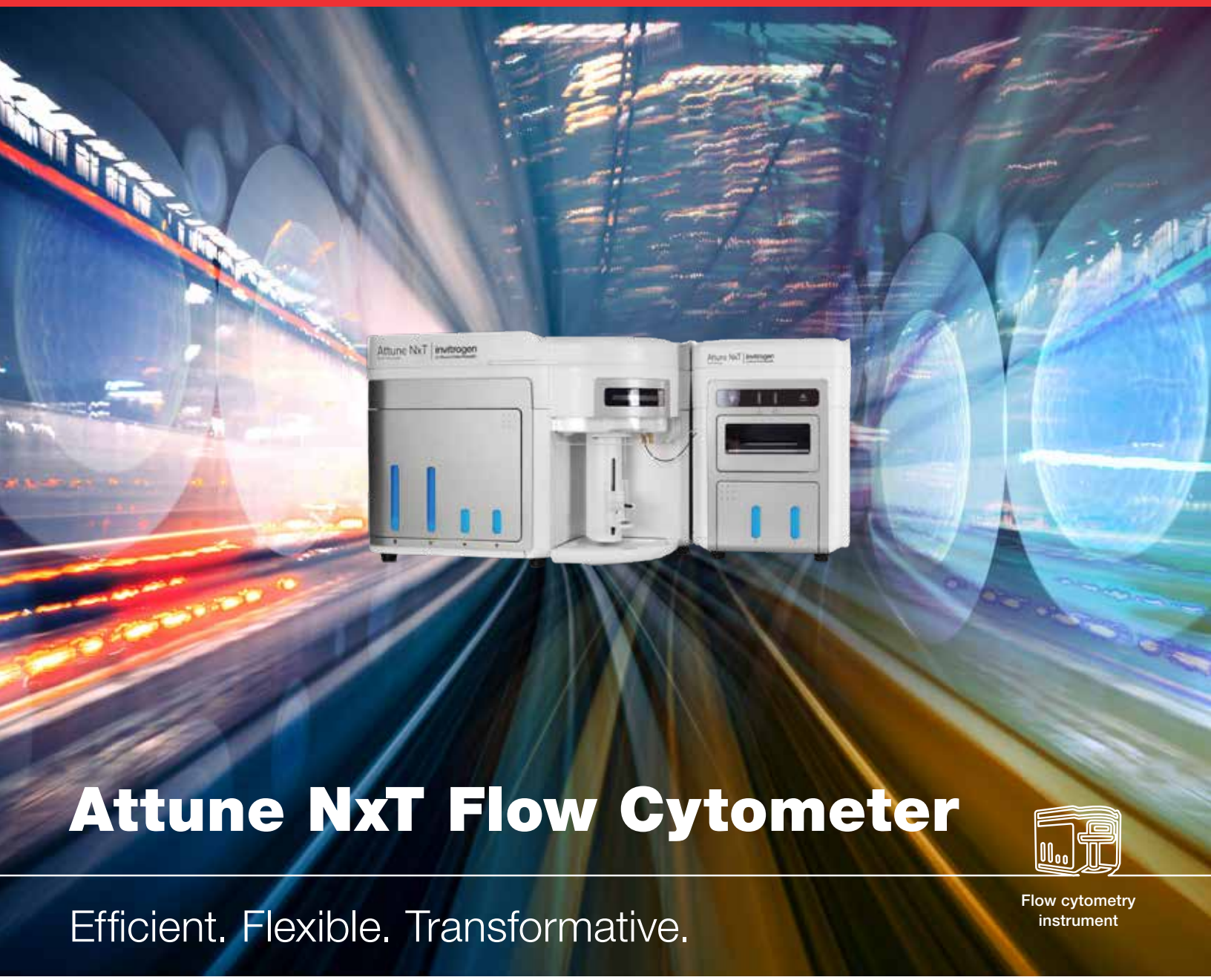
invitrogen



Attune NxT Flow Cytometer

Efficient. Flexible. Transformative.

Flow cytometry instrument

ThermoFisher SCIENTIFIC

Building a legacy of advancing flow cytometry technology

Never settle for average when striving for significant breakthroughs

Technological advancements in flow cytometry are creating an entirely new lineage of reagents and instruments. They break the mold of traditional products found in flow cytometry.

Innovative flow cytometry solutions enable research studies in academia, pharma and biopharma, and across various industries to create more effective, efficient, and economical solutions.

Thermo Fisher Scientific is committed to developing products that fuel groundbreaking research, ensuring uncompromising quality and enabling greater efficiency.

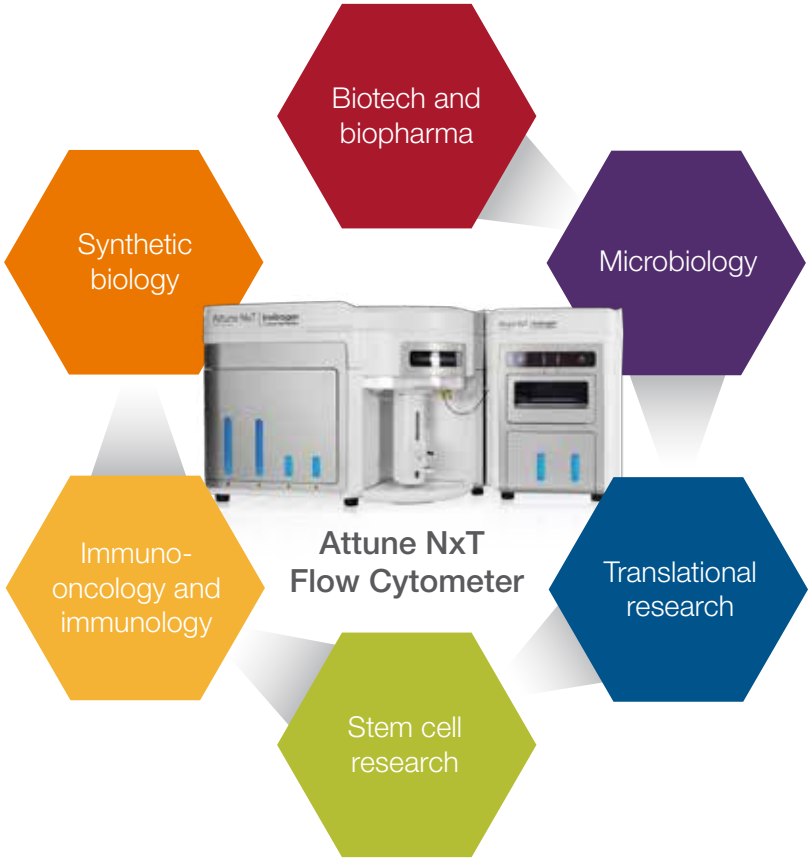
With the industry's largest research and development budget, our suite of comprehensive flow cytometry solutions, including Invitrogen™ Attune™ NxT Flow Cytometer together with the Invitrogen™ cell health reagent portfolio and the Invitrogen™ eBioscience™ antibody conjugates, help drive discovery of new biological insights for many applications.

Find out more at
thermofisher.com/attune

Antibodies for
>10,000
flow cytometry

>195
Attune NxT Flow Cytometer citations

>642
original Attune citations



denotes environmentally friendly feature

Contents

Explore the Attune NxT Flow Cytometer

Designed for efficiency

Speed and accuracy

Technology

With acoustic-assisted hydrodynamic focusing, the Attune NxT Flow Cytometer (Figure 1) avoids compromise between data quality and higher sample rates by uncoupling cell alignment from sheath flow. Acoustic-assisted hydrodynamic focusing precisely aligns cells using ultrasonic radiation pressure (>2 MHz) to transport particles into the center of the sample stream. This prefocused stream is then injected into the sheath stream (Figure 2). This results in a narrow particle stream and uniform laser illumination, regardless of the sample input rate.

The instrument's speed specifications include:

- Sample input flow rate ranges from 12.5 to 1,000 µL/min
- Data acquisition speed up to 35,000 events/second with 34 parameters, based on a 10% coincidence rate per Poisson statistics
- Maximum electronic speed is 65,000 events/second with all parameters

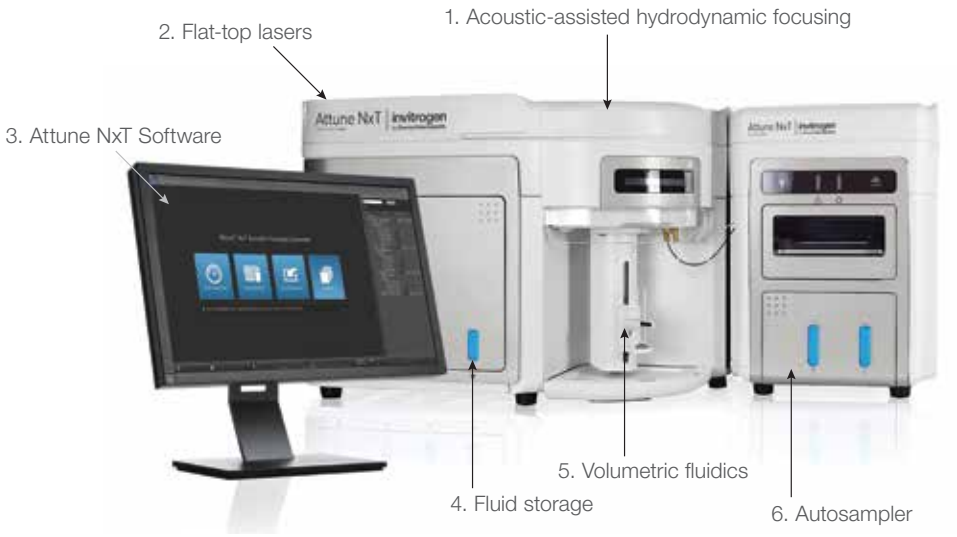


Figure 1. The Attune NxT Flow Cytometer components. (1) Patented acoustic-assisted hydrodynamic fluidics increase sample input speed while maintaining data integrity. **(2)** Flat-top lasers deliver more even application of light to each cell. **(3)** Invitrogen™ Attune™ NxT Software designed to guide users through complex flow cytometry experiments. **(4)** Fluid storage designed for minimal waste. **(5)** Volumetric fluidics provides cell counting and a resistance to clogging. **(6)** Autosampler provides easy 1-click transition from tube to plate.

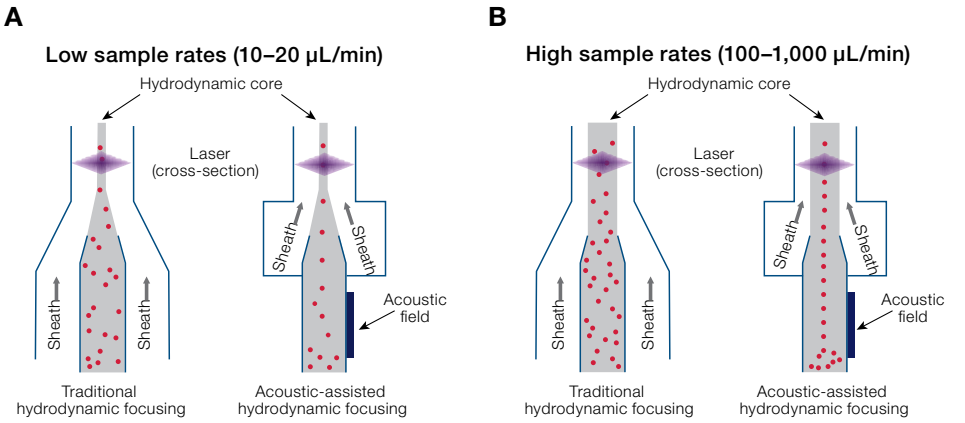


Figure 2. Acoustic focusing vs. traditional hydrodynamic focusing as particles pass through the laser. (A) In acoustic focusing, cells remain in tight alignment even at higher sample rates, resulting in less signal variation and improved data quality. **(B)** In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream, resulting in increased signal variation and compromised data quality.

Benefits

- Greater reproducibility and consistency in data
- Maintain consistent concentration results across all flow rates (Figure 3)
- Process very dilute or concentrated samples while maintaining low coefficient of variations (CVs) (Figure 4)

35,000
events/sec

Up to
1,000 µL/min

10x
faster*

Data

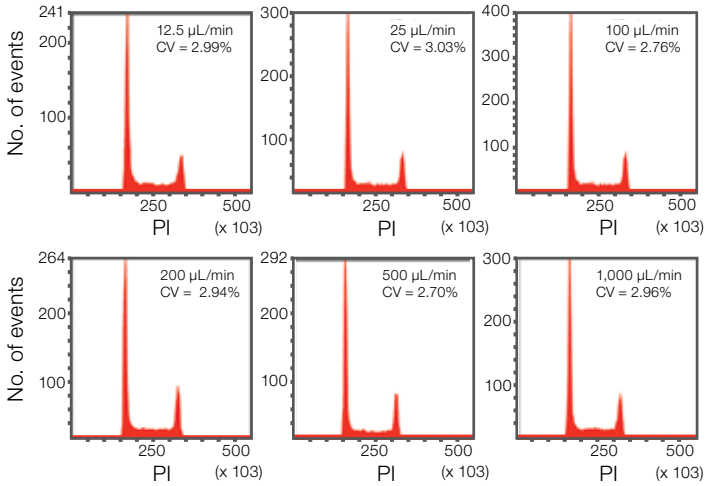


Figure 3. Minimal data variation at high sample rates with the Attune NxT Flow Cytometer. Jurkat cells were fixed and stained with propidium iodide, treated with RNase, and analyzed at a concentration of 1 x 10^6 cells/mL at different sample rates. The left peak in all graphs reflects cells in G_0/G_1 phase, while the right peak reflects cells in G_2/M phase. Regardless of sample rate, the width of the G_0/G_1 and G_2/M peaks, and the CVs remain consistent, even at the highest sample rate of 1,000 µL/min.

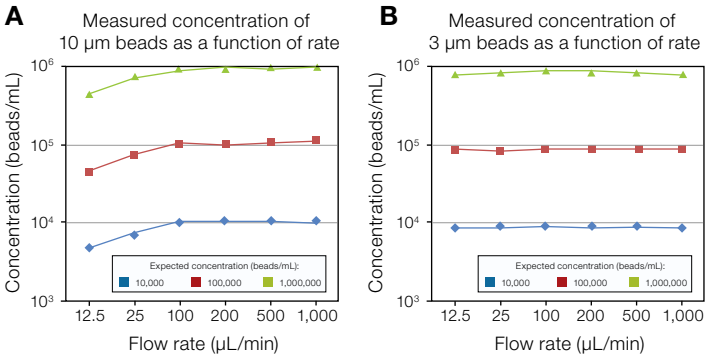


Figure 4. Data demonstrating the measured vs. expected concentration as a function of flow rate. (A) Measured concentration of 10 µm beads as a function of rate. Larger particles (e.g., 10 µm) show consistent results across the flow rate range 100–1,000 µL/min. **(B)** Measured concentration of 3 µm beads as a function of rate. Smaller particles (e.g., 0.2–3 µm) show consistent concentration results across all flow rates for the three concentrations of beads/mL tested.

"The ability to run very dilute samples is quite amazing and might be a life saver on many occasions where you have little-to-no sample left."

– J. P. Robinson, PhD
Purdue University

* Than traditional hydrodynamic focusing systems.

Smooth flow delivery for accurate counts

The Attune NxT Flow Cytometer delivers samples into the instrument with minimal variation (Figure 5). Smoother delivery of samples provides more confidence when presenting cell counting data.

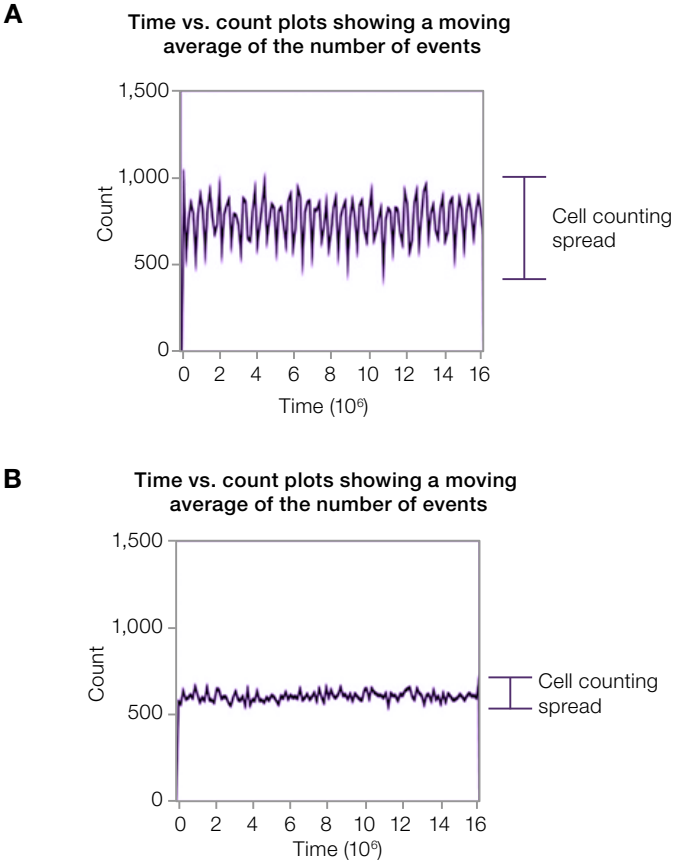
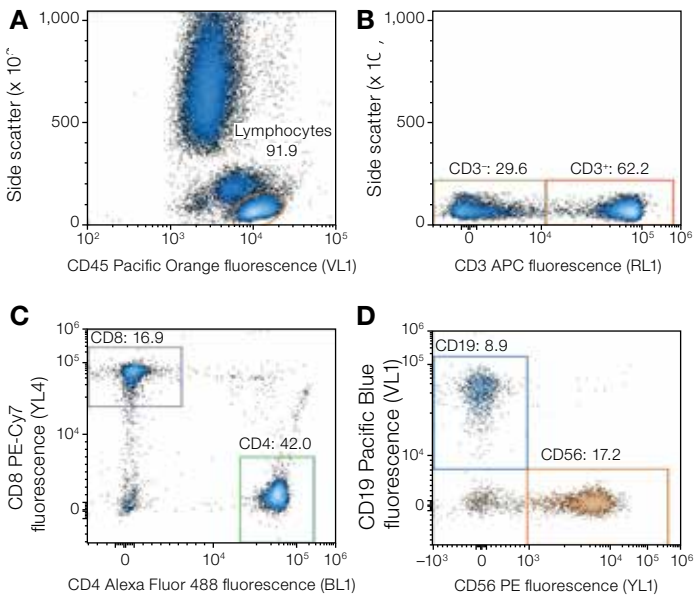


Figure 5. Time vs. count plot to obtain a moving average of the number of events showing up at a given time. (A) Data from a flow cytometer with a peristaltic pump, showing fluid pulsation ±33% of the average count and a total spread of 66% of the average count. **(B)** Time vs. count data from Attune NxT Flow Cytometer with a non-peristaltic pump, showing fluid pulsation of ±5%.

Technology

Samples on the Attune NxT Flow Cytometer are delivered by a positive-displacement syringe pump for volumetric analysis meaning that all events are automatically counted, and particle counts or concentrations can be viewed with the simple click of a button. Figure 6 shows the scatter plots and cell concentrations for all lymphocyte subpopulations.

Data



Name	Count	%Total	%Gated	Concentration
All Events	66,906	100.00	100.00	297.4
Lymphs	20,683	30.91	30.91	91.9
CD3⁺	13,990	20.91	67.64	62.2
CD4	9,439	14.11	67.47	42.0
CD8	3,811	5.70	27.24	16.9
CD3⁻	6,655	9.95	32.18	29.6
CD19	2,001	2.99	30.07	8.9
CD56	3,869	5.78	58.14	17.2

Figure 6. Lymphocyte subset analysis. A 100 µL aliquot of normal human whole blood was labeled with fluorophore-conjugated antibodies against CD surface markers, followed by red blood cell (RBC) lysis using 2 mL of Invitrogen™ High-Yield Lyse Fixative-Free Lysing Solution (Cat. No. HYL250), resulting in a 1:21 dilution of the blood. **(A)** Lymphocytes are identified on a density plot of CD45 vs. side scatter with an oval gate around the lymphocyte ($CD45^+$) population. **(B)** Cells in the lymphocyte gate are displayed on a density plot of CD3 vs. side scatter. Rectangle gates surround the $CD3^+$ T cell and $CD3^-$ B and natural killer (NK) cell populations. **(C)** Cells in the $CD3^+$ gate are then displayed on a density plot of CD4 vs. CD8 to quantify $CD4^+$ helper T cells ($CD4^+ CD3^+ CD45^+$) and $CD8^+$ cytotoxic T cells ($CD8^+ CD3^+ CD45^+$). **(D)** $CD3^-$ cells are displayed on a density plot of CD56 vs. CD19 to distinguish $CD56^+$ NK cells from $CD19^+$ B cells. The statistics table shows the gating and measured concentrations (cells/µL).

Benefits

- Syringe easily removed for cleaning or replacement
- Consistent cell concentration results across all flow rates (Figure 7)
- Precise counts without the need for expensive beads

Tips

Useful for samples that are inherently low in concentration, such as cerebrospinal fluid (CSF), and stem cell samples with low cell numbers.

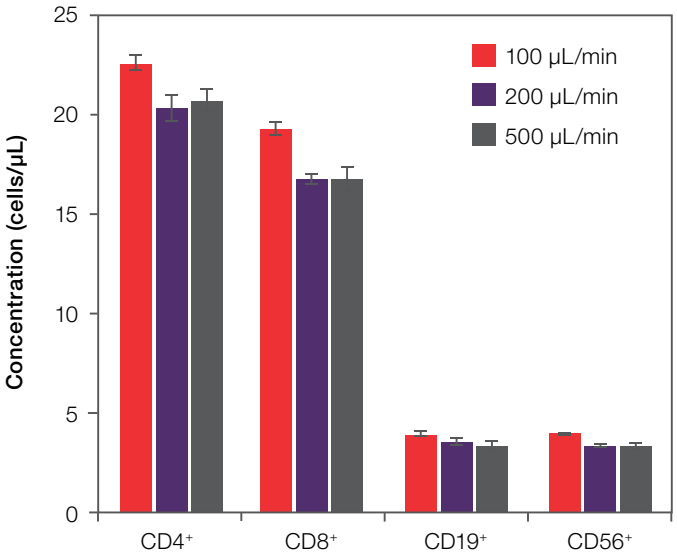


Figure 7. Replicate samples collected at three flow rates on the Attune NxT Flow Cytometer. Cell concentrations were measured using three different flow rates: 100, 200, and 500 µL/min. The Attune NxT Flow Cytometer provides similar concentration measurements for each lymphocyte subpopulation, regardless of the flow rate. Each bar represents the mean cells/µL ±standard deviation of three samples run at each indicated flow rate for each population.

Reduce clogging from difficult samples

Your research samples are precious as they are often difficult to produce. The Attune NxT Flow Cytometer is less prone to clogging, allowing challenging samples such as cardiomyocytes, heterogeneous blood cells, and cancer cells to flow with confidence.

Technology

Engineered to actively resist clogging, a syringe-driven system (Figure 8) and larger flow cell help prevent the loss of precious sample such as cancer stem cells from primary pancreatic tumors (Figure 9), and is drastically less susceptible to clogs. The Attune NxT Flow Cytometer employs a non-pressurized system that mechanically decreases the occurrence of clogging.

Benefits

- Easy flow of difficult samples such as large or sticky cells
- Sample recovery feature built into software
- Comparatively lower fluid consumption (~1.8 L/day)

Tips

The higher the flow rate a sample is run on the Attune NxT Flow Cytometer, the lower the amount of sheath fluid that is used

"We have yet to clog the machine with our debris-rich primary tumor samples. Of course, the acoustic technology greatly facilitates the identification of small populations, like cancer stem cells, increasing our capacity to detect and quantify these rare events with high efficiency and reliability."

– Bruno Sainz Jr, PhD
Autónoma University of Madrid, School of Medicine

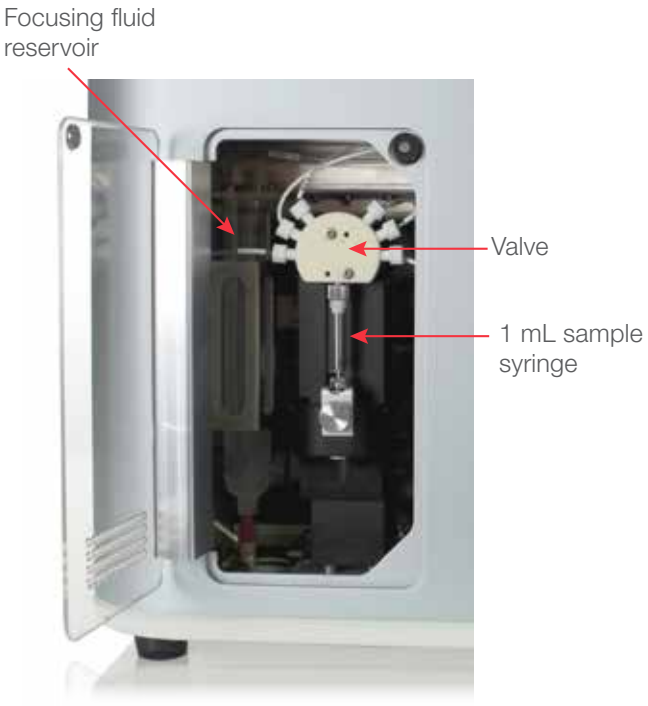


Figure 8. Positive-displacement syringe pump. Syringe easily removed for cleaning or replacement.

Data

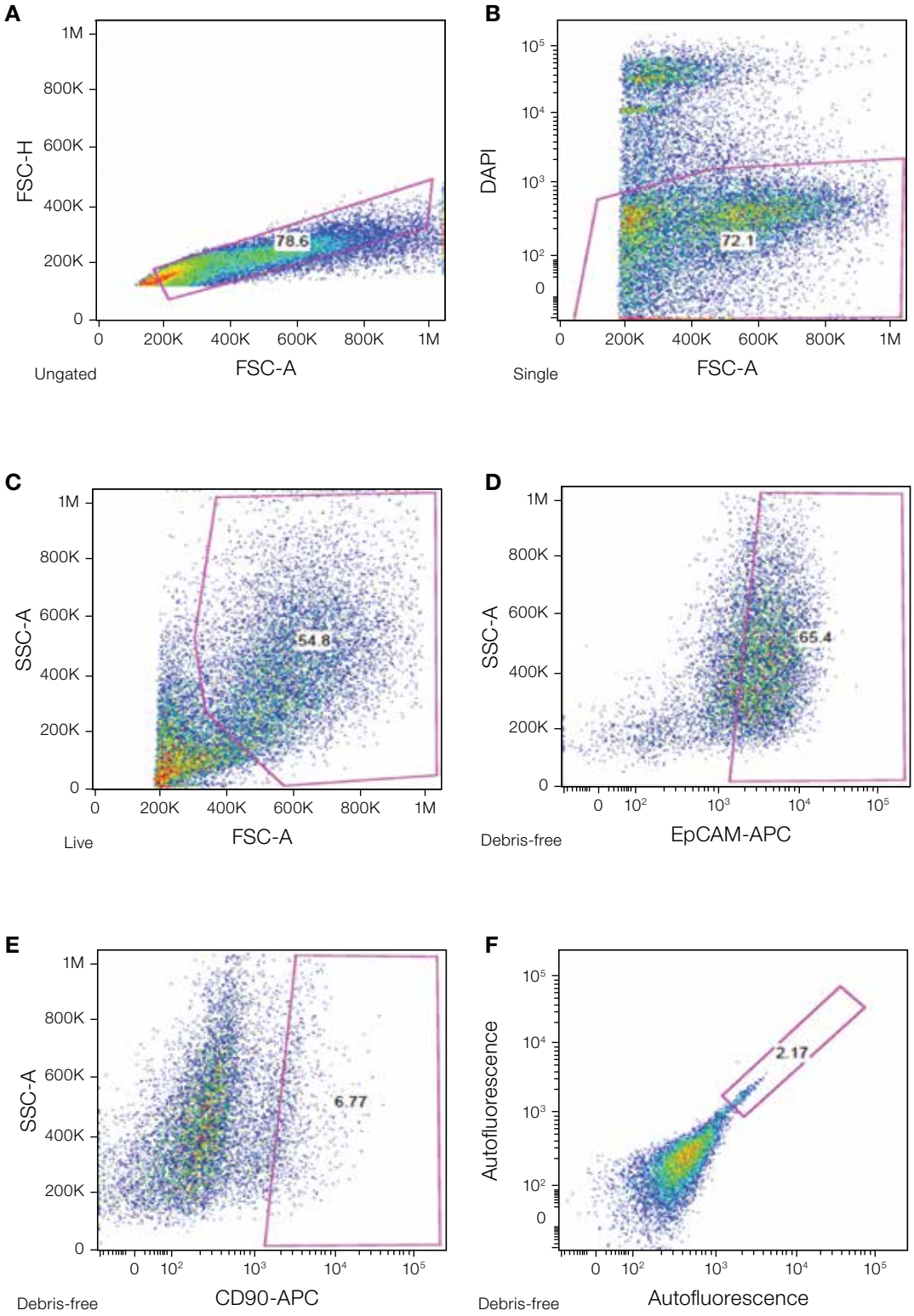


Figure 9. The Attune NxT Flow Cytometer detects autofluorescent and CD90⁺ rare cancer stem cells from primary pancreatic tumors without clogging. Tumors were minced and enzymatically digested with collagenase, followed by an overnight incubation with 30 µM riboflavin in RPMI medium with 10% FBS. Cells were then blocked with flebogamma and stained with anti-EpCAM or anti-CD90 antibodies. **(A–C)** Single, live, and debris-free cell gating strategy. **(D)** EpCAM⁺, **(E)** CD90⁺, and **(F)** autofluorescence-positive cells within the tumor population. Data courtesy Bruno Sainz Jr, PhD.

Application highlight

Improved data resulting from less trauma to cells

Acoustic focusing allows the Attune NxT Flow Cytometer to deliver a no-wash, no-lyse protocol (Figure 10) to minimize cell loss, significantly reduce time, and simplify sample preparation.

> "Multiplexing and compensation are much easier and extremely efficient with the Attune NxT."
>
> – Bruno Sainz Jr, PhD
> Autónoma University of Madrid, School of Medicine

Benefits

- Improve lab safety with reduced sample handling with no-wash protocol
- Completely cut out time-consuming centrifugation steps
- Save countless hours running dilute samples and reduce reagent costs
- Eliminate cell loss due to wash steps or RBC removal procedure
- Ideal for limited sample volumes and for functional live-cell assays (Figure 11)

Protocol

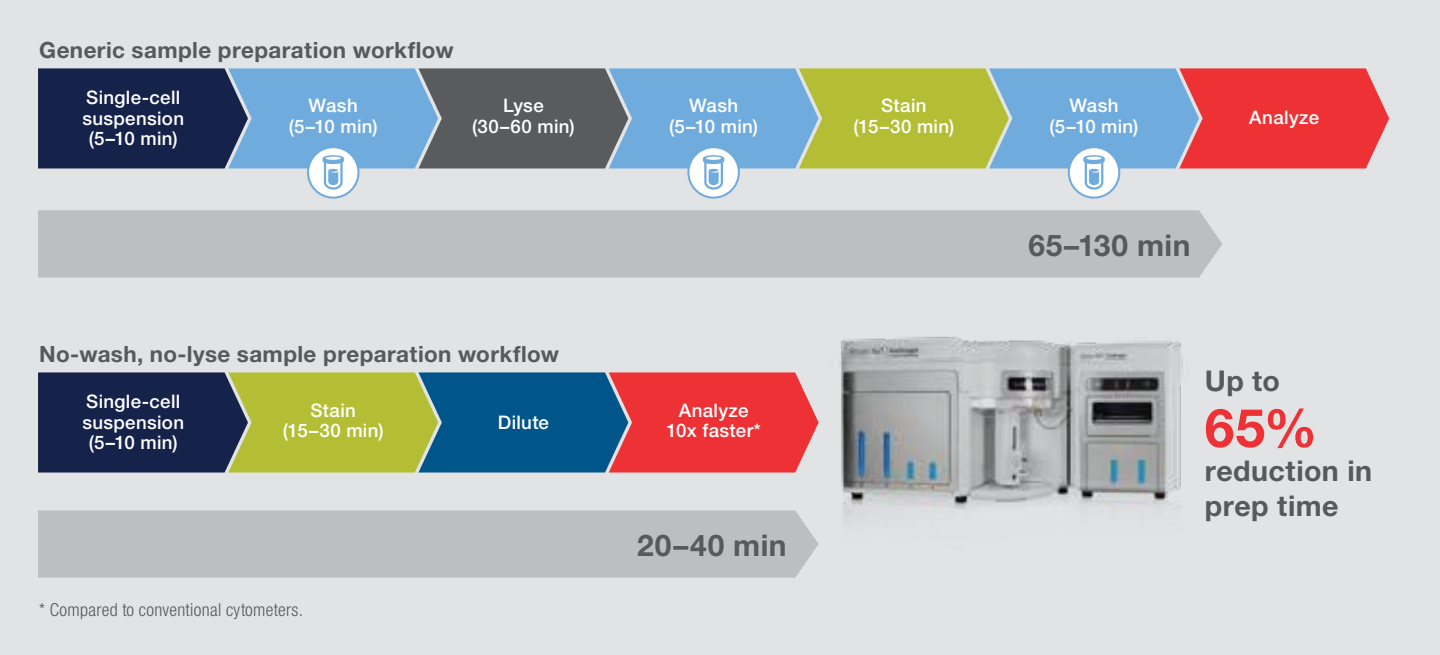


Figure 10. No-wash, no-lyse sample preparation workflow.

Data

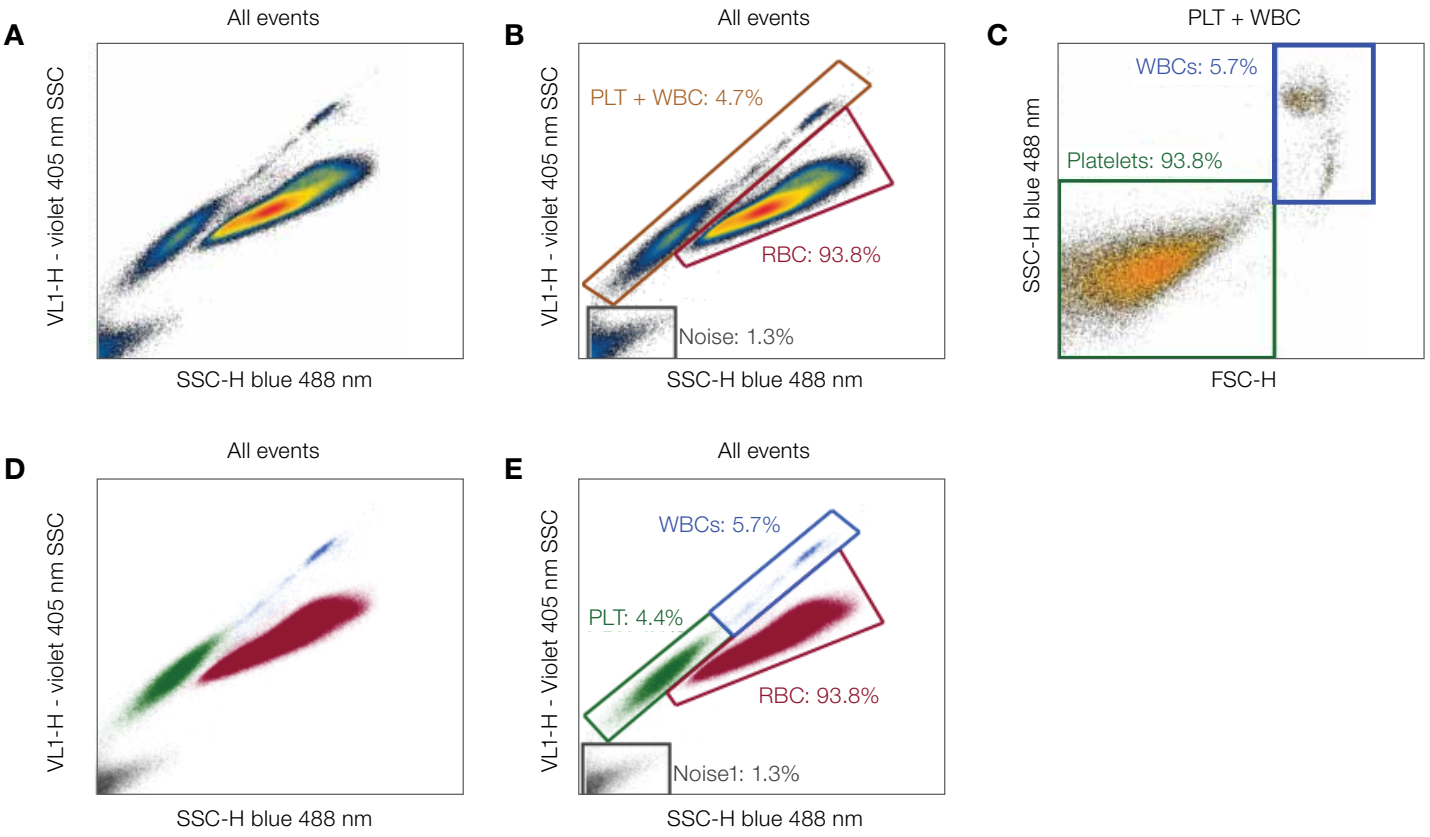


Figure 11. Forward scatter (FSC) and side scatter (SSC) analysis with blue (488 nm) and violet (405 nm) lasers on intact whole blood (no-lyse, no-wash). (A, B) RBCs, white blood cells (WBCs), and platelets are separated on the basis of light scatter only by using a combination of blue and violet laser 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 show the dominant cell type in whole blood is the RBC, while the WBCs and platelets are relatively rare events.

Precision optical performance

Minimize instrument downtime with the Attune NxT optical system. The Attune NxT lasers are designed to last the life span of a flow cytometer and provide a wider area of light intensity.

Technology

The Attune NxT Flow Cytometer uses flat-top lasers with an intensity profile that allows a much wider window of alignment (Figure 12). This innovative design helps ensure precise fixed alignment of 4 spatially separated solid-state lasers onto the sample stream (Figure 13), minimizing the effects of changes in fluidics or optics. The stability of the optical system leads to increased data consistency over time, superior performance, and first-class reliability.

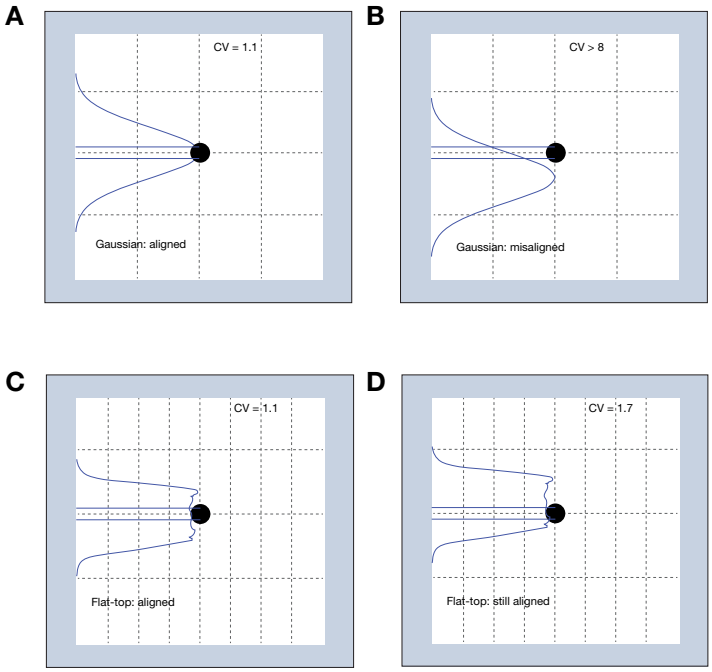


Figure 12. Emission profiles of lasers used in flow cytometers. **(A)** Gaussian laser profile with proper alignment, **(B)** Gaussian laser profile with misalignment, **(C)** flat-top laser profile with proper alignment, and **(D)** flat-top laser profile still in proper alignment.

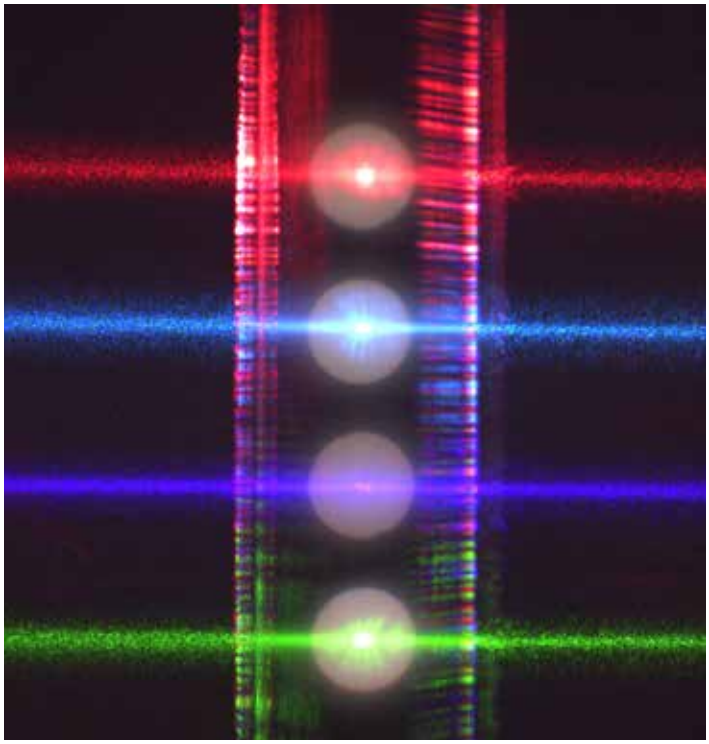


Figure 13. The Attune NxT Flow Cytometer can be configured with up to 4 spatially separated lasers.

Benefits

- No warm-up delay: fiber isn't affected by instrument warm-up
- Simmer mode: automatic shutoff prolongs laser usage lifetime up to 10x
- Lasers are only turned on when acquiring samples

"Having evaluated the instrument over several months, I would say the Attune NxT Flow Cytometer fits the superior category of flow cytometers."

– J. P. Robinson, PhD
Purdue University

Attune NxT Autosampler

For even more efficiency

Improve workflow efficiency with the high-throughput option—the Invitrogen™ Attune™ NxT Autosampler. Built-in compatibility switches between tubes and plates with a single click in the Attune NxT Software. The Attune NxT Autosampler is compatible with many different plate formats, including 96-well, 384-well, and deep-well plates. The system is designed to provide minimal variation regardless of sampling method (tube vs. plate) and collection rate (Figures 14 and 15).

> "The dual tube-to-plate operation—instant change from tubes to plates is really an excellent feature."
>
> – J. P Robinson, PhD
> Purdue University

Technology

Acquisition time*

- <42 min for 96-well plate
- <180 min for 384-well plate

Carryover

- <0.5% in "Plate Loader" format—standard mode, 2 wash cycles
- Ultralow carryover—multiple rinse capability

Extended fluidics option

- Optional external fluid tank with 10 L fluid capacity.

* Using one rinse and one mix (aspiration) and full analysis of a 40 µL sample.

Benefits

- One-click transition from tubes to plates using Attune NxT Software
- Performs automated cleaning when the instrument is shutting down
- Mixes sample by aspiration instead of shaking, ensuring homogeneity of the sample and maintenance of cell viability

> "We looked at several metrics and compared the Attune NxT 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."
>
> – E. M. Meyer
> University of Pittsburgh Cancer Institute

Data

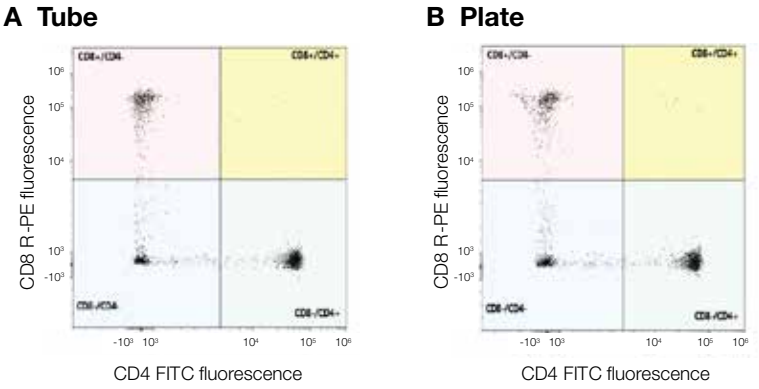


Figure 14. Consistent results are achievable regardless of sampling method. Whole blood lysed with ammonium chloride was labeled with Invitrogen™ mouse anti–human CD45 Pacific Orange™, mouse anti–human CD4 FITC, and mouse anti–human CD8 R-PE antibody conjugates. Labeled samples were analyzed on a blue and violet laser–configured Attune NxT Flow Cytometer equipped with a 488 nm laser for fluorescence excitation of FITC (530 BP) and R-PE (574/24 BP), and a 405 nm laser for Pacific Orange dye (603/48 LP). Identical samples, including compensation controls, were analyzed using either **(A)** tube mode or **(B)** plate mode with a standard collection rate of 200 µL/min. Lymphocytes were gated using a CD45 vs. side scatter plot and analyzed for expression of CD4 and CD8 antigens. Minimal variation was observed between analysis in a tube alone and on a plate running on the Attune NxT Autosampler.

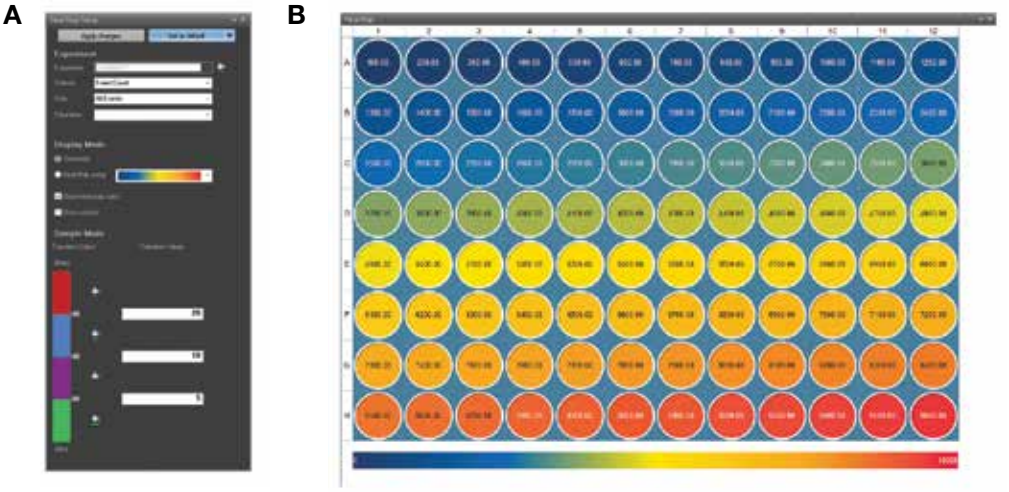


Figure 15. Consistent well-to-well results: the Attune NxT Autosampler heat-map function identifies variation within a parameter across a 96-well plate. Live and heat-killed THP-1 cells were stained with 2 µg/mL propidium iodide, dispensed into a 96-well V-bottom plate, and run at a standard collection rate of 500 µL/min with 2 mix cycles per well and 2 rinse cycles between wells. Propidium iodide was excited using a 488 nm laser (640 LP). **(A)** On the heat map, a color gradient graphically represents the percentage of propidium iodide–positive cells (dead cells). Red-colored wells indicate 0% propidium iodide–positive cells (live cells) within the sample analyzed from that well; magenta-colored wells indicate a sample containing 100% propidium iodide–positive cells. **(B)** The values overlaid on each well in the heat map are the measured percentages of dead cells in the individual wells. Minimal variation is observed in propidium iodide fluorescence across each row of the entire plate, with a CV of 1.44% for the entire data set (96 wells).

Flexibility to create a practical instrument

Detect the full range of fluorescence

The Attune NxT Flow Cytometer accommodates up to 14 color panels. The filter and laser are configurable and field upgradable, giving the freedom to upgrade up to 4 lasers and 16 detection channels (Tables 1 and 2).

"The problem now is not finding the needle in the haystack, but deciding which haystack to look at."

– Prof. Andrea Cossarizza
University of Modena, Italy

Table 1. The Attune NxT Flow Cytometer system configurations.

Lasers	Laser configuration	Cat. No.	Violet 405 nm	Blue 488 nm	Yellow 561 nm	Green 532 nm	Red 637 nm	Total detection channels*
1	Blue	A24864	Available as upgrade	4	Available as upgrade	Available as upgrade	Available as upgrade	6
2	Blue/green	A28995	Available as upgrade	3	–	4	Available as upgrade	9
	Blue/yellow	A24861	Available as upgrade	3	4	–	Available as upgrade	9
	Blue/red	A24863	Available as upgrade	4	Available as upgrade	Available as upgrade	3	9
	Blue/violet	A24862	4	4	Available as upgrade	Available as upgrade	Available as upgrade	10
	Blue/violet 6	A29002	6	3	Available as upgrade	–	Available as upgrade	11
3	Blue/green/red	A28997	Available as upgrade	3	–	4	3	12
	Blue/red/yellow	A28993	Available as upgrade	3	4	–	3	12
	Blue/green/violet	A28999	4	3	–	4	Available as upgrade	13
	Blue/violet/yellow	A24859	4	3	4	–	Available as upgrade	13
	Blue/red/violet	A24860	4	4	Available as upgrade	Available as upgrade	3	13
	Blue/red/violet 6	A29003	6	3	Available as upgrade	–	3	14
4	Blue/red/violet/green	A29001	4	3	–	4	3	16
	Blue/red/yellow/violet	A24858	4	3	4	–	3	16
	Blue/red/yellow/violet 6	A29004	6	2	3	–	3	16

* Includes forward scatter (FSC) and side scatter (SSC).

Up to **16** parameters

Up to **4** lasers

Up to **14** colors

Benefits

- Field upgradeability to accommodate expanding needs
- Use more lasers for expanded multicolor panel design options
- Use less reagents

Table 2. 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	7	9	10	10	11	11	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		

Figure 16. The optical filters in the Attune NxT Flow Cytometer are user-exchangeable, easily slotted in and out of the optical bench to maximize your capacity.

Expand the range of performance for your violet laser

The Attune NxT Flow Cytometer is easily upgradable to 6-channel detection for the violet (405 nm) laser (Table 3). The Attune NxT Flow Cytometer with violet 6 channel configuration is 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 available Super Bright dyes at **thermofisher.com/superbright**

Benefits

- Modular expansion options for growth when needed, not before
- Facilitate application development with fewer restrictions on fluorochrome detectors
- Enhanced capability to perform a variety of applications on a single instrument

Download the poster at: **thermofisher.com/attune-14C**

Technology

Table 3. Attune NxT Flow Cytometer configuration using 6 fluorescence detectors for the violet laser.

Laser	Fluorescence detectors		
	2-laser	3-laser	4-laser
Violet, 405 nm	6	6	6
Blue, 488 nm	3	3	2
Yellow, 561 nm	NA	NA	3
Red, 637 nm	NA	3	3
Total fluorescence detectors available	9	12	14
Total parameters per configuration*	11	14	16

* Includes FSC and side scatter SSC.

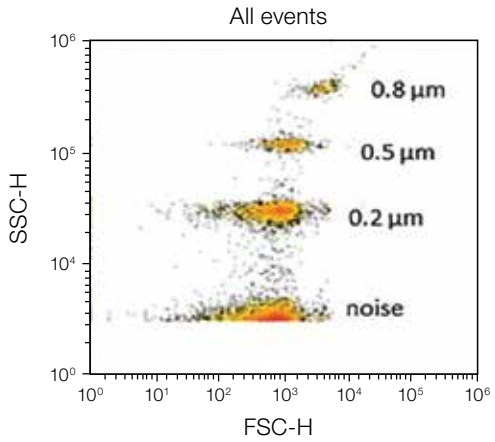


Figure 17. FSC and SSC discrimination of 0.2 µm, 0.5 µm, and 0.8 µm particles using the Submicron Bead Calibration Kit from Bangs Laboratory.

Table 4. Fluorophore guidelines for the 6 fluorescence detectors off the violet laser in the Attune NxT Flow Cytometer.

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
VL2	525/50	eFluor 506, LIVE/DEAD™ Fixable Aqua, CFP, VioGreen™, Brilliant Violet™ 510, Pacific Green™, BD Horizon™ V500
VL3	610/20	Super Bright 600, LIVE/DEAD™ Fixable Yellow, Qdot™ 605, Pacific Orange™, Brilliant Violet™ 605
VL4	660/20	Super Bright 645, Brilliant Violet™ 650
VL5	710/50	Super Bright 702, Qdot™ 700, Brilliant Violet™ 711
VL6	780/60	Brilliant Violet™ 786

* List is not inclusive of all available fluorophores.

Violet laser for small-particle detection

The Attune NxT Flow Cytometer can be configured with optional violet side scatter for better small-particle resolution. With optimal fluorescence sensitivity and up to 16-parameter detection capability on particle sizes as small as 0.2 µm (Figure 17), the Attune NxT Flow Cytometer supports a large variety of multiparameter applications (Figure 18).

Data

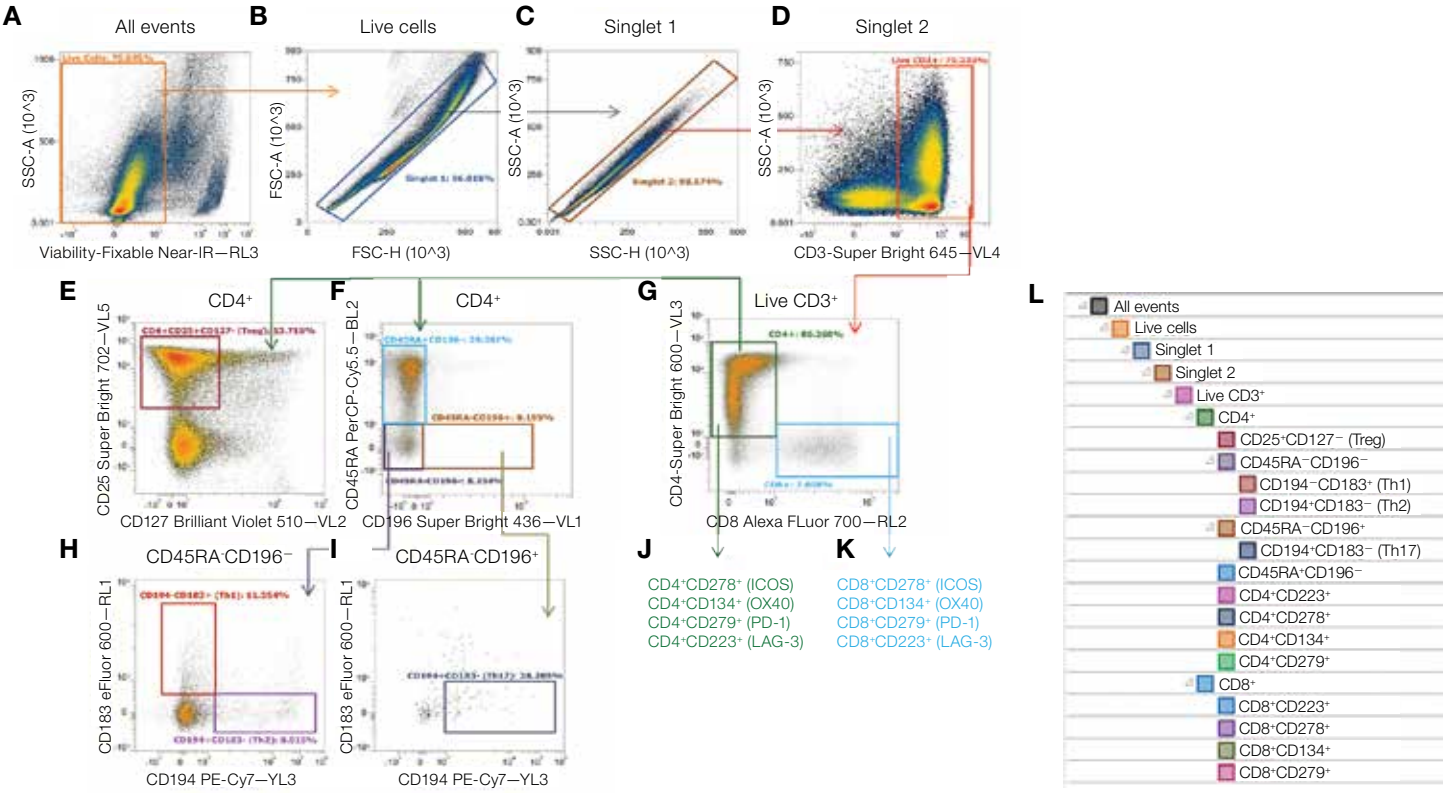


Figure 18. T lymphocyte immunophenotyping: 14-color flow cytometry panel design using the Attune NxT violet 6 channel option and Super Bright fluorescent dyes gating strategy. (A) A region is placed around live peripheral blood mononulcear cells (PBMCs) as identified by the Invitrogen™ LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit. **(B, C)** Live cells are analyzed through sequential singlet gating. A region is then placed on the **(D)** $CD3^+$ population for gating on **(E)** $CD4^+$ and $CD8^+$ populations. The $CD4^+$ population is used to gate on **(F)** CD127 vs. CD25, for **(G)** CD45RA vs. CD196, and **(J)** CD278, CD134, CD279, and CD223 populations. The $CD45RA^-/CD196^-$ population from **(G)** is gated on **(H)** CD183 vs. CD194. The $CD45RA^-/CD196^+$ population from **(G)** is gated on **(I)** CD183 vs. CD194. The $CD8^+$ population from **(E)** is used for gating **(K)** CD278, CD134, CD279, and CD223 populations. **(L)** The entire gating strategy is displayed in hierarchical format using the Attune NxT violet 6 channel option and v2.6 software for easy visualization.

Attune NxT Software

Feature-rich, researcher-inspired software that performs to your specifications

The Attune NxT Software was masterfully developed to offer user-focused functionality with many automated, user-definable, and administrative features to provide powerful data acquisition and analysis simple enough for users at any experience level (Figure 19).

Speed

- Increase productivity with live-streaming update of statistics during acquisition of events
- Fast refresh rates for large data sets of up to 20 million events per sample with option to append

Guided functionality

- Automated maintenance: “Startup”, “Shutdown”, “Rinse”, “Sanitize Attune™ SIP”, “Deep Clean”, “Sanitize”, “Decontaminate”, “Autosampler Calibration”
- “Automated Backup Options” to ensure data redundancy
- Heat map for easy setup of plate-based assays
- “Sample Recovery” to return unused sample to save precious samples
- Hierarchy view of plots to instantly view complex gating strategies
- “Autosampler Calibration” of the Attune NxT Autosampler every 30 days to ensure optimal performance

> “One of the more impressive aspects of the Attune NxT Software is its “ease of use”. As a shared facility manager, I instruct a wide variety of users how to run a wide variety of instruments. It is often a challenge to teach a new, and at times, veteran cytometrist how to operate a new system. User-friendly software is a must. Several of my facility users picked up on the Attune NxT Software right away. Many were able to run complex multi-parameter experiments in their first session; some of them were doing this on their own without any assistance from the facility staff. If only all of our instruments were so easy to use!”
>
> – E. M. Meyer
> University of Pittsburgh Cancer Institute

Customizable

- User customizable “Wait-to-Record” function
- Ability to set user options for default settings for gates, plots, fonts, colors, and group/sample names
- Visual appearance of the plots is completely customizable; fonts, colors, titles legends, and much more can appear exactly the way you want

Publication-quality data

- Smart gate naming to customize quad gate names and target names
- Add text, statistics, and even images to make your data pop
- 1-click saving of high-resolution plots in a variety of file formats
- Overlay module to perform comparative analysis of single- and dual-parameter data
- Preview plots to instantly view all combinations of parameters in a file

Figure 19. Intuitive, user-friendly software interface with familiar workflow.

Compensation tools

- Both negative and unstained gating parameters are available
- On-plot compensation for fine-tune adjustment
- Modification of compensation to add or remove parameters as needed after compensation is set up
- Set up and collect compensation controls directly from a plate

User management

- Levey-Jennings and "Performance History" reports of baseline and performance tests to monitor trends
- Ability to create and manage multiple user accounts
- System access based on user-account privileges

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

Tips

Need to delay the time between sample acquisition and when it is recorded from a plate? No problem with "Wait-to-Record" function.

Synthetic biology solutions—CRISPR

Flow cytometry fits into the CRISPR analysis workflow, enabling researchers to monitor the efficiency of genome editing experiments. Fluorescent protein reporters allow measurement of transfection rates, optimization of your conditions, and rapid analysis using flow cytometry.

Technology

The Invitrogen™ GeneArt™ Genomic Cleavage Selection Kit, which enables measurement of the percentage and the mean fluorescence intensity (MFI) of orange fluorescent protein (OFP)–expressing cells using a flow cytometer, contains a vector with the *OFP* gene for a quick visual check on the functionality of the engineered nuclease.

Benefits

When screening libraries or large sample populations of edited cells, flow cytometry enables razor-precision analysis. With appropriate antibodies, fluorescent proteins, or functional probes, complex phenotypes can be unraveled through multiplexing (Figure 20).

- Process optimization using fluorescent protein reporters allow you to quickly measure transfection efficiencies
- Rapid library screening
- Save time and money using flow cytometry for genome editing

Data

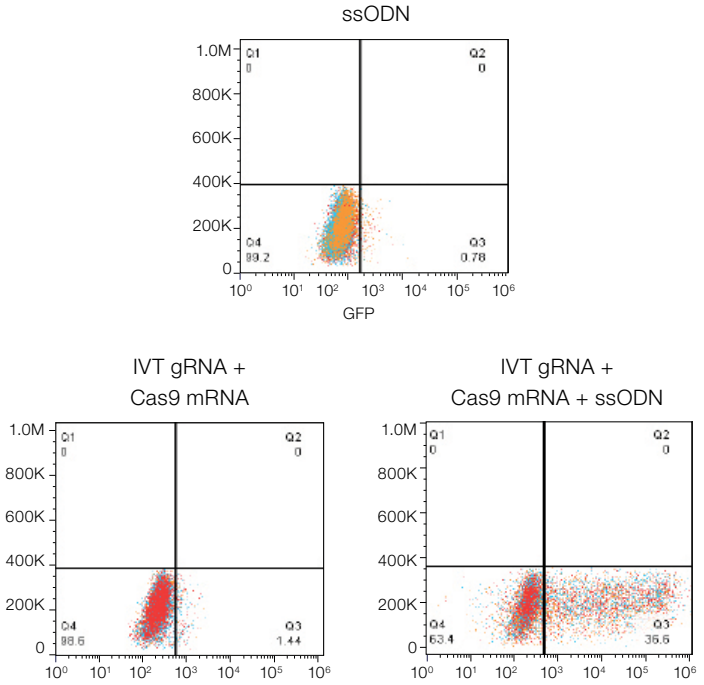


Figure 20. Tracking blue fluorescent protein (BFP) converting to green fluorescent protein (GFP) by homologous recombination using the CRISPR-Cas9 system. Single-stranded oligodeoxynucleotides (ssODN) assist in making large genomic changes following cleavage by Cas9 nuclease and *in vitro*–transcribed guide RNA (IVT gRNA).

Stem cell solutions

Flow cytometry analysis of transcription factors during cardiomyocyte differentiation

The ability to direct human pluripotent stem cells (hPSCs) toward differentiated cell phenotypes offers tremendous potential for personalized and regenerative medicine. Quantification of the dynamic expression patterns of transcription factors that underlie cardiomyocyte differentiation often relies on detection of mRNA transcripts via quantitative reverse transcription PCR (RT-qPCR) in cell and tissue lysates made from heterogeneous populations of cells.

Benefits

- Accelerate discovery and screening workflows
- Ideally suited for use with fragile and large cell types like stem cells and cardiomyocytes (Figure 21)
- Gentle and safe analysis without clogging the instrument or wasting cells

Read the *BioProbe Journal* article:
thermofisher.com/attune-cardiomyocyte

Data

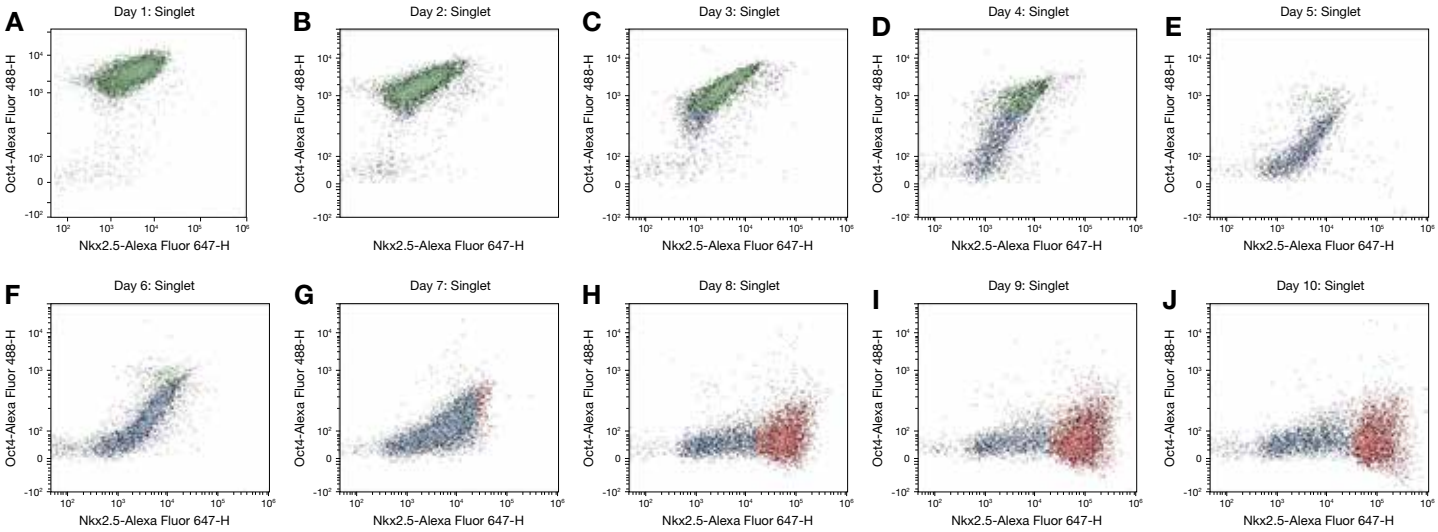


Figure 21. Two-parameter plots representing staining profiles for Oct4 and Nkx2.5 in H9 hPSC cells during cardiomyocyte differentiation. All plots were gated on singlet cells. **(A)** At day 1, nearly all cells are Oct4$^+$ and Nkx2.5$^-$, consistent with a pluripotent state. **(B–J)** During the time course of differentiation, with data shown for each day of differentiation, cells lose Oct4 expression and begin to express the cardiac marker Nkx2.5. The precedence-density plot display is used, with the red-colored population representing Nkx2.5$^+$ cells, and the green-colored population representing Oct4$^+$ cells.

Research solutions

Fluorescent proteins

The Attune NxT Flow Cytometer supports a method for detecting multiple fluorescent proteins for simultaneous analysis within the same cell, thus overcoming a broad emission spectrum and resulting spectral overlap. Excite and detect GFP and YFP with the same set of laser and bandpass filters. As shown in Figure 24, with an appropriate filter set, the 488 nm laser efficiently excites both fluorescent proteins simultaneously. The GFP and YFP signals can be appropriately discriminated using the Invitrogen™ Attune™ NxT Fluorescent Protein Filter Kit (Figure 25).

Benefits

- Achieve effective transfection efficiency detection, and expression of one to many fluorescent proteins using flow cytometry
- No-hassle labeling with ready-to-use kits for fluorescent protein detection (Figure 26)
- Simultaneous detection of GFP and YFP

Data

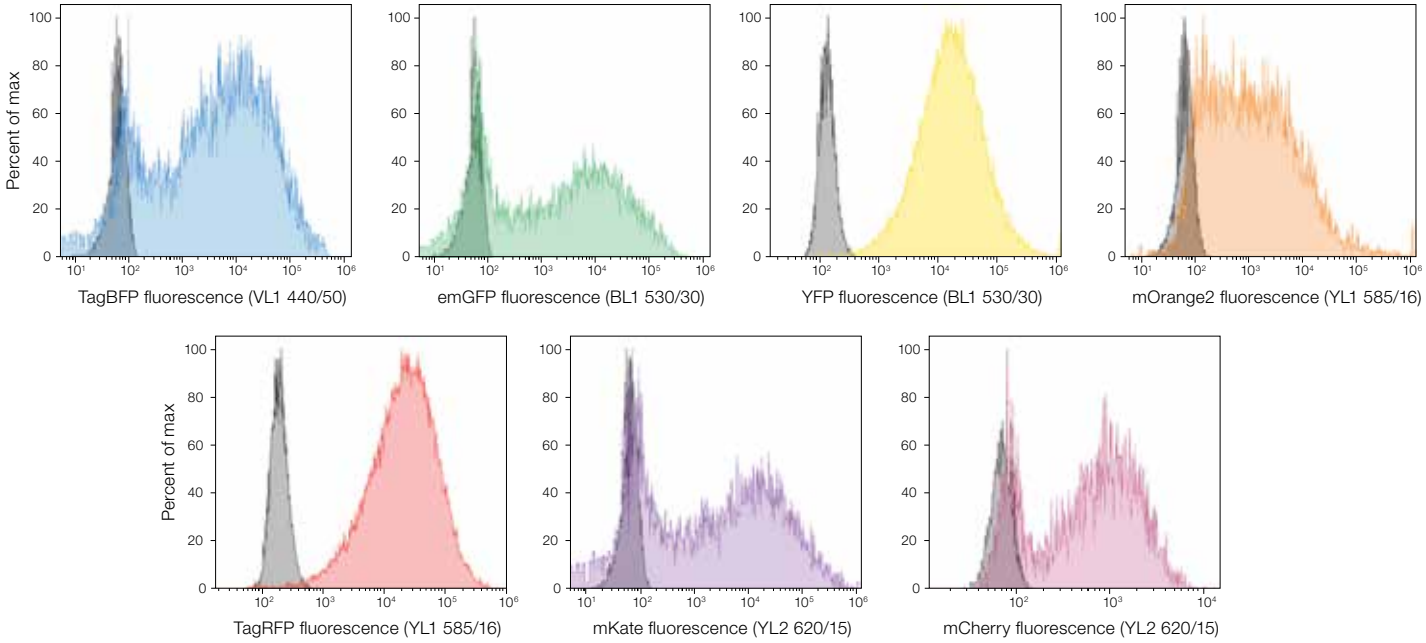


Figure 24. Detection of a palette of fluorescent proteins using the Attune NxT Flow Cytometer. Cells were transfected or transduced with vectors expressing different fluorescent proteins. Samples were acquired at a flow rate of 100 µL/min using 405 nm, 488 nm, or 561 nm excitation sources. The gray peaks represent control cells that do not express fluorescent proteins.

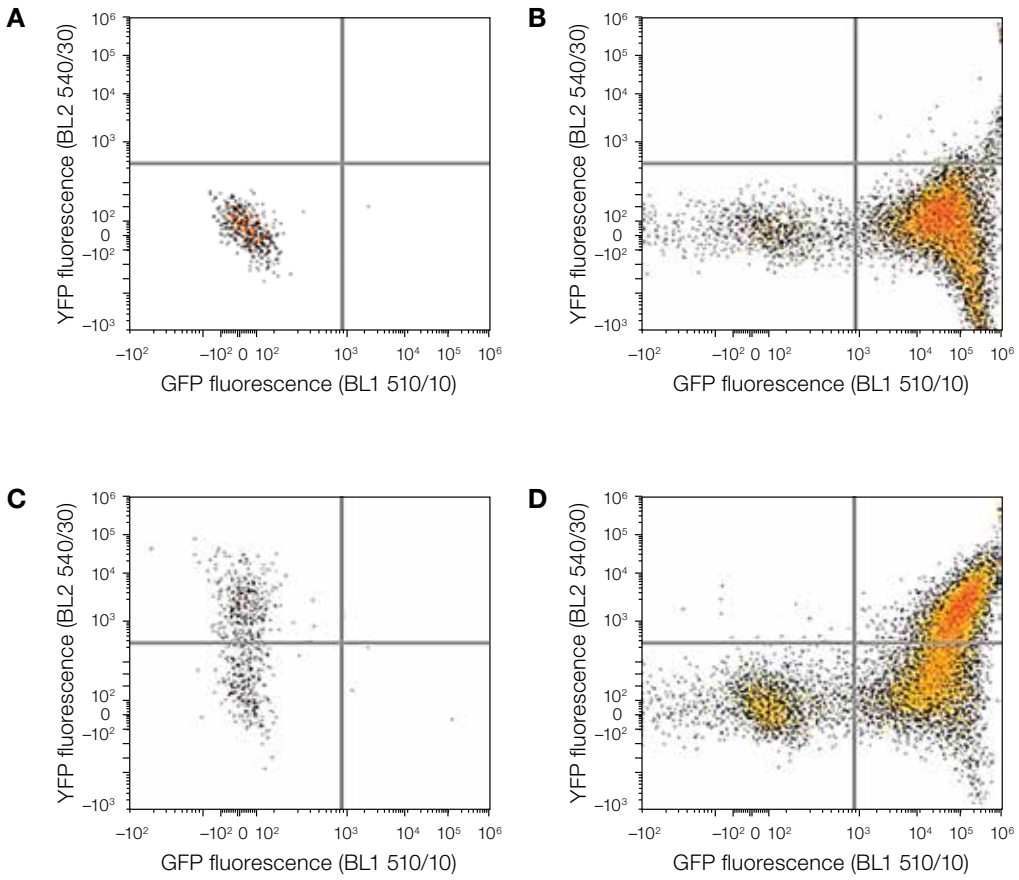


Figure 25. Flow cytometric detection of dual expression of GFP and YFP. (**A)** Shows untransfected cells. U2OS cells were transfected with vectors encoding GFP or YFP, either **(B, C)** individually or in **(D)** combination. Samples were acquired and analyzed using the Attune NxT Flow Cytometer at a flow rate of 200 µL/min. A total of 400,000 cells were collected for the sample coexpressing both fluorescent proteins, and a minimum of 5,000 events were collected for each control sample. The 488 nm laser was used for excitation of both fluorescent proteins. Coexpression of GFP and YFP is shown in the upper-right quadrant of **(D)**, and the lower-right quadrant shows cells expressing only GFP.

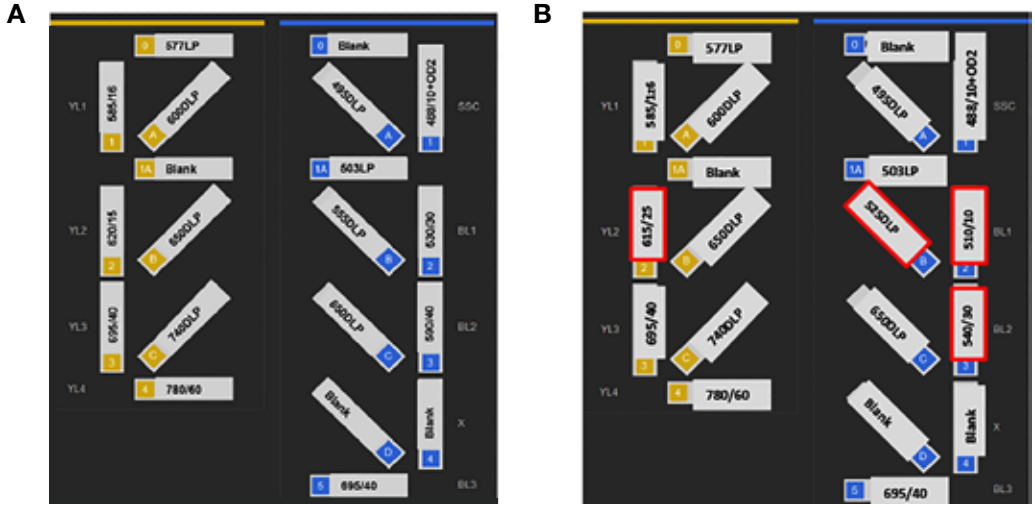


Figure 26. Use of the Attune NxT Fluorescent Protein Filter Kit. The standard configuration for the 561 nm yellow and 488 nm blue laser optical filter blocks is shown in **(A)**, and the same optical filter blocks using the Attune NxT Fluorescent Protein Filter Kit are shown in **(B)**, with changes outlined in red.

Microbiology research solutions

Bacterial analysis

The Attune NxT Flow Cytometer enables well-separated bacterial populations. For researchers working with *E. coli*, the available green 532 nm laser delivers distinct separation of double-live populations of dividing cells (green) and dead (red) *E. coli* cells (Figures 27 and 28).

Benefits

- Easily, reliably, and quantitatively distinguish live and dead bacteria in minutes
- Handle bacteria samples with improved clogging resistance
- Run dilute samples in minutes

Data

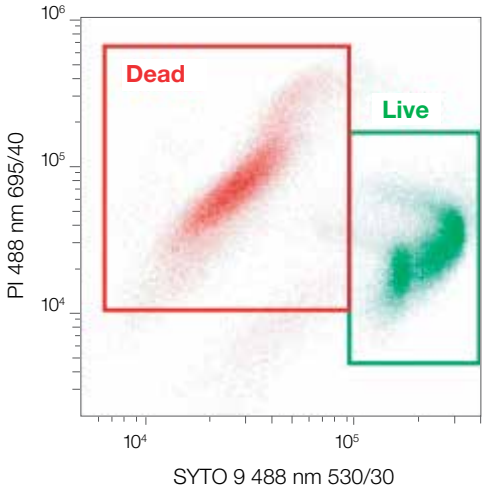


Figure 27. Staining of *E. coli* cells using the Invitrogen™ BacLight™ LIVE/DEAD Bacterial Viability Kit and blue laser excitation of Invitrogen™ SYTO™ 9 dye and propidium iodide (PI). Samples were analyzed on the Attune NxT Flow Cytometer at the 12.5 µL/min flow rate with an event rate of approximately 5,000 events/second. Instrument settings (voltages, threshold, and advanced settings) were set using single-color controls. The blue 488 nm laser was used for fluorescence excitation of both SYTO 9 dye and PI. SYTO 9 fluorescence emission was collected using a 530/30 nm emission (BL1), whereas propidium iodide fluorescence emission was collected using the 695/40 nm emission (BL2). In this example, a BL1 and SSC threshold was used, and results are shown without compensation. The live, SYTO 9–positive population is shown in green, and dead cells (Dead) are shown in red. Live and dead cell populations are easily distinguished.

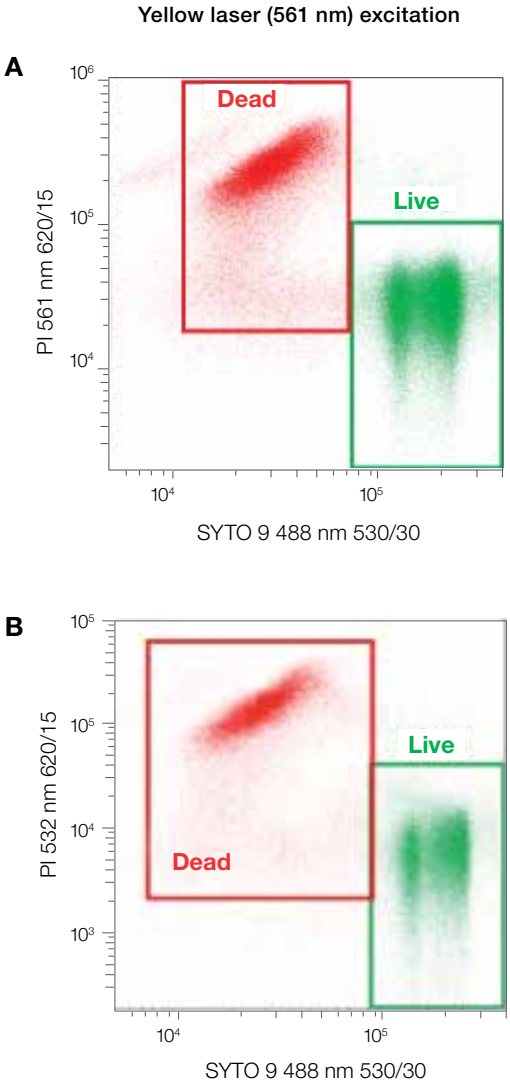


Figure 28. Staining of *E. coli* cells using the BacLight LIVE/DEAD Bacterial Viability Kit using either 561 nm or 532 nm excitation of PI. *E. coli* cells were grown in lysogeny broth (LB) and harvested. Samples were analyzed on the Attune NxT Flow Cytometer at the 12.5 µL/min flow rate with an event rate of approximately 5,000 events/second using the blue 488 nm laser and 530/30 nm emission (BL1) for SYTO 9 detection, and **(A)** yellow 561 nm laser 620/15 nm emission (YL2) for detection of propidium iodide (PI). **(B)** Green 532 nm laser 620/15 nm emission (GL2) was used for detection of PI. BL1 and SSC thresholds were set and no compensation was performed. The live population of dividing cells (Live) is shown in green; dead cells (Dead) are shown in red. Excitation of SYTO 9 and PI with different lasers results in better separation of the populations.

Oncology research solutions

13-color human lymphocyte immunophenotyping panel

Flow cytometry is the method of choice for identifying cells within complex populations, as it allows for multiparameter analysis of thousands to millions of cells in a short time. Lymphocyte, monocyte, and granulocyte populations were distinguished with FSC and SSC; and monocyte, T cell, B cell, and natural killer (NK) cell populations were identified using fluorescently labeled antibodies against surface antigens specific for the different immunological populations (Figure 29).

Benefits

- Easier design of multicolor panels—improve choices of reagents
- Excellent cell population resolution for 13-color human lymphocyte immunophenotyping experiments
- Strong signal separation for more data clarity

Data

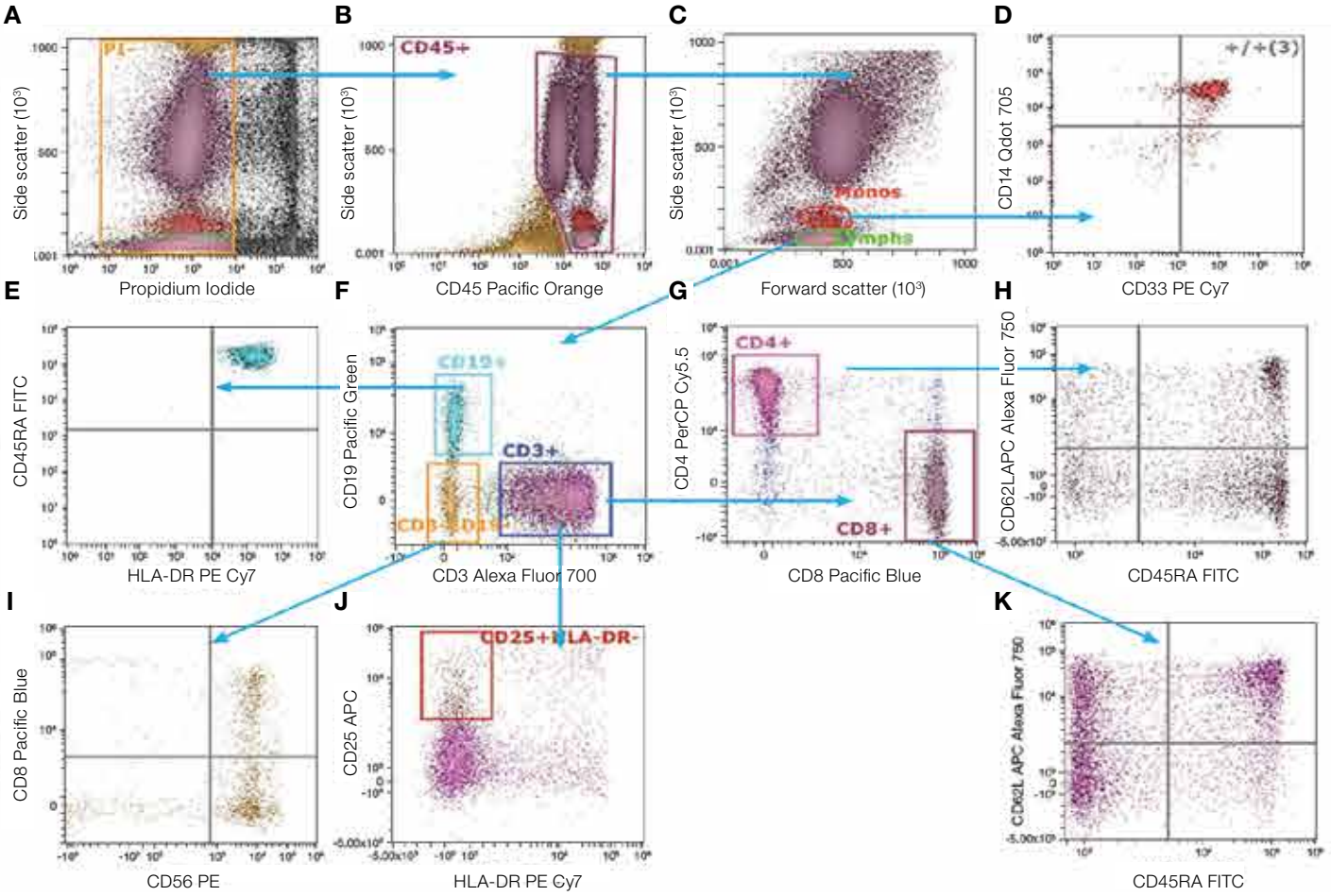


Figure 29. Gating strategy. (A) Dead cells were excluded from the analysis by gating on live cells in a dot plot. **(B)** CD45⁺ cells were gated on to select the leukocyte population from the lysed whole blood. **(C)** Lymphocytes and monocytes were gated based on forward and side scatter profiles. **(D)** Monocytes are found above the lymphocytes based on scatter profiles and express both CD14 and CD33. **(E)** B cells can be further characterized by HLA-DR and CD45RA expression. **(F)** Within the lymphocyte gate, T cells can be isolated based on their expression of CD3 and **(G)** further subdivided into CD4 (T helper cell) and CD8 (cytotoxic T cell) subpopulations. **(J)** In addition, regulatory T cells express CD4 and CD25. **(H and K)** CD62L identifies naïve (TN) CD4 and CD8 T cells, whereas HLA-DR is expressed by activated T cells (TA). **(I)** NK cells can be identified as they lack B cell (CD19) and T cell (CD3) markers, and express CD56.

Immuno-oncology research solutions

Innate lymphoid cells (ILCs) are rare populations of cytokine-producing lymphocytes that express no unique cell surface markers. ILCs can however be identified by combinations of multiple cell surface markers, making flow cytometry the best method for their detection.

Benefits

- Run large sample volumes in a fraction of the time for rare-cell detection
- No need to concentrate your sample
- Achieve a reliable measure of accuracy for detection of cell populations comprising less than 1% of the total cells by easily collecting millions of events (Figure 30)

Data

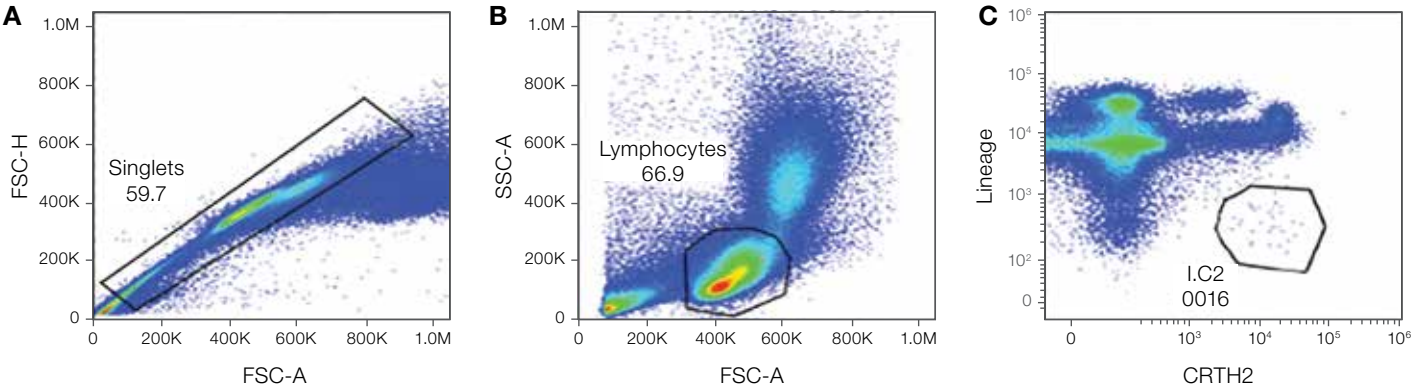


Figure 30. Detection of rare ILC2 population in PBMCs. (A) Labeling of 1 x 10^6 PBMCs resuspended in 100 µL PBS (+10% FBS). The antibodies used were a lineage cocktail containing CD2, CD3, CD14, CD16, CD19, CD56, and CD235a conjugated to Invitrogen™ FITC, CD123-FITC, and CRTH2-Alexa Fluor™ 647 conjugates. The ILC2 cells are then defined as the lineage (BL1)-negative, CRTh2 (RL1)-positive populations. **(B)** CRTH2 cells expressing the chemoattractant receptor–homologous molecule expressed on Th2 cells. CRTH2, is a seven-transmembrane protein coupled with heterotrimeric G proteins. CRTH2 is the prostaglandin D2 receptor and is expressed by Th2 cells, eosinophils, and basophils. CD294 prevents the apoptosis of Th2 cells and mediates the chemotaxis of CRTH2-expressing cells to the sites of allergic inflammation, such as the asthmatic lung. **(C)** The ILC2 cells are defined as lineage-negative and CRTH2-positive. In this example, the ILC2 population is 0.016% of the parent gate. Data courtesy David Cousins, University of Leicester.

Flow cytometry reagents

Enable and explore a bright, expanded world of flow cytometry with Invitrogen™ fluorescence detection molecules and probes, which are backed by 40 years of pioneering R&D. From conjugated antibodies through functional dyes and cell functional assays, our flow cytometry products exist to pioneer your research.

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

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 over 15,000 flow-specific conjugated antibodies with multiple fluorophore options, including the new Super Bright violet-excitable polymer dyes.

Buffers—The use of appropriate buffers is crucial to the success of your flow cytometry experiments. We offer a wide variety of buffers to suit your research needs, whether your experiment calls for extracellular, intracellular, and/or nuclear cell staining.

Reagents—At the forefront of invention and development of fluorescent probes for over 40 years, we offer a comprehensive variety of cell functional assays for studying viability, apoptosis, cell cycle, and cell proliferation.

Flow support products—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 from limited sample. As with all high-performance instrumentation, flow cytometers must also be calibrated regularly to ensure accuracy and reliability. The stability, uniformity, and reproducibility of Invitrogen™ microsphere products make them excellent tools for flow cytometer instrument setup and calibration.

We are focused on advancing meaningful discoveries and partnering to make tools for cellular analysis widely accessible, affordable, and powerful for all life scientists. On the quest for significant breakthroughs, we know that you never settle for average, and neither will we.

Robotic automation solutions

Orbitor RS Microplate Mover

Maximize operating capacity, mitigate human operator error, and enable rich, reproducible data with the Thermo Scientific™ Orbitor™ RS Microplate Mover as part of a comprehensive, multicomponent workcell for robotically automated flow cytometry.

Extended
10 L
fluidics

Available
4–40°C
storage

Up to
19.5 hours
continuous runtime*

Technology

The robotic arm offers active and passive protective safety features, demonstrated reliability, and flexible configuration options for arrangement and storage. Operation is managed by Thermo Scientific™ Momentum Scheduling Software, established with instrument drivers available for over 200 instruments. The dashboard facilitates dynamic scheduling for active prioritization, visualized progress, and plate tracing. Extended-run fluidics allow for up to 19.5 hours of unattended continuous runtime under specific run conditions.

Benefit

- Robust performance, precise motion, and consistent performance
- Compatible with a diverse range of plate types
- Works with both lidded and unlidded plates

The Attune NxT Flow Cytometer configured for robotic automation with the Orbitor RS Microplate Mover.

* Under specific run conditions.

Walk away with confidence

Labs optimized with robotic handling will benefit from the performance, software design, engineering, and safety features of the Orbitor RS Microplate Mover.

- **Temperature sensitivity**—optional, temperature-controlled Thermo Scientific™ SmartStor™ benchtop microplate storage device with a temperature range of 4–40°C
- **Flexible capacity**—plate capacity of 20 standard microplates or 9 deep-well blocks, and self-scanning internal inventory
- **Mitigate evaporation**—the Orbitor RS Microplate Mover can de-lid and re-lid plates as they are loaded, unloaded, and stored
- **Protect from light exposure**—opaque and lidded plates protect samples in the random access hotel storage tower

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

Transforming capabilities

Options that resonate

With additional lasers, more detection channels, increased flexibility, and design modifications that further improve the performance, reliability, and robustness, the Attune NxT Flow Cytometer continues to offer more options and added functionality. Since its initial unveiling, the compact system with innovative acoustic technology is moving ahead with added new functionalities and capabilities.

"I knew I had learned a lot during 25 years of experience doing research with flow cytometry. Now I am surprised to see how much I can learn doing research with the Attune NxT Flow Cytometer, and how this new technology can be very helpful to make the invisible visible."

– Jordi Petriz, PhD
José Carreras Leukaemia Foundation

Timeline—stay tuned for what's next

2014

Invitrogen™ Attune™ NxT
Flow Cytometer introduced

2015

Invitrogen™ Attune™ NxT Software
support for deep-well plates
(2 mL) and sample recovery

Invitrogen™ Attune™ NxT
No-Wash No-Lyse Filter Kit

Invitrogen™ Attune™ NxT
Fluorescent Protein Filter Kit

2016

Invitrogen™ Attune™ NxT
Green laser (532 nm) launched

2017

Invitrogen™ Attune™ NxT
(Violet 6-channel configuration)

2018

Preview Thermo Scientfic™
Orbitor™ RS Microplate Mover

Aftermarket care

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

- **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.

Ordering information

Product*	Description	Cat. No.
Attune NxT 1-Laser System	AB Maintenance including 1 planned maintenance (PM)	ZG51SCATTUNEB
Attune NxT 1-Laser System	AB Assurance including 1 PM	ZG11SCATTUNEB
Attune NxT 2-Laser System	AB Maintenance including 1 PM	ZG51SCATTUNEBRBVBY
Attune NxT 2-Laser System	AB Assurance including 1 PM	ZG11SCATTUNEBRBVBY
Attune NxT 3-Laser System	AB Maintenance including 1 PM	ZG51SCATTUNEBRVBVY
Attune NxT 3-Laser System	AB Assurance including 1 PM	ZG11SCATTUNEBRVBVY
Attune NxT 4-Laser System	AB Maintenance including 1 PM	ZG51SCATTUNEBVRY
Attune NxT 4-Laser System	AB Assurance including 1 PM	ZG11SCATTUNEBVRY
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
Orbitor RS	AB Protection Orbitor Robot NxT	ZG30SCORBROBNXT

* Instrument needs to be networked.

Ordering information

Unit type	Configuration	Parameter	Cat. No.
4-laser	Blue/red/yellow/violet	16	A24858
4-laser	Blue/red/violet/green	16	A29001
4-laser	Blue/red/yellow/violet 6	16	A29004
3-laser	Blue/red/violet 6	14	A29003
3-laser	Blue/red/violet	13	A24860
3-laser	Blue/violet/yellow	13	A24859
3-laser	Blue/red/yellow	12	A28993
3-laser	Blue/green/violet	13	A28999
3-laser	Blue/green/red	12	A28997
2-laser	Blue/violet 6	11	A29002
2-laser	Blue/violet	10	A24862
2-laser	Blue/red	9	A24863
2-laser	Blue/yellow	9	A24861
2-laser	Blue/green	9	A28995
1 laser	Blue	6	A24864

invitrogen

ThermoFisher SCIENTIFIC

Ordering information

Product	Cat. No.
Attune NxT accessories	
Attune NxT Autosampler	4473928
Attune NxT External Fluid Supply	A28006
Attune NxT Software, Single License	A25554
Attune NxT Software, 5 Licenses	A24856
Attune NxT Software, 10 Licenses	A24855
Orbitor RS Microplate Mover Stack	A33007
Orbitor RS Microplate Mover Hotel	A33008
Orbitor RS Microplate Mover Stack/Hotel	A35220
Attune NxT upgrades	
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 No-Wash No-Lyse Filter Kit	100022776
Attune NxT Custom Filter Holder Kit	A27784
Attune NxT 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 Performance Tracking Beads	4449754