Comparing technical specification documents

Introduction

Flow cytometer manufacturers provide technical specification sheets (tech specs or spec sheets) that describe the instruments' key performance characteristics, and these documents can contain a wealth of information for those interested in purchasing a flow cytometer. Comparing various tech specs, however, can be challenging because their values may have been calculated in different ways, despite sharing the same terminology.

Technical specification sheets

The purpose of the spec sheet is to help you identify design attributes of the flow cytometer, such as performance, size, environment, and software, to determine if the instrument is a good fit for your research.

Comparing specifications from multiple manufacturers

Technical specifications can be used as a basis for comparison, helping you assess the value of different instruments for the price. The spec sheet is also a guide to the performance that the manufacturer will warrant. For this reason, you should have a good understanding of the stated values and how they pertain to your intended use of the instrument. When using the spec sheet as a comparison guide across platforms, be inquisitive. There are many performance values that appear comparable across instruments but in reality are quite different. A specification is derived from a specific test or calculation, but these tests are not standardized across instrument developers and may be misleading in a side-by-side comparison.

Sections within a technical specification sheet vary from one manufacturer to another, adding additional variability. Commonly published categories of information are described in Figures 1–8 using the spec sheet for the Invitrogen™ Attune™ NxT Flow Cytometer as an example. Key specifications that require special attention during flow cytometer evaluations are discussed, including helpful hints about how to decipher the variations.

Instrument specifications

Optics
- **Laser power** (as shown in table below)

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

* Amount of measured usable laser power after light has gone through the beam optics and shaping filters.
** Vendor-specified theoretical maximum.

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 tubes from 17 x 100 mm to 8.5 x 45 mm
- **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:** Configuration for 10 L fluid
- **Nominal fluid consumption:** 1.8 L/day
- **Automated maintenance cycles:** ≤15 min startup and shutdown—deep clean, sanitize, and debubble modes

Figure 1. Fluidics. The fluidics system of a flow cytometer transports the sample from the sample tube to the flow cell. Once through the flow cell (and past the laser and detector), the sample is transported to waste. The fluidics section in a spec sheet provides information about the sample, volume, flow rates, flow cell, and fluidic capacity.

Optics

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Red	637	100	140

* Amount of measured usable laser power after light has gone through the beam optics and shaping filters.
** Vendor-specified theoretical maximum.

- **Laser excitation:** Optimized excitation for minimized stray laser-line noise and losses to reflection
- **Laser profile:** 10 x 50 μm flat-top laser providing robust alignment
- **Emission filters:** Up to 14 color channels with wavelength-tuned photomultiplier tubes (PMTs); user-changeable, keyed filters
- **Laser separation:** 150 μm
- **Optical alignment:** Fixed alignment with prealigned welded fiber; no user maintenance required
- **Onboard thermoelectric cooler:** No warm-up delay; fiber isn't affected by on/off
- **Simmer mode:** Instant on/off reduces usage and/or aging by 10x; only keep it "on" when acquiring samples; reports hours of usage
- **Flat top specified at the flow cell:** Coefficient of variation (CV) <3% over width of flat top
- **Upgradable:** Convenient field changes

Figure 2. Optics. As an analysis platform, flow cytometry relies on interrogation of individual cells by laser light and the collection of the resulting fluorescence and scatter. The optics system handles illumination and light collection within the instrument. The optics section in the sheet provides overviews of optical specifications such as laser type and power, laser profile, alignment, and photomultiplier tubes (PMTs).

Pay special attention to: laser power
Specified in mW
The power specification is most frequently defined as the laser's output as published by the laser manufacturer (Figure 2). However, the power specification stated does not necessarily correspond with the power that is actually delivered at the point of interrogation, because light losses in the optical components between the laser and the flow cell can be large. This light loss varies from system to system. High light loss means that some of the laser's power is not being fully utilized in the experiment; reduced light loss means higher laser intensity on the flow cell, leading to greater excitement of the fluorophores and greater sensitivity. Because of this variability, a higher number for laser power indicated on the spec sheet does not mean that the instrument is more sensitive than another.

How to compare
Be cautious in how much you rely on this specification when comparing instruments. Most manufacturers don't report the actual power that is reaching the flow cell, but this is where the comparison should be made.

Pay special attention to: pinholes and laser alignment design
This specification, which is not reported by every manufacturer, refers to the number of pinholes that determine if the fluorescence signal generated by each laser is separated at the detectors (also called PMTs). You should know if the system's lasers are spatially separated by internal pinholes or whether the system is collinear. Pinholes allow for maximal excitation of fluorophores and minimal crosstalk between the laser lines.

Several flow cytometer manufacturers utilize collinear lasers. When lasers are aligned in a collinear fashion through a single pinhole, there is a collection of signal from more than one laser in the same optical path. This configuration means that the response of several dyes excited by different lasers is measured by the same detector, which can affect compensation values and lead to difficulty in analysis. Other risks in this design include susceptibility to alignment issues (increased coincidence rate if the beams are not exactly collinear) or reduced sensitivity for dim labels. An alternate design is a spatially separated system (Figure 2). This configuration has several benefits, including resistance to alignment problems, more choices for laser colors, and improved compensation for multicolor panels.

How to compare
Be sure you have a clear understanding of the type and quantity of lasers that are assigned to each pinhole, and ask if the system under consideration uses spatially separated or collinear lasers.

Instrument specifications

| Optics | • Laser power (as shown in table below) |

Laser	Wavelength (nm)	Beam-shaping optics (BSO)* (mW)	Diode power** (mW)
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Green	532		
Yellow	561		
Red	637		

* Amount of measured usable laser power after light has gone through the beam optics
** Vendor-specified theoretical maximum.

- **Laser excitation:** Optimized excitation for minimiz...
- **Laser profile:** 10 x 50 µm flat-top laser providing r...
- **Emission filters:** Up to 14 color channels with wav...
- **Laser separation:** 150 µm
- **Optical alignment:** Fixed alignment with prealigne...
- **Onboard thermoelectric cooler:** No warm-up de...
- **Simmer mode:** Instant on/off reduces usage and/...
- **Flat top specified at the flow cell:** Coefficient of v...
- **Upgradable:** Convenient field changes

Fluidics
- **Flow cell:** Quartz cuvette gel coupled to 1.2 nume...
- **Sample analysis volume:** 20 µL to 4 mL
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- **Fluid storage:** All fluids stored within instrument
- **Extended fluidics option:** Configuration for 10 L f...
- **Nominal fluid consumption:** 1.8 L/day
- **Automated maintenance cycles:** ≤15 min startup...

Performance
- **Fluorescence sensitivity:** ≤80 molecules of equiv...
- **Fluorescence resolution:** CV <3% for the singlet...
- **Data acquisition rate:** Up to 35,000 events/sec, 3...
- **Maximum electronic speed:** 65,000 events/sec with all parameters
- **Carryover:** Single-tube format: <1%
- **Forward and side scatter sensitivity:** Able to discriminate 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 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

Performance
- **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:** Able to discriminate 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 nm bandpass filter
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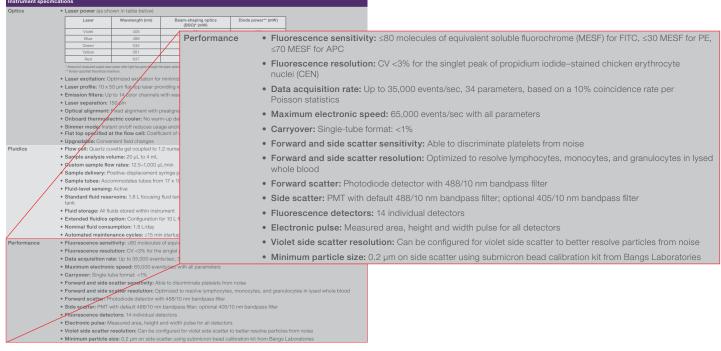
Figure 3. Performance. Of the typical specifications published widely among manufacturers, the performance section of a spec sheet contains several common features, such as MESF calculations, data acquisition rate, and parameters for forward scatter (FSC) and side scatter (SSC).

Pay special attention to: maximum event rate or theoretical maximum event rate

Specified as events/sec

The event rate is the physical count of the cells or particles as they pass through the instrument's interrogation point. Two basic yet quite different methods are used to determine what the value is, even though this value is generally cited in the same way (i.e., events/sec). The two methods for calculating event rate are:

• Assessing how fast the electronics can process events

• Assessing the maximum event rate once a specific coincidence rate has been reached

It's important to understand how the manufacturer arrived at this value in the spec sheet.

Method 1

The first method is theoretical and disregards the rate of coincidence and other instrument design features such as the system fluidics. Thus, while the electronics may be able to process events at 10,000–100,000 events/sec, this event rate may correspond to a coincidence rate well above the generally accepted 10% rate limit according to Poisson distribution. This way of presenting the specification for event rate has become more popular in recent years due to the advent of faster (though not necessarily higher-quality) electronic circuitry; however, event rates calculated this way don't take coincidence into consideration.

Method 2

The second method of determining an instrument's event rate is based on a 10% level of coincidence, which is more relevant to researchers (Figure 3). Lower coincidence rates indicate higher data integrity. Therefore, using this method best represents acceptable coincidence at an actual event rate that can be used to generate high-quality data.

Users can be more confident that the data will be within acceptable coincidence rate levels at a given event rate when manufacturers report their specification for event rate using this method.

How to compare

When deciding between instruments, be sure that you know how the manufacturer arrived at the specification for event rate. If this value was calculated using the first methodology (speed of electronics only), you should carefully examine the coincidence rates when running your samples.

Pay special attention to: carryover

Carryover refers to the amount of an original sample that carries over and contaminates the next sample, resulting in data inaccuracies (Figure 3). The percent-carryover specification is usually measured by acquiring a fixed volume of sample, followed by acquiring a fixed volume of a particle-free, buffer-only solution such as phosphate-buffered saline (PBS). Events representing the contaminating cells are then identified in the buffer-only solution. Many manufacturers run a specific cell line or set of beads to determine this carryover value under defined conditions.

How to compare

It's important to find out what these defined conditions are and what sample was used to determine a carryover value. For example, the number of washes or the size or type of particles or cells used for the specification test may be vastly different from what a researcher would use. Ask the manufacturer how they arrived at their stated carryover value. Knowing how different manufacturers calculate this value can help you make a direct comparison of carryover just by referring to the specification.
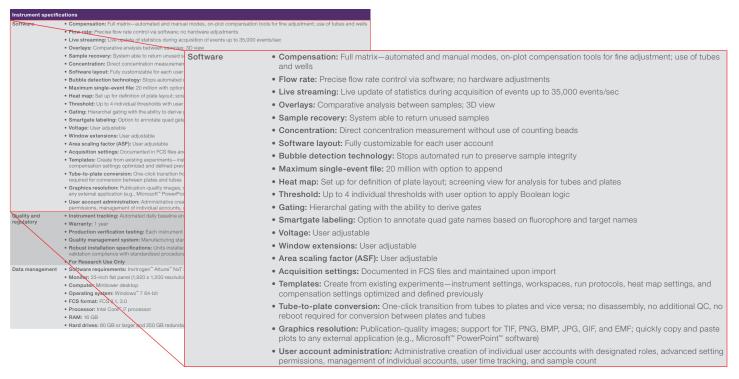
Instrument specifications

Software

- **Compensation:** Full matrix—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 acquisition of events 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:** Hierarchal gating with the ability to derive gates
- **Smartgate labeling:** Option to annotate quad gate names based on fluorophore and target names
- **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 images; support for TIF, PNG, BMP, JPG, GIF, and EMF; 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, advanced setting permissions, management of individual accounts, user time tracking, and sample count

Figure 4. Software. The software section of a spec sheet enables researchers to identify key capabilities and features built into the system. This section also indicates the extent of functionality, user-definable features, maintenance features, and user account administration. Software features vary among manufacturers and some systems have unique, exclusive options.

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; and performance validation compliance with standardized procedure
- **For Research Use Only**

Data management

- **Software requirements:** Invitrogen™ Attune™ NxT Software
- **Monitor:** 23-inch flat panel (1,920 x 1,200 resolution); dual-monitor capability
- **Computer:** Minitower desktop
- **Operating system:** Windows™ 7 64-bit
- **FCS format:** FCS 3.1, 3.0
- **Processor:** Intel Core™ i7 processor
- **RAM:** 16 GB
- **Hard drives:** 80 GB or larger and 250 GB redundant array of independent disks (RAID)–compatible hard drives

Figure 5. Quality and regulatory. Quality and regulatory specifications indicate manufacturing integrity, warranty, field engineering procedures, and ISO credentials.

Instrument specifications

Software
- **Compensation:** Full matrix—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 acquisition of events up to 35,000 events/sec
- **Overlays:** Comparative analysis between samples; 3D view
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- **Concentration:** Direct concentration measurement without use of counting beads
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- **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:** Hierarchal gating with the ability to derive gates
- **Smartgate labeling:** Option to annotate quad gate names based on fluorophore and target names
- **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—[...] compensation settings optimized and defined prev[...]
- **Tube-to-plate conversion:** One-click transition fr[...] required for conversion between plates and tubes
- **Graphics resolution:** Publication-quality images; [...] any external application (e.g., Microsoft™ PowerPo[...]
- **User account administration:** Administrative crea[...] permissions, management of individual accounts, [...]

Quality and regulatory
- **Instrument tracking:** Automated daily baseline an[...]
- **Warranty:** 1 year
- **Production verification testing:** Each instrument [...]
- **Quality management system:** Manufacturing sta[...]
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Figure 6. Data management. This section is key for successful computer installation and setup. Specifications within this section detail RAM, hard drives, and FCS format.

Instrument specifications

Installation requirements
- **Electrical requirements:** 100–240 VAC, 50/60 Hz, <150 W
  Thermo Fisher Scientific certifies that the Attune NxT Flow Cytometer conforms to relevant directives to bear the CE mark. The instrument also conforms to the UL and CAN/CSA general requirements (61010.1). The Attune NxT Flow Cytometer is a Class I laser product per Center for Devices and Radiological Health (CDRH) regulations and EN/IEC 60825.
- **Heat dissipation:** <150 W
- **Temperature operating ranges:** 15–3[...]
- **Operating humidity:** 10–90%, noncon[...]
- **Audible noise:** <65 dBA at 1.0 m
- **Instrument size (H x W x D):** ~40 x 58[...]
- **Weight:** ~29 kg (64 lb)
- **Available configurations** (as shown in[...]

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- **Heat dissipation:** <150 W
- **Temperature operating ranges:** 15–30°C (59–86°F)
- **Operating humidity:** 10–90%, noncondensing
- **Audible noise:** <65 dBA at 1.0 m
- **Instrument size (H x W x D):** ~40 x 58 x 43 cm (16 x 23 x 17 in.), including fluid bottles
- **Weight:** ~29 kg (64 lb)
- **Available configurations** (as shown in table below)

Lasers	Laser configuration	Cat. No.	Violet 405 nm					
1	Blue	A24864	Available as upgrade					
	Blue/green	A28995	Available as upgrade					
2	Blue/yellow	A24861	Available as upgrade					
	Blue/red	A24863	Available as upgrade					
	Blue/violet	A24862						
	Blue/violet 6	A29002	6				upgrade	
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

Figure 7. Installation requirements. A typical requirements section denotes information about the environmental impact of the instrument, operating conditions, and footprint.

Pay special attention to: operating temperature
Specified in °C or °F

This specification is often overlooked, yet can be important to the lifetime value of your instrument because optical alignment and fluidics are highly coupled to these values (Figure 7).

How to compare

Determine if the lab remains at relatively constant temperature and if the instrument will be used in a variety of places. Instrument performance is tested and warranted only within the specified temperature range, so be sure to consider the conditions of operation.

Pay special attention to: size and weight
Specified as H x W x D in cm or in.; instrument weight in kg or lb

Consider whether the instrument will fit in the desired space or area available in a fume hood. Be sure to include the space requirements for accessories such as an autosampler and find out if any space will be necessary for the fluidics (Figure 7). An external fluidics system is common for many flow cytometers and can add substantially to the overall space needed for the instrument. If the instrument will be moved periodically, think about how difficult this might be. Be aware that not all benchtop instruments have the same space requirements or portability.

How to compare

When a demo is being performed, request to see the full system with all components set up so that you're able to directly compare the space needed for each system.

Instrument specifications

Performance	• **High-throughput mode acquisition time:** <42 min for 96-well plate, <180 min for 384-well plate (using one rinse and one mix, and full analysis of a 40 µL sample) • **Carryover:** <0.5% in plate loader format (standard mode, 2 wash cycles); multiple-rinse capability for ultralow carryover • **Sample mixing:** Mixing optimized to preserve cell viability; mixing cycles optimized to sample analysis volume • **Mixing method:** Each well mixed via full aspiration (no shaking) • **Wash cycle:** User-defined number of wash cycles, dependent on plate-processing protocol and time to acquire plates • **Minimum dead volume (single draw):** 30 µL for 12.5–200 µL/min, 50 µL for 50–1,000 µL/min • **Sample window:** Protectively coated window allows visibility to well progress while preventing exposure to ambient light during acquisition • **Auto-calibration:** Regular, 30-day interval, system-initiated function
Fluidics	• **Plate and tube compatibility:** One-click transition from tubes to plates and vice versa; no disassembly, no additional QC, no reboot required for conversion between plates and tubes • **Compatible plate types:** 96 deep-well (flat, round, and V-bottom), 96-well standard depth (flat, round, and V-bottom), 384-well standard depth (flat, round, and V-bottom), 384 deep-well (flat, round, and V-bottom) • **Cleaning cycles:** Automated daily and monthly cleaning protocols • **Fluidics requirements:** 800 mL total of onboard fluid tanks, capable of running four 96-well plates • **Extended fluidics option:** Optional external fluid tank with 10 L fluid capacity

Figure 8. Sampler specifications. A sampling device is an accessory to the flow cytometer. Specifications of this device include acquisition time, carryover, and mixing method. Additional information regarding compatible plate types and fluidics options may also be included.

Pay special attention to: plate analysis speed
Specified in minutes per 96- or 384-well plate

Plate-speed values are typically defined as the time required to complete analysis of a 96- or 384-well plate (Figure 8). There are two aspects to this definition: one is the sample volume, the other is the sample-processing rate. Be aware that the time value reported in this specification may represent a sacrifice in data quality. Also, be sure to get clarity on what volume of sample was analyzed and what sample processing rate was used to determine the value in this specification. Some manufacturers choose to show a specification figure that minimizes plate times by collecting low-volume samples. However, in practice this could require samples to be highly concentrated, resulting in data quality issues like higher coincidence and abort rates. Conversely, if samples aren't concentrated enough, a low volume may lead to a lack of enough events to be statistically significant.

The sample-processing rate (the rate at which the sample is being introduced to the fluidics system) can also affect the data quality. In flow cytometers that rely solely on hydrodynamic focusing, the sample is spread across a wider core stream as the flow rate increases. Higher sample rates produce greater variability, less precise measurements, and compromised data quality. With instruments that utilize acoustics-assisted hydrodynamic focusing, the cells remain tightly aligned in the center of the stream regardless of the sample rate, resulting in less signal variation and improved data quality. Therefore, if you

choose to increase the sample input rate in order to lower plate times, you should use a system that offers acoustic focusing to avoid loss of data quality.

Another variable in calculating the plate analysis speed is whether the probe is rinsed between samples. Probes that are not rinsed introduce the potential for higher carryover—a tradeoff that should be considered before making a decision to run experiments at the given specification for plate analysis time.

How to compare
Make sure you understand the tradeoff in data quality that may be incurred to achieve the times the manufacturer represents with this specification.

Conclusion
Always inquire about the tests associated with pertinent specification values, so you can be confident that you're making accurate comparisons between the features of the instruments under consideration.

Resource

Investigating flow cytometry instrument capabilities to maximize your research
The 40-page Flow Cytometer Evaluation Guide will enable a better understanding of the components and capabilities of various flow cytometers, for objective comparison of instruments from several manufacturers.

Get the guide at **thermofisher.com/compareflow**

Decide for yourself at **thermofisher.com/compareflow**

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