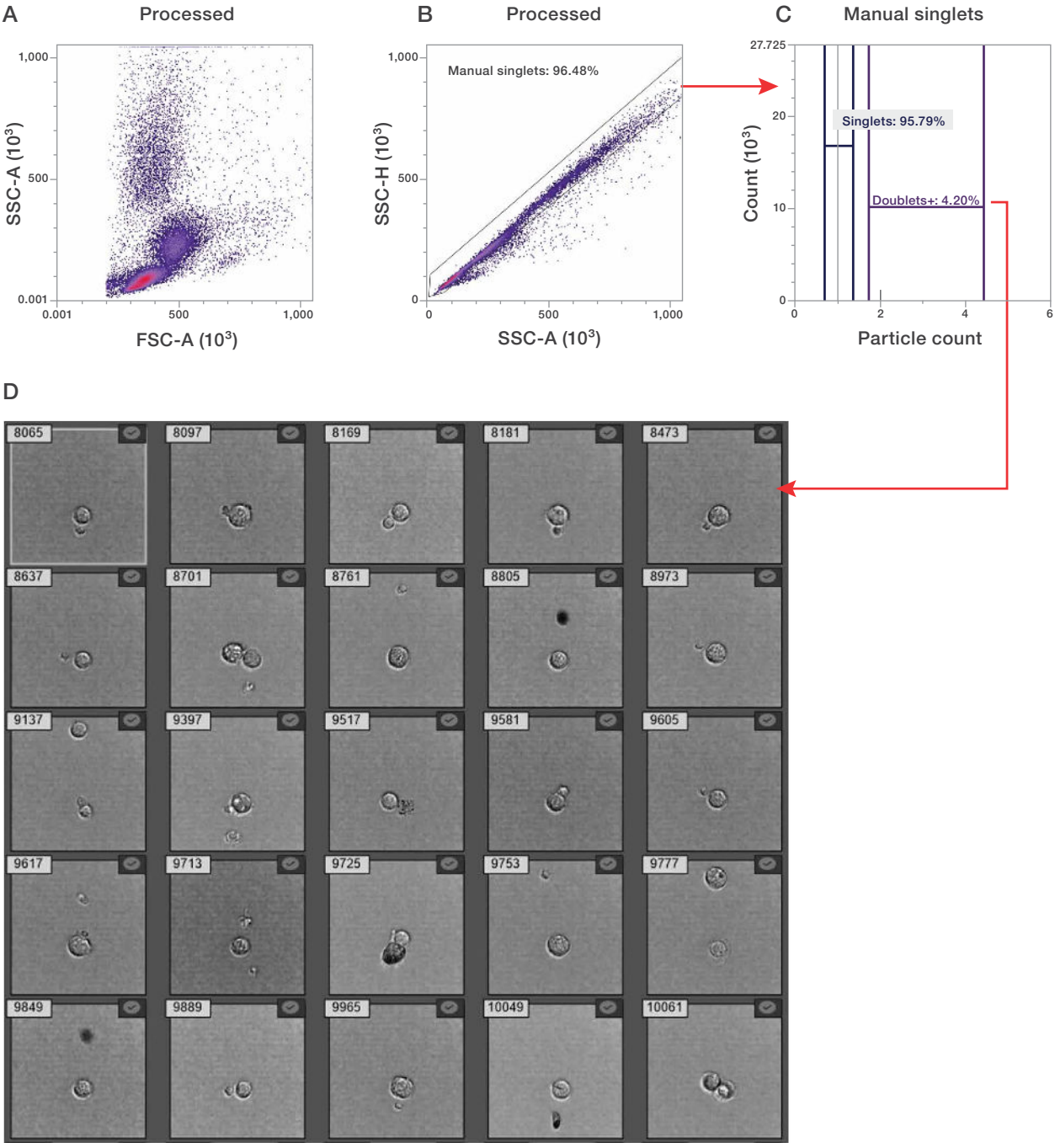
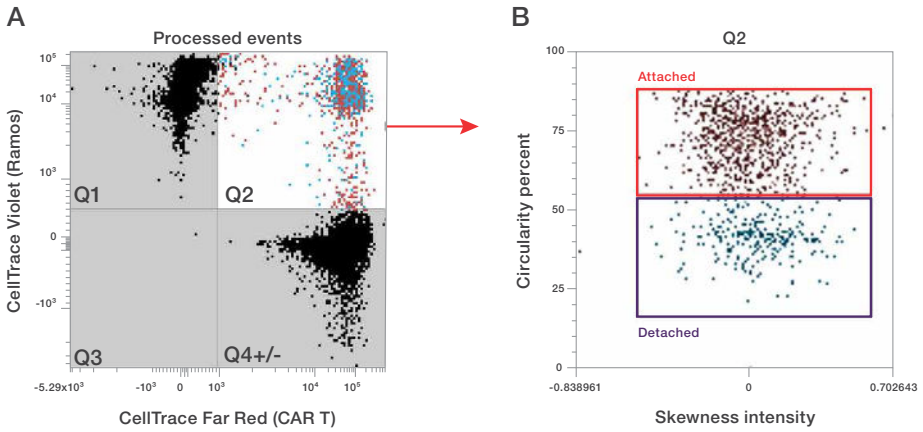



**Figure 9. Aged whole human blood lysed with ammonium chloride lysis buffer.** Image processing was done using the “Cells Half Resolution” model. Statistics shown are % gated.

# Gain new insights into cell biology with image-derived parameters

Imaging CAR T/Ramos cell interactions is possible with the Attune CytPix Flow Cytometer. Users can implement extended image-derived parameters (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.



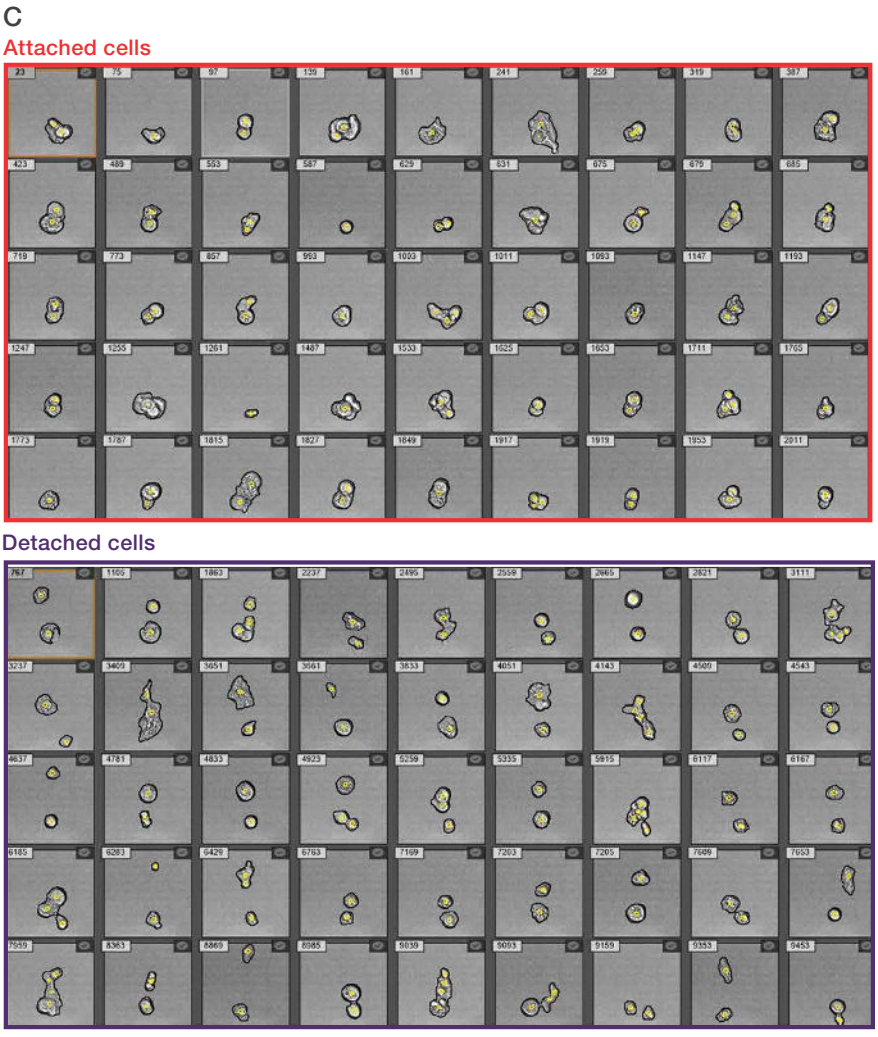




**Figure 10. Visualization of CAR T cells targeting lymphoma cells.** CAR T and Ramos cells were labeled with Invitrogen™ CellTrace™ Far Red and Violet stains, respectively, and incubated at a 1:1 ratio for 1 hour at 37°C. Unfiltered samples were acquired on the Attune CytPix Flow Cytometer at 200 µL/minute, >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 (cell 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.

# Efficient—rapid, accurate acquisition

## Acoustic focusing can run ~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. This means you can process difficult samples—including low-concentration and precious samples—more quickly and accurately with minimal loss in quality.

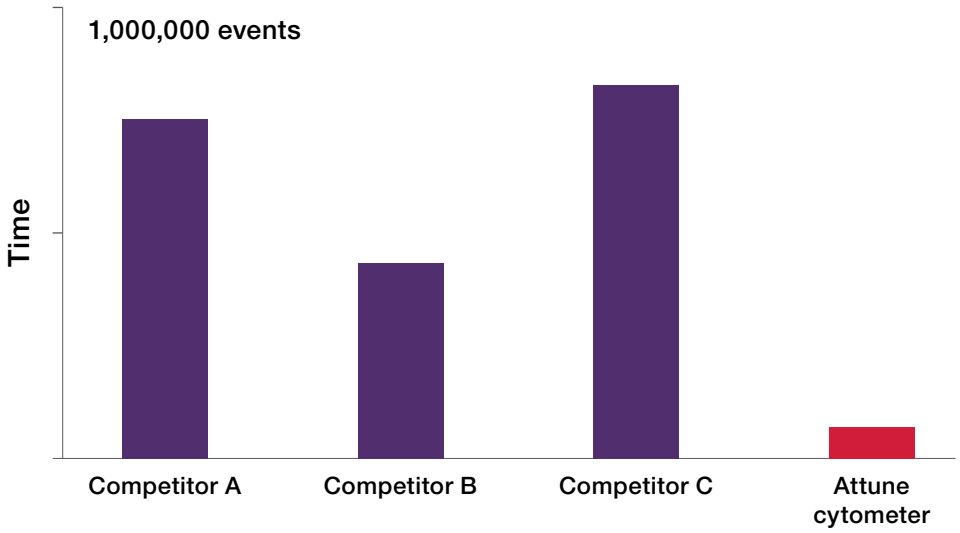



**Figure 11. Rapid data acquisition.** The time required to acquire 1,000,000 events is compared to three competitor instruments running at maximum sample rates.

"What I like most about the Attune NxT Flow Cytometer is its accuracy and its speed of processing. I could list a range of other things that I like about it because we are building up that list as we discover more aspects of its functions, but the core things are speed and accuracy that are not available with hydrodynamic flow cytometers."

— Tim Inglis, BM, DM, PhD, FRCPath, FRCPA, DTM&H
School of Medicine, University of Western Australia, PathWest Laboratory Medicine

## 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 a unique combination of speed and quality, cutting the time to collect rare events significantly over long acquisition times.

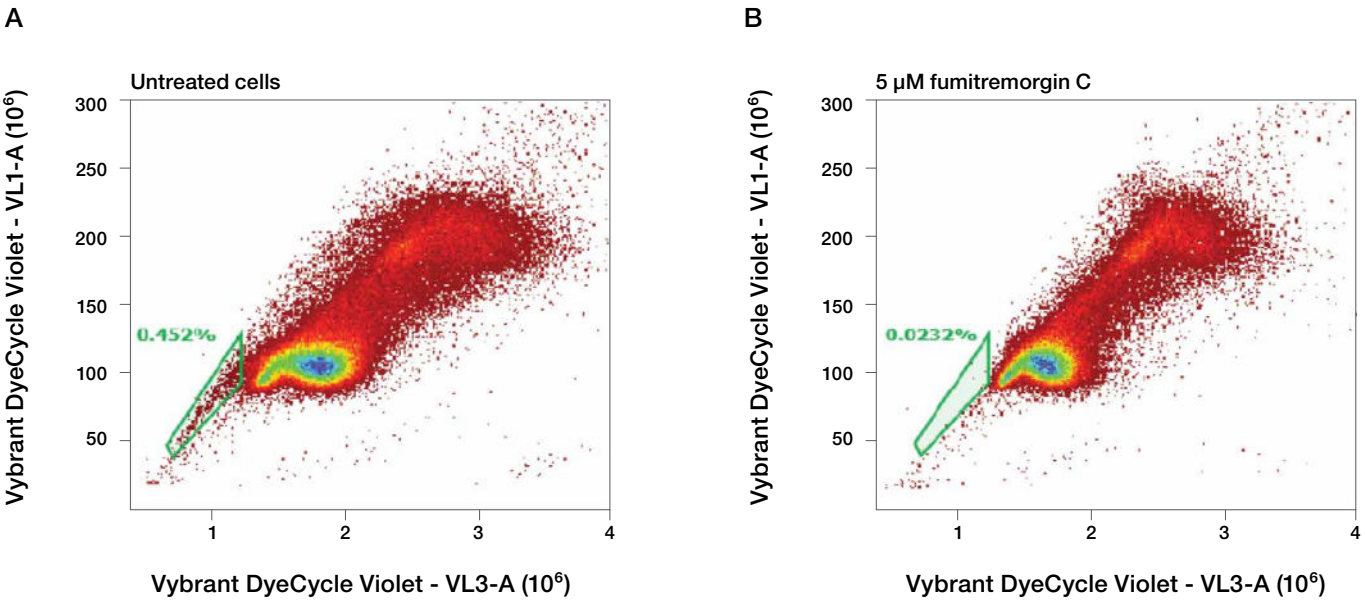



**Figure 12. Identifying limbal stem cells (LSC) in a population of differentiated corneal cells. (A)** Untreated cells have a side population of limbal stem cells (0.452% of total cells) with decreased Invitrogen™ Vybrant™ DyeCycle™ Violet fluorescence due to ABCG2-mediated dye efflux. **(B)** The percentage 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 DyeCycle Violet dye.

> "I can't believe how quick and easy it is to collect a large body of empirical evidence on the Attune NxT Flow Cytometer to fully support our hypothesis."
>
> — Jordi Petriz, PhD
> Group Leader, Functional Cytomics Group;
> Josep Carreras Leukaemia Research Institute, Barcelona, Spain

# High-performance automation and robotic solutions

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

### CytKick Autosamplers allowing 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 CytKick autosamplers.**

| Category | CytKick Autosampler | CytKix 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<br>96-well standard depth<br>384 deep-well<br>384-well standard depth | 96 deep-well<br>96-well standard depth<br>384 deep-well<br>384-well standard depth<br>1.5 mL and 2 mL microcentrifuge tube rack (up to 24 per rack)<br>96-well foil covered<br>384-well foil covered customizable to accept additional plate types |

"We looked at several metrics and compared the CytKick autosampler to other 96-well plate readers. The autosampler proved to have very good stability and very low carryover. We were most impressed by the way that the autosampler took advantage of the Attune NxT Flow Cytometer's fluidics and high-volume throughput. Without compromising stability or precision, the autosampler was able to run plates much faster than any other plate reader."

– EM Meyer
University of Pittsburgh Cancer Institute

## Robotic automation

Extend your unmanned runtime settings and scalability with 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. You can leverage both scheduling and integration to get the most from your solution.

Comprehensive specifications available at
thermofisher.com/flowautomation

**Figure 13. Optional automation configuration.** Maximize operating capacity, mitigate human operator error, and enable rich, reproducible data with the 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.

# Integrated, powerful, and intuitive software

## Attune Cytometric Software

The Attune Cytometric Software is designed for compatibility with Thermo Scientific™ 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.

A

B

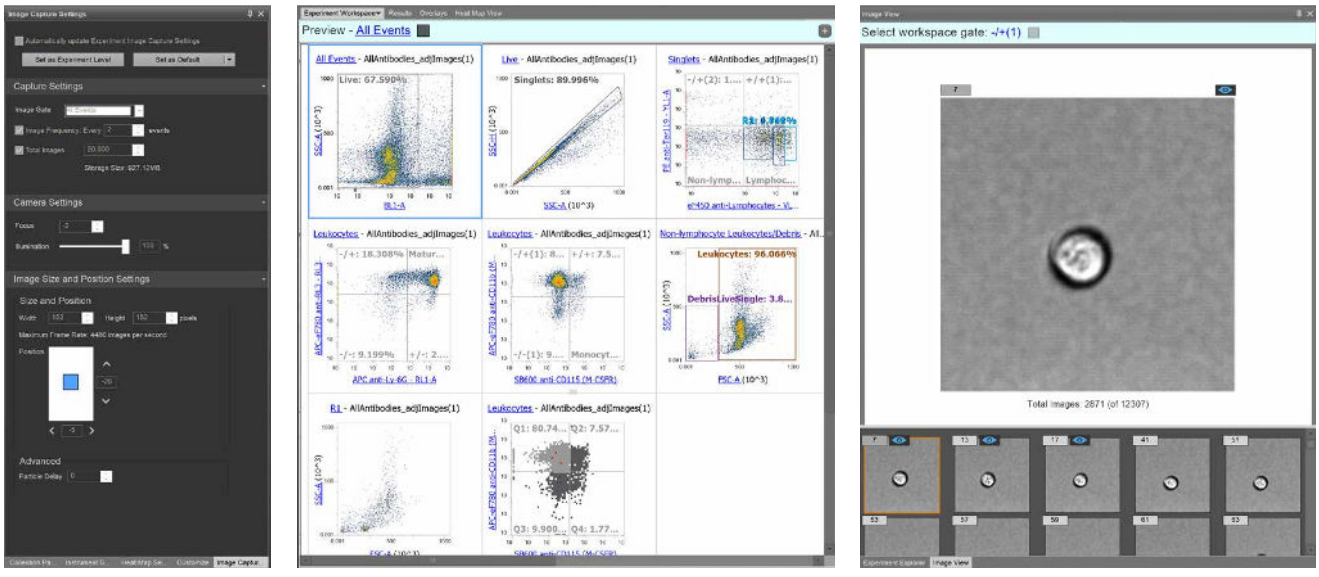



Image analysis settings | Dot plot view | Image view for brightfield image capture

**Figure 14. Intuitive, user-friendly software interface with familiar workflow. (A)** Typical Attune NxT interface and **(B)** typical Attune CytPix interface.

## Imaging analysis software feature

Attune Cytometric Software automates image analysis with a processing rate 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. These measurements consist of more than 25 parameters that include quantification of singlets (particle count), roundness (circularity), size (area square), shape (eccentricity), complexity (entropy), and others (Table 6).

Gating on these image-based parameters allows you to analyze populations of interest and confirm or enhance 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.

Using image parameters enables researchers to improve and quality control gating strategy, as well as make new discoveries about their sample biology.

**Table 6. Image-derived parameters available for image analysis.**

| Image-derived parameters | |
|---|---|
| **Intensity features** | |
| Maximum intensity | Intensity skewness |
| Minimum intensity | Intensity kurtosis |
| Total intensity | Intensity entropy |
| Average intensity | Average normalized intensity |
| Intensity standard deviation (SD) | Normalized intensity SD |
| Intensity %CV | Normalized intensity %CV |
| **Shape features** | |
| Area ($\mu m^2$) | Major axis (µm) |
| Perimeter area (µm) | Minor axis (µm) |
| Circularity (%) | Minor to major axis ratio (%) |
| Pseudo diameter (µm) | Eccentricity (%) |
| **Object feature** | |
| Particle count | |
| **Pixel feature** | |
| Pixel count | |
| **System features** | |
| On border | Processed |
| Confidence score | Processable |

**Figure 15. The Attune Cytometric Software is intuitive and easy to use.**

## Momentum Integration Software

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 ensuring 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 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.

## 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

# Flow cytometry reagents

Accelerate your science with a comprehensive suite of solutions for the analysis of cells and their function with Invitrogen™ eBioscience™ flow cytometry antibodies and Invitrogen™ cell health reagents.

**Antibodies**—Build and expand your panels using our extensive portfolio of antibodies conjugated to over 30 types of fluorophores, including traditional, 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 (Figure 16). Now there are more colors to choose from, which offers you flexibility when designing multicolored panels.

**Instrument and compensation**—Beads are essential to perform quantitative measurements on individual cells and other particles with high precision, speed, and accuracy, especially when performing flow cytometry using multiple channels, markers that are poorly expressed, or samples of limited quantity. Select from our wide range of easy-to-use beads for your experimental needs.

**Buffers**—We offer a wide variety of buffers to suit your research needs, whether your experiment calls for extracellular, intracellular, and/or nuclear cell staining.

**Compensation and instrument beads**—Build flow cytometry panels with more accurate compensation using Invitrogen™ UltraComp eBeads™ Plus Compensation Beads. When a fluorophore-conjugated antibody is added to the beads, both positive and negative populations result (Figure 17).

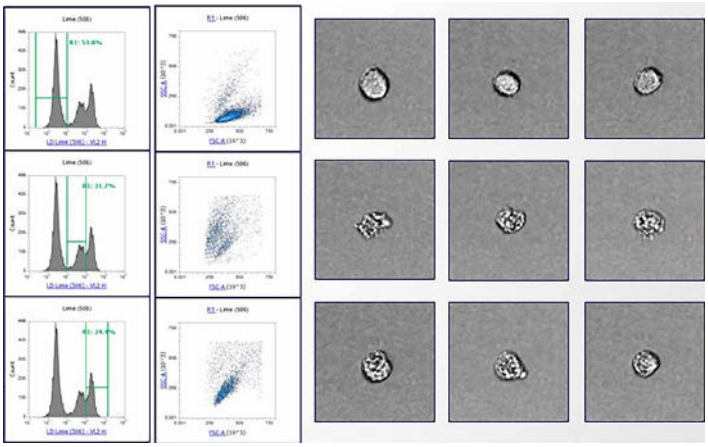


**Figure 16. LIVE/DEAD dyes have minimal spillover and allow for larger and more accurate flow cytometry experiments.** Incubate cells with the Invitrogen™ LIVE/DEAD™ Fixable Lime (506) Viability Kit for 405 nm excitation to distinguish viable from dead cells. Viable cells show rounded and intact cell membranes; dead and dying cells show compromised membranes.

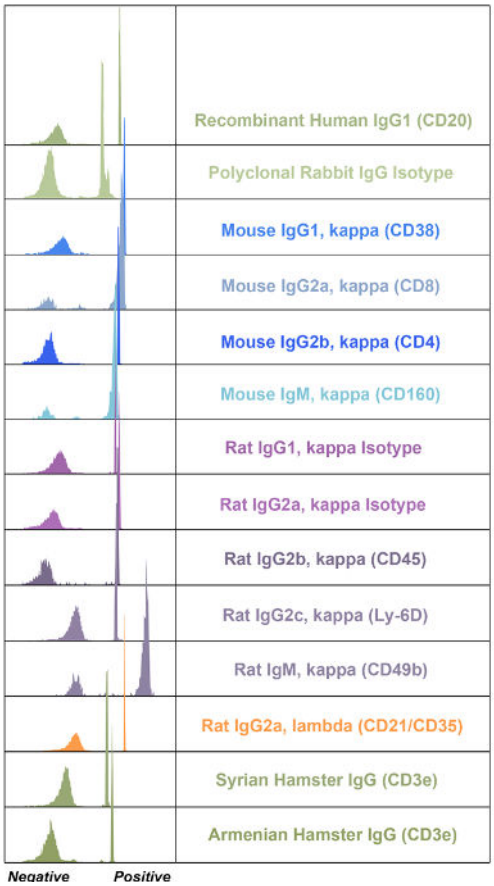



**Figure 17. Staining of UltraComp eBeads Plus Compensation Beads with 14 different Invitrogen™ PE-conjugated monoclonal antibodies, including one of each subclass commonly used in flow cytometry.** Beads were stained with 0.25 µg of each antibody and analyzed by flow cytometry. Each histogram represents one staining antibody.

## Flow Cytometry Panel Builder

Design your panel for your Attune Flow Cytometer using the Invitrogen™ Flow Cytometry Panel Builder:

- A quick and easy-to-use web tool (Figure 18)
- Allows incorporation of antibodies using the configuration of your Attune Flow Cytometer
- Fluorophore selection built on spectral visualization of all fluorophores per laser

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

**Figure 18. Simplified panel design with a 5-step panel design strategy.** The Flow Cytometry Panel Builder offers a customizable panel building process to fit your flow cytometry experimental needs, whatever your experience level.


Get free, one-on-one, customized assistance with a live specialist for panel design with our Free Panel Design Service

Get more information at
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

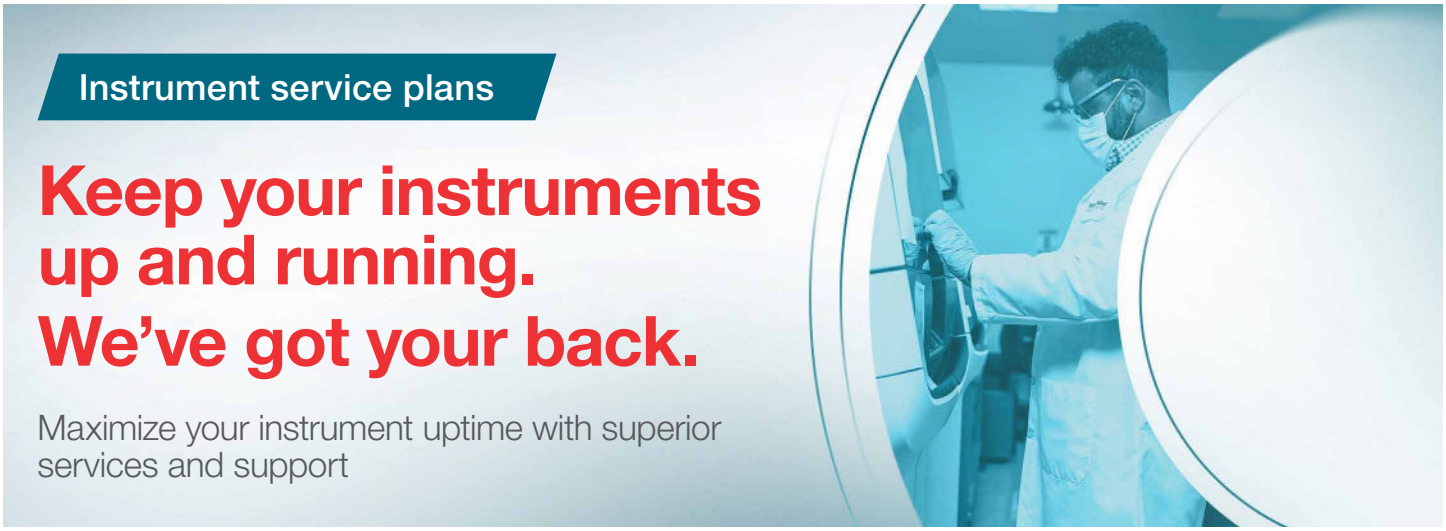
Our technical services, field engineering, and training teams are fully committed to your success using Attune Flow Cytometers for your research. Instrument service plans, consulting, and training programs are designed to ensure instrument performance, team readiness, and overall optimal research outcomes 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
- **AB Assurance plan and extended warranty**—covers all costs associated with instrument repairs

Table 7. Comparison of 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. |
|---|---|
| **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 |
| **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 Wash Solution, 250 mL | A24974 |
| Attune Shutdown Solution (1X), 250 mL | A24975 |
| Attune NxT No-Wash No-Lyse Filter Kit | 100022776 |
| Attune Performance Tracking Beads | 4449754 |
| **Software** | |
| Attune Software, single license | A25554 |
| Attune Software, 5 licenses | A24856 |
| Attune Software, 10 licenses | A24855 |
| Attune Software 21 CFR Part 11, single license | A47288 |
| Attune Software 21 CFR Part 11, server license, 5 users | A47289 |
| Attune Software 21 CFR Part 11, server license, 10 users | A47290 |
| Attune IQ/IPV, Attune Operation Qualification and Instrument Performance Qualification (IQ/IPV) | 4465413 |
| Attune IQ/OQ, Attune Installation Qualification and Operation Qualification (IQ/OQ) | 4465445 |

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

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