Flow Cytometry

Flow Cytometer Buyer's Guide

From Instrument to Configuration

Visit bio-rad.com/cellanalysis for more information.

BIO-RAD

What to Consider When Buying a Flow Cytometer

A flow cytometer is a significant investment for your laboratory and there are many considerations to ensure that you choose an appropriate instrument to meet your users' needs. Things like the number of parameters it can measure, the throughput requirements, and sample handling will all need to be factored into your decision. This Buyer's Guide highlights some key considerations to enable you to narrow down your instrument and configuration options.

Define Your Needs

Having a clear idea of what your users' needs are will pay huge dividends. Not only will it guarantee that you approach your purchase in a practical way, but it will help you justify your budget for new equipment. If you are in a multiuser environment, consider identifying gaps in the current service by surveying your userbase. As a flow cytometer is a substantial investment, make sure you think long-term; anticipating your future needs is almost as important as the here and now.

The Bio-Rad ZE5 Cell Analyzer is designed with flexibility in mind to help adapt to future use. Its integrated plate loader can handle 1.5 and 5 ml tubes as well as plates, and it can be upgraded with the addition of extra lasers and detectors if your needs change. It is automation-ready and ideal for high-throughput screening and routine laboratory applications.

Prioritize Your Applications

Anticipate the different types of applications that your flow cytometer will be routinely used for so you can identify essential features and prioritize laser combinations. For example, if immunophenotyping is a priority, the inclusion of violet (405 nm) and ultraviolet (UV; 355 nm) lasers will help expand the number of compatible fluorophores and enables more flexible panel design.

The ZE5 Cell Analyzer offers a host of features that ensure it is suitable for a wide range of applications, including immunophenotyping, exosome detection, fluorescence resonance energy transfer (FRET), bead arrays, and high-throughput screening. The system can be preconfigured with different laser combinations based on your priority applications (Table 1).

Table 1. Laser priority by application. Larger circles represent higher priority.

Application	Laser					
	355 nm*	405 nm	488 nm	561 nm	640 nm	Explanation
Immunophenotyping	●	●	Included	•	•	UV* and violet lasers provide the highest number of colors.
Small particles**	•	●	Included	●	•	The ZE5 Cell Analyzer can be equipped with a small particle detector triggered off the violet laser. The brightness of phycoerythrin (PE), which is maximally excited by the yellow (561 nm) laser, is also useful for small particle detection.
FRET	●	●	Included	●	•	The yellow and violet lasers give the best choice of compatible FRET pairs.
Novel markers	●	●	Included	•	●	Generally, markers with only a few conjugates will be available in only fluorescein isothiocyanate (FITC), PE, and allophycocyanin (APC). The blue laser (488 nm) covers FITC and PE and the red laser APC.
Bead assays	•	●	Included	●	●	The red laser is often needed for bead arrays and the violet laser gives maximum flexibility for other applications.

* Only available for 4 and 5 laser configurations.
** Also consider the small particle detector.
APC, allophycocyanin; FITC, fluorescein isothiocyanate; FRET, fluorescence resonance energy transfer; PE, phycoerythrin; UV, ultraviolet.

The small particle detector is an additional scatter detector triggered from the violet laser and calibrated to give enhanced resolution of small particles such as bacteria, yeast, and extracellular vesicles. It is included as standard on all five-laser ZE5 Cell Analyzer configurations. Four- and three-laser configurations that have a violet laser are also available with a small particle detector. It is also an option as a field upgrade for four- and three-laser systems equipped with a violet laser.

Count Your Parameters

How many parameters do you need to measure? The number will determine which laser configuration is best for you:

- For 17 or fewer, consider a three-laser configuration
- For 17–24, consider a four-laser configuration
- For 24–27, consider a five-laser configuration that includes a small particle detector

For complex immunophenotyping assays, think about expanding the number of parameters available off the UV laser from five to seven. Note that, in this configuration, yellow laser parameters are reduced from seven to five. Refer to the ZE5 Cell Analyzer Laser and Filter Configuration Guide on pages 6–7 when configuring your ZE5 Cell Analyzer.

Think About Throughput

Ensure that your instrument can handle the volume of samples that you need to process for your current workload, but also factor in a 50% increase in throughput to ensure it can also meet your needs if they evolve. Avoid having to double up to meet throughput demands by choosing a flow cytometer that has additional capabilities, ready for future applications and experiments.

The Bio-Rad ZE5 Cell Analyzer can collect data at a higher rate than any other instrument currently available. The electronics and fluidics make it capable of sampling data at 100,000 events per second without compromising data quality, making rare event detection super-fast. Additionally, in high-throughput mode plates can be processed at speeds previously only available with instruments dedicated to screening: a 96 well plate may take less than 15 minutes and a 384 well plate less than 50 minutes. Combining these two advantages makes it possible to perform complex immunophenotyping panels quickly and maximize productivity.

Consider Automation

The ability to screen more and analyze faster while maintaining high quality, accurate results is key to progressing research. Automating your flow cytometry workflow can dramatically increase productivity and lead to greater progress. As not all instruments are suitable for automation, future proof your workflow by making sure that you opt for an instrument that was designed with automation in mind. Robotic integration and automation features simplify operations, save time, and improve lab performance. Automation providers will be happy to offer further assistance with setting up your workstation.

External Fluidics are especially useful if you are considering automation. The house deionized (DI) connection allows the ZE5 Cell Analyzer to be connected directly to a supply of DI water. The external bulk carboys replace the internal tanks to give up to 24 hr uninterrupted run time.

Automation-ready, the ZE5 Cell Analyzer has been integrated by many providers into a wide range of automation workcells. Large capacity fluidics options ensure uninterrupted 24 hour operation.

Visit bio-rad.com/htscreening for more information on ZE5 Cell Analyzer automation.

Identify Your Installation Site

Laboratory space is often at a premium, especially in more established labs. Think about space below, as well as above, the bench and don't forget to factor in access for servicing. If considering automation, the flexibility of modern robotics allows for workcells to be built in more limited spaces than previously so automation can be possible even in smaller spaces. It's a good idea to contact your local instrument representative as well as your automation provider to seek advice if space is limited.

Unlike many other systems, the ZE5 Cell Analyzer does not need a separate fluidics cart, all the fluidics can be contained within the instrument itself, although larger external tanks are available as well. With a remarkably small footprint, it can be installed on a standard benchtop.

Next Steps

Make a Shortlist and Use Your Network

Once you have a clear idea of what you need, make a shortlist of instruments that match your requirements. Then use your network to gain experience from your peers. Speak to your colleagues as well as the wider userbase on social media, at meetings, and at conferences to get a broad opinion of the instruments on your list.

Talk with a Specialist

Contact your local sales teams and speak with a specialist in flow cytometry within the company. They should ensure that the instrument you have selected meets your requirements and is the right configuration for you. This is your chance to obtain more detailed information, so make sure that you have a list of questions as well as providing as much detail as possible on what you need from the instrument. For a neutral appraisal of the technology, talk to other researchers using the same system you are considering.

Request a Demo

Once you have identified a flow cytometer that meets your requirements, put it through its paces by arranging a demonstration. Involve key stakeholders in the demonstration and perform your most important assays to ensure the machine will deliver what you hope. Plan to have suitable samples ready for your demonstration.

5-Laser (27 colors)

Laser	Filter	Fluorochrome
355	387/11	SBUV400, BUV395
	447/60	SBUV445, AF350, DAPI
	525/50	SBUV510, BUV496
	670/30	SBUV665, BUV661
	700LP	SBUV740, SBUV795, BUV737
405	420/10	BV421
	460/22	SBV440, Pacific Blue
	525/50	SBV515, BV510, CFP
	615/24	SBV610, BV605
	670/30	SBV670, BV650
	720/60	SBV710, BV711
	750LP	SBV760, SBV790, BV786
488	488/10	Side Scatter
	525/25	FITC, GFP, YFP, AF488, Kiravia520
	593/52	SBB580
	692/80	SBB675, SBB700, PerCP-Cy5.5
	750LP	SBB765, SBB810
561	577/15	SBY575, PE
	589/15	DsRed, tdTomato
	615/24	SBY605, PE-Dazzle, PE-CF594, mCherry
	640/20	mPlum
	670/30	SBY665, PE-Cy5, PE-AF647
	720/60	SBY720, PE-Cy5.5
	750LP	SBY800, PE-Cy7, PE-AF750
640	670/30	APC, AF647
	720/60	AF700
	775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810

5-Laser 7 Off UV Option A (27 Colors)

Laser	Filter	Fluorochrome
355	387/11	SBUV400, BUV395
	509/24	SBUV510, DAPI, Zombie UV
	577/15	SBUV575, BUV536
	615/24	SBUV605, BUV615
	670/30	SBUV665, BUV661
	747/33	SBUV740, BUV737
	780 LP	SBUV795, BUV805
405	420/10	BV421
	460/22	SBV440, Pacific Blue
	525/50	SBV515, BV510, CFP
	615/24	SBV610, BV605
	670/30	SBV670, BV650
	720/60	SBV710, BV711
	750LP	SBV760, SBV790, BV786
488	488/10	Side Scatter
	525/35	FITC, GFP, YFP, AF488, Kiravia520
	593/52	SBB580
	692/80	SBB675, SBB700, PerCP-Cy5.5
	750LP	SBB765, SBB810
561	583/30	SBY575, PE, DsRed, tdTomato, RFP
	615/24	SBY605, PE-Dazzle, PE-CF594, mCherry
	670/30	SBY665, PE-Cy5, PE-AF647
	720/60	SBY720, PE-Cy5.5
	750LP	SBY800, PE-Cy7, PE-AF750
640	670/30	APC, AF647
	720/60	AF700
	775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810

5-Laser 7 Off UV Option B (27 Colors)

Laser	Filter	Fluorochrome
355	387/11	SBUV400, BUV395
	460/22	SBUV445, DAPI, Zombie UV, L/D Blue
	509/24	SBUV510, BUV496
	577/15	SBUV575, BUV563
	670/30	SBUV665, BUV661
	747/33	SBUV740, BUV737
	780 LP	SBUV795, BUV805
405	420/10	BV421
	460/22	SBV440, Pacific Blue
	525/50	SBV515, BV510, CFP
	615/24	SBV610, BV605
	670/30	SBV670, BV650
	720/60	SBV710, BV711
	750LP	SBV760, SBV790, BV786
488	488/10	Side Scatter
	525/35	FITC, GFP, YFP, AF488, Kiravia520
	593/52	SBB580
	692/80	SBB675, SBB700, PerCP-Cy5.5
	750LP	SBB765, SBB810
561	583/30	SBY575, PE, DsRed, tdTomato, RFP
	615/24	SBY605, PE-Dazzle, PE-CF594, mCherry
	670/30	SBY665, PE-Cy5, PE-AF647
	720/60	SBY720, PE-Cy5.5
	750LP	SBY800, PE-Cy7, PE-AF750
640	670/30	APC, AF647
	720/60	AF700
	775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810

Note: This is not a comprehensive list of fluorophores and dyes that can be used with the ZE5 Cell Analyzer.

continues

ZE5 Cell Analyzer Laser and Filter Configuration Guide, continued

4-Laser (24 colors)*,**

Laser	Filter	Fluorochrome
405	420/10	BV421
	460/22	SBV440, Pacific Blue
	525/50	SBV515, BV510, CFP
	615/24	SBV610, BV605
	670/30	SBV670, BV650
	720/60	SBV710, BV711
	750LP	SBV760, SBV790, BV786
488	488/10	Side Scatter
	509/24	FITC, GFP, AF488, Kiravia520
	549/15	YFP
	583/30	SBB580, PE
	615/24	SBB615, PE-CF594, PE-Dazzle
	692/80	SBB675, SBB700, PerCP-Cy5.5, PE-Cy5
	750LP	SBB765, SBB810, PE-Cy7, PE-AF750
561	577/15	SBY575, PE
	589/15	DsRed, tdTomato
	615/24	SBY605, PE-Dazzle, PE-CF594, mCherry
	640/20	mPlum
	670/30	SBY665, PE-Cy5, PE-AF647
	720/60	SBY720, PE-Cy5.5
	750LP	SBY800, PE-Cy7, PE-AF750
640	670/30	APC, AF647
	720/60	AF700
	775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810

3-Laser (17 Colors)*,**

Laser	Filter	Fluorochrome
405	420/10	BV421
	460/22	SBV440, Pacific Blue
	525/50	SBV515, BV510, CFP
	615/24	SBV610, BV605
	670/30	SBV670, BV650
	720/60	SBV710, BV711
	750LP	SBV760, SBV790, BV786
488	488/10	Side Scatter
	509/24	FITC, GFP, AF488, Kiravia520
	549/15	YFP
	583/30	SBB580, PE
	615/24	SBB615, PE-CF594, PE-Dazzle
	692/80	SBB675, SBB700, PerCP-Cy5.5, PE-Cy5
	750LP	SBB765, SBB810, PE-Cy7, PE-AF750
640	670/30	APC, AF647
	720/60	AF700
	775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810

3-Laser Option 2 (17 colors)**

Laser	Filter	Fluorochrome
488	488/10	Side Scatter
	509/24	FITC, GFP, AF488, Kiravia520
	549/15	YFP
	583/30	SBB580, PE
	615/24	SBB615, PE-CF594, PE-Dazzle
	692/80	SBB675, SBB700, PerCP-Cy5.5, PE-Cy5
	750LP	SBB765, SBB810, PE-Cy7, PE-AF750
561	577/15	SBY575, PE
	589/15	DsRed, tdTomato
	615/24	SBY605, PE-Dazzle, PE-CF594, mCherry
	640/20	mPlum
	670/30	SBY665, PE-Cy5, PE-AF647
	720/60	SBY720, PE-Cy5.5
	750LP	SBY800, PE-Cy7, PE-AF750
640	670/30	APC, AF647
	720/60	AF700
	775/50	APC-Cy7, AF750
	800LP	AF790, APC/Fire810

3-Laser (20 Colors)*,**

Laser	Filter	Fluorochrome
405	420/10	BV421
	460/22	SBV440, Pacific Blue, mTurquoise
	525/50	SBV515, BV510, CFP, Cascade Yellow
	615/24	SBV610, BV605
	670/30	SBV670, BV650
	720/60	SBV710, BV711
	750LP	SBV760, SBV790, BV786
488	488/10	Side Scatter
	509/24	FITC, eGFP, AF488, Kiravia520, mClover
	549/15	eYFP
	583/30	SBB580, PE
	615/24	SBB615, PE-CF594, PE-Dazzle
	692/80	SBB675, SBB700, PerCP-Cy5.5, PE-Cy5
	750LP	SBB765, SBB810, PE-Cy7, PE-AF750
561	577/15	SBY 575, PE
	589/15	DsRed, tdTomato, RFP
	615/24	SBY605, PE-Dazzle, PE-CF594, mCherry
	640/20	mPlum
	670/30	SBY665, PE-Cy5, PE-AF647
	720/60	SBY720, PE-Cy5.5
	750LP	SBY800, PE-Cy7, PE-AF750

Note: This is not a comprehensive list of fluorophores and dyes that can be used with the ZE5 Cell Analyzer.

* Small particle detector can be included in configuration or omitted from configuration with the option to add later.

** Eligible for an upgrade with a UV laser.

AFxxx, Alexa Fluor; APC, allophycocyanin; BV, Brilliant Violet; BUV, Brilliant UltraViolet, CFP, cyan fluorescent protein; Cy, cyanine; dsRed, Discosoma Red Fluorescent Protein; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; eGFP, enhanced green fluorescent protein; eYFP, enhanced yellow fluorescent protein; LP, long pass; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; RFP, red fluorescent protein; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet; SBY StarBright Yellow; UV, ultraviolet; YFP, yellow fluorescent protein.

Visit bio-rad.com/cellanalysis for a wide range of videos, technical reports, published findings, and other resources describing the Bio-Rad ZE5 Cell Analyzer.

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