

# Attune flow cytometer systems

Table 1. Invitrogen™ Attune™ flow cytometer specifications.

Instrument specification		Attune™ CytPix™ Flow Cytometer		Attune™ NxT Flow Cytometer	
Optics: fluorescence detection	Laser power	Laser	Wavelength (nm)	Beam-shaping optics (BSO)* (mW)	Diode power** (mW)
		Violet	405	50	100
		Blue	488	50	100
		Green†	532	100	140
		Yellow	561	50	100
		Red	637	100	140
	Laser excitation	Optimized excitation for minimized stray laser-line noise and losses to reflection			
	Laser profile	10 x 50 µm flat-top laser provides robust alignment			
	Emission filters	Up to 14 color channels with wavelength-tuned photomultiplier tubes (PMTs); user-changeable, keyed filters			
	Laser separation	100 µm			150 µm
Optical alignment	Fixed alignment with prealigned welded fiber; no user maintenance required				
Onboard thermoelectric cooler	No warm-up delay; fiber unaffected by on/off				
Simmer mode	Instant on/off reduces wear up to 10-fold; only on during data acquisition; hours of usage reported				
Flat-top laser specified at the flow cell	Coefficient of variation (CV) <3% over the width of the flat-top laser				
Upgradable	Expand capability with laser upgrade purchase installed by field service				
Optics: imaging	Laser excitation	405 nm			NA
	Pulse width	<50 ns			NA
Fluidics	Flow cell	Quartz cuvette gel coupled to 1.2 numerical aperture (NA) collection lens, 200 x 200 µm			
	Sample analysis volume	20 µL to 4 mL			
	Custom sample flow rates	12.5–1,000 µL/min			
	Sample delivery	Positive displacement syringe pump for volumetric analysis			
	Sample tubes	Accommodates 17 x 100 mm to 8.5 x 45 mm tubes			
	Fluid level sensing	Active			
	Standard fluid reservoirs	1.8 L focusing fluid tank, 1.8 L waste tank, 175 mL shutdown solution tank, and 175 mL wash solution tank			
	Fluid storage	All fluids stored within instrument			
	Extended fluidics option	10 L fluid configuration			
	Nominal fluid consumption	1.8 L/day			
Automated maintenance cycles	≤15 min start-up and shutdown; deep clean, sanitize, and debubble modes				

\* Amount of measured usable laser power after light has gone through the beam optics and shaping filters.  
 \*\* Vendor-specified theoretical maximum.  
 † Green laser not available on the Attune CytPix Flow Cytometer.

Table 1. Attune flow cytometer specifications (continued).

Instrument specification		Attune CytPix Flow Cytometer	Attune NxT Flow Cytometer
Performance: fluorescence detection	Fluorescence sensitivity	≤80 molecules of equivalent soluble fluorochrome (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)	
	Data acquisition rate	Up to 35,000 events/sec, 34 parameters, based on a 10% coincidence rate per Poisson statistics	
	Maximum electronic speed	65,000 events/sec with all parameters	
	Carryover	Single tube format: <1%	
	Forward and side scatter sensitivity	Can distinguish platelets from noise	
	Forward and side scatter resolution	Optimized to resolve lymphocytes, monocytes, and granulocytes in lysed whole blood	
	Forward scatter	Photodiode detector with 488/10 nm bandpass filter	
	Side scatter	PMT with default 488/10 nm bandpass filter; optional 405/10 + OD2 bandpass filter	PMT with default 488/10 nm bandpass filter; optional 405/10 nm bandpass filter
	Fluorescence detectors	14 individual detectors	
	Electronic pulse	Measured area; height and width pulse for all detectors	
	Violet side scatter resolution	Can be configured for violet side scatter to better resolve particles from noise	
	Minimum particle size	0.2 μm on side scatter using submicron bead calibration kit from Bangs Laboratories, or 0.1 μm on side scatter under following conditions: use an Attune NxT Flow Cytometer with standard 0.5 mm blocking configuration, an Invitrogen™ Attune™ NxT Small Particle Side-Scatter Filter (488/10 filter) (Cat. No. 100083194), and Invitrogen™ Attune™ Focusing Fluid (Cat. No. 4488621, 4449791, A24904, or J106627) that has been passed through a 0.025 μm filter	
Performance: imaging	Pixel resolution	0.3 μm/pixel	NA
	Objective magnification	20x	NA
	Objective numerical aperture	0.45	NA
	Theoretical resolution	0.6 μm	NA
	Detection limit	Visually detect 800 nm particles	NA
	Image capture rate	Up to 6,000 images/second, depending on image size and event rate	NA
	Image size	96 x 96 pixels to 248 x 248 pixels	NA
	Field of view	29 x 29 μm <sup>2</sup> to 74 x 74 μm <sup>2</sup>	NA

Table 1. Attune flow cytometer specifications (continued).

Instrument specification		Attune CytPix Flow Cytometer	Attune NxT Flow Cytometer
Flow cytometry software features	Compensation	Full matrix in automated and manual modes; on-plot compensation tools for fine adjustment; use of tubes and wells	
	Flow rate	Precise flow rate control via software; no hardware adjustments	
	Live streaming	Live update of statistics during event acquisition up to 35,000 events/sec	
	Overlays	Comparative analysis between samples; 3D view	
	Sample recovery	System able to return unused samples	
	Concentration	Direct concentration measurement without use of counting beads	
	Software layout	Fully customizable for each user account	
	Bubble detection technology	Stops automated run to preserve sample integrity	
	Maximum single-event file	20 million with option to append	
	Heat map	Set up for definition of plate layout; screening view for analysis for tubes and plates	
	Threshold	Up to 4 individual thresholds with user option to apply Boolean logic	
	Gating	Hierarchical gating with the ability to derive gates	
	Voltage	User-adjustable	
	Window extensions	User-adjustable	
	Area scaling factor (ASF)	User-adjustable	
	Acquisition settings	Documented in FCS files and maintained upon import	
	Templates	Create from existing experiments—instrument settings, workspaces, run protocols, heat map settings, and compensation settings optimized and defined previously	
	Tube-to-plate conversion	One-click transition from tubes to plates and vice versa; no disassembly, no additional QC, no reboot required for conversion between plates and tubes	
	Graphics resolution	Publication-quality data plots; supports TIF, PNG, BMP, JPG, GIF, and EMF files; quickly copy and paste plots to any external application (e.g., Microsoft™ PowerPoint™ software)	
	User account administration	Administrative creation of individual user accounts with designated roles; permissions for advanced settings; management of individual accounts; user time tracking and sample count	
Keyword management	User-defined or custom		
Imaging software features	Image capture settings	Set total number of recorded images, image frequency, image capture gate, image size, image position, focus, and illumination for control over experiment design and data footprint	NA
	Image view	Image view option allows overview of image gallery; cell image option allows viewing of individual images in the workspace for any cell population	NA
	Image backgating	Correlates images to flow cytometry data by backgating all or only selected images onto supported workspace plots	NA
	Image measurement tool	Elliptical tool to measure event areas in images in $\mu\text{m}^2$	NA
	Image export options	Exports images as 8-bit and 16-bit TIF, PNG, GIF, BMP, JPG, or EMF files	NA

Table 1. Attune flow cytometer specifications (continued).

Instrument specification		Attune CytPix Flow Cytometer	Attune NxT Flow Cytometer
Automated image processing	Derived parameters	<b>Object features</b> —particle/cell count	NA
		<b>Pixel features</b> —pixel count	
		<b>Shape features</b> —area ( $\mu\text{m}^2$ ), perimeter area ( $\mu\text{m}$ ), circularity (%), pseudo diameter ( $\mu\text{m}$ ), major axis ( $\mu\text{m}$ ), minor axis ( $\mu\text{m}$ ), minor to major axis ratio (%), eccentricity (%)	
		<b>Intensity features</b> —maximum intensity, minimum intensity, total intensity, average intensity, intensity standard deviation, intensity %CV, intensity skewness, intensity kurtosis	
		<b>System features</b> —on border, confidence score, processed, processable	
	Model types	Leukocytes; beads	
	Particle/cell size	5–20 $\mu\text{m}$	
	Processing speed	Up to 1,000 images/second (varies with image complexity)	
	Image size	16-bit	
	Image dynamic range	10-bit	
Image processing results	Feature data as described above; image mask data as JSON file		
File types	FCS; JSON; TIF		
Performance	$\leq 10\%$ error on shape features $\leq 10\%$ error in singlet counting		
Quality and regulatory	Instrument tracking	Automated daily baseline and performance test with Levey-Jennings plots	
	Warranty	1 year	
	Production verification testing	Each instrument is tested and verified for assembly integrity and performance to specifications	
	Quality management system	Manufacturing standards comply with the requirements of ISO 13485:2003	
	Robust installation specifications	Units installed by engineer; preplanning checklist, delivery, and installation; performance validation compliance with standardized procedure	
	Regulatory status	For Research Use Only	
Computer	Software requirements	Invitrogen™ Attune™ Cytometric Software	
	Monitor	27 in. flat panel (1,920 x 1,080 resolution); dual-monitor capability	23 in. flat panel (1,920 x 1,080 resolution); dual-monitor capability
	Computer	Minitower desktop	
	Operating system	Microsoft™ Windows™ 10 software, 64-bit	
	FCS format	FCS 3.1, 3.0	
	Processor	Intel™ Core™ i7 processor	
	RAM	64 GB	32 GB
	Hard drives	2 x 8 TB SSD, 560 MB/sec; controller RAID1, integrated	2 x 2 TB SATA 3.0 GB/sec, 8 MB data burst cache; controller RAID1, integrated
	GPU	NVIDIA™ Quadro™ P2200	NA
Installation requirements	Electrical requirements	100–240 VAC, 50/60 Hz, <150 W Thermo Fisher Scientific certifies that the Attune flow cytometers conform to relevant directives to bear the CE mark. The instruments also conform to the UL and CAN/CSA general requirements (61010.1). The Attune flow cytometers are class I laser products per Center for Devices and Radiological Health (CDRH) regulations and EN/IEC 60825.	
	Heat dissipation	<150 W	
	Temperature operating ranges	15–30°C (59–86°F)	
	Operating humidity	10–80%, noncondensing	10–90%, noncondensing
	Audible noise	<65 dBA at 1.0 m	
	Instrument size (H x W x D)	~49 x 58 x 43 cm (19 x 23 x 17 in.), including fluid bottles	~40 x 58 x 43 cm (16 x 23 x 17 in.), including fluid bottles
	Weight	~33 kg (73 lb)	~29 kg (64 lb)

## Autosamplers for Attune flow cytometers

Table 2. Technical specifications.

Specification	Invitrogen™ CytKick™ Autosampler	Invitrogen™ CytKick™ Max Autosampler
Acquisition time	<ul style="list-style-type: none"> <li>&lt;42 min per 96-well plate in high-throughput mode</li> <li>&lt;70 min per 96-well plate in standard mode with wash cycles</li> <li>&lt;145 min per 384-well plate in standard mode; one mix, one rinse, and full analysis for each 20 µL sample at 500 µL/min</li> </ul>	<ul style="list-style-type: none"> <li>~22 min per 96-well plate in Boost mode; one rinse, one mix, and full analysis for each 20 µL sample at 1,000 µL/min</li> <li>~88 min per 384-well plate in Boost mode; one rinse, one mix, and full analysis for each 20 µL sample at 1,000 µL/min</li> </ul>
Carryover	<ul style="list-style-type: none"> <li>&lt;0.5% carryover for 100 µL, 200 µL, 500 µL, and 1,000 µL samples with one mix and one rinse in standard mode</li> <li>&lt;1.0% carryover for 12.5 µL and 25 µL samples</li> </ul>	<ul style="list-style-type: none"> <li>&lt;0.5% carryover for 100 µL, 200 µL, 500 µL, and 1,000 µL samples with one mix and one rinse in standard mode</li> <li>&lt;1.0% carryover for 12.5 µL and 25 µL samples</li> <li>&lt;1.0% carryover for 500 µL and 1,000 µL samples in Boost mode with one mix and one rinse</li> </ul>
Mixing optimization	Mixing optimized to preserve cell viability; number of mixing cycles optimized to match sample analysis volume	
Mixing method	Each well mixed via aspiration and dispensation of sample (no shaking)	
Number of wash cycles	Up to 10 wash cycles (user-defined)	
Minimum dead volume (single draw)	30 µL for 12.5–200 µL/min; 50 µL for 1,000 µL/min	
Sample window	Window allows viewing of well progress; protective coating prevents exposure to ambient light during acquisition	
Autocalibration	Regular 30-day intervals with system-initiated function	
Plate and tube compatibility	One-click transition from tubes to plates and vice versa; no disassembly, no additional QC, no reboot	
Compatible plate types	<ul style="list-style-type: none"> <li>96 deep-well (flat, U-bottom, and V-bottom)</li> <li>96-well standard depth (flat, U-bottom, and V-bottom)</li> <li>384-well standard depth (flat, U-bottom, and V-bottom)</li> <li>384 deep-well (flat, U-bottom, and V-bottom)</li> </ul>	<ul style="list-style-type: none"> <li>96 deep-well (flat, U-bottom, and V-bottom)</li> <li>96-well standard depth (flat, U-bottom, and V-bottom)</li> <li>384-well standard depth (flat, U-bottom, and V-bottom)</li> <li>384 deep-well (flat, U-bottom, and V-bottom)</li> <li>Customizable to accept other plate types</li> <li>1.5 mL and 2 mL microcentrifuge tube racks (up to 24 tube racks per vessel)</li> <li>Foil-covered 96-well (U-bottom) and 384-well (U-bottom and V-bottom)</li> </ul>
Fluidics requirements	<b>Fluid storage:</b> external <b>Total fluid volume:</b> two 2 L tanks	
Extended fluidics	Available with Invitrogen™ Attune™ NxT External Fluid Supply (EFS); optional external fluid tank with 10 L fluid capacity	
Size (W x D x H)	~43 x 33 x 41 cm (17 x 13 x 16 in.)	
Space requirements	<b>Minimum width:</b> 43 cm (17 in.); total width is 99 cm (39 in.) when attached to an Attune flow cytometer <b>Minimum depth:</b> 39.5 cm (15.6 in.); allow 33 cm (13 in.) for the cytometer unit and 6.5 cm (2.6 in.) behind the unit for ventilation <b>Minimum clearance height:</b> 74 cm (29.1 in.) above the mounting	
Mounting	Mounted on side or placed behind	
Weight	16.9 kg (37.2 lb) with empty focus and waste bottles 20.9 kg (46 lb) with focus and waste bottles at full capacity	
Operating range (environmental conditions)	15–30°C (59–86°F)	
Operating humidity	<80%, noncondensing	
Electrical requirements	100–240 VAC, 50/60 Hz, <300 W	
Sample cooling	NA	Passive cooling available for 96-well U-bottom plates and microcentrifuge tube racks
Evaporation protection (foil cover)	NA	Yes
Service	Field service or rapid exchange option	
Warranty	1-year standard warranty; extended warranty options available	

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