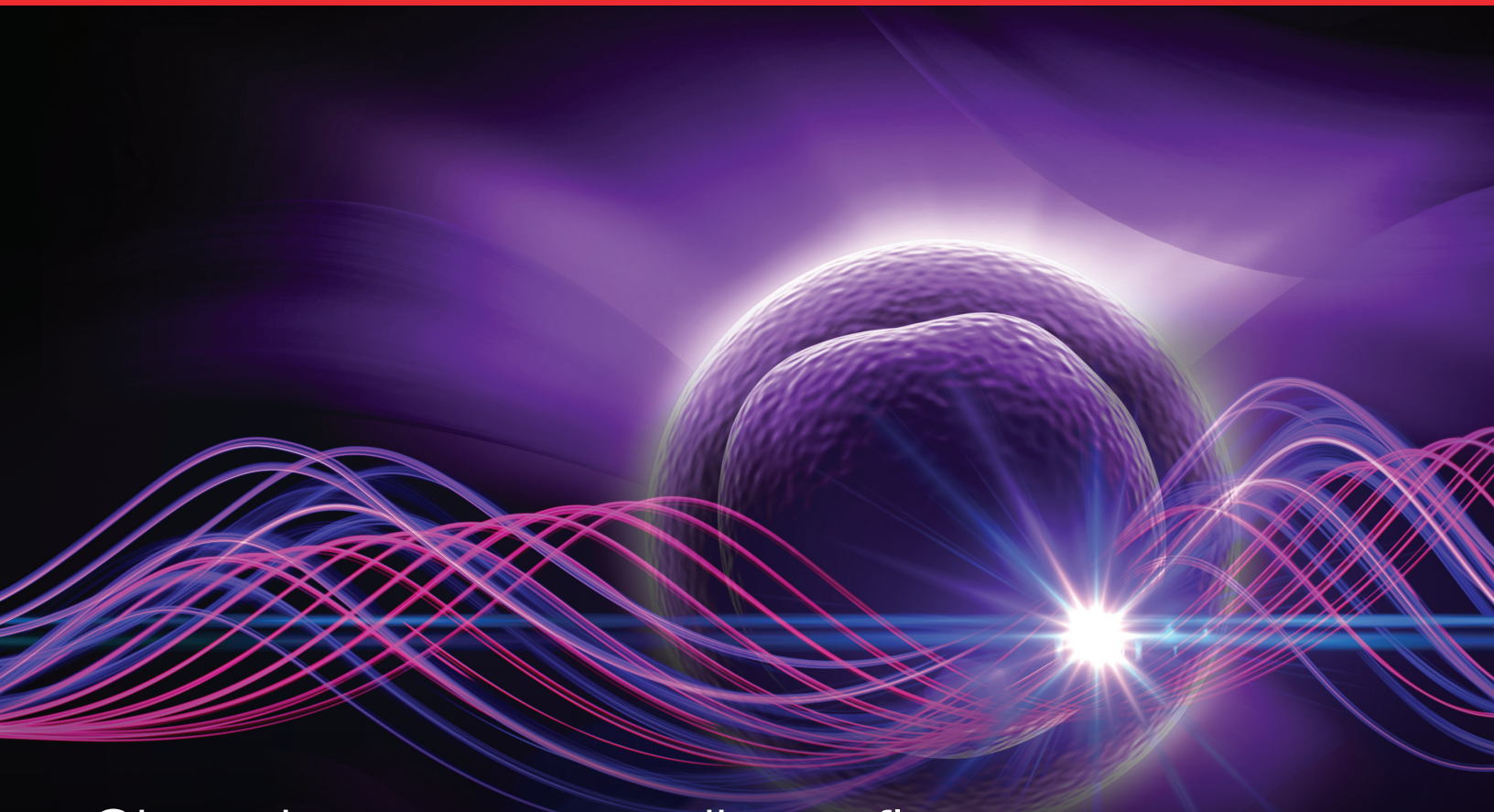
invitrogen



Choosing an extraordinary flow cytometer

A decision workbook for measuring performance across instruments from multiple manufacturers

ThermoFisher SCIENTIFIC

This workbook is intended as a guide for deciding on the purchase of a flow cytometer. When evaluating different instruments, it's best to conduct the comparison in a similar fashion as your research experiments: the comparisons should be as standardized as possible. We've gathered a set of tools and checklists to simplify this process for you, and we've broken them down into four steps: define, document, determine, and decide.

Define your needs for an instrument and how you will go about comparing multiple platforms.

- Exercise 1: Identify your decision team
- Exercise 2: Characterize your user base
- Exercises 3a–3f: Establish research areas and focus planning
  - 3a: Research area
  - 3b: Topic of interest
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- Exercise 6: Schedule demo and evaluation participants

Gather technical specifications, feedback from colleagues, and capabilities on multiple instruments.

- Exercise 7: Create your list of candidate instruments
- Exercises 8a–8c: Complete technical specification comparison table
  - 8a: Optics specifications
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Determine performance of each candidate instrument by taking measurements of controlled variables across platforms.

- Exercise 10: Plan in-lab demos
- Exercise 11: Plan sample types and prep
- Exercise 12: Aquire fluorescence sensitivity plots
- Exercise 13: Determine number of peaks and resolution
- Exercise 14: Verify multicolor immunophenotype
- Exercise 15: Complete plot comparison grid
- Exercise 16: Perform acquisition of dilute samples

Decide on an instrument based on the data and your experiences with each instrument.

- Exercise 17: Debrief
- Exercise 18: Determine subjective level of support vs. opposition rating scale
- Exercise 19: Tally subjective scale scores
- Exercise 20: Finalize decision

Define your needs for an instrument and how you will go about comparing multiple platforms.			

Exercise 1: Identify your decision team

An invaluable first step in the process is to identify your decision team. While this may sound formal, gathering a group who will assess each candidate instrument and offer feedback is crucial. Remember that multiple stakeholders will be involved in funding the new instrument and operating it, and there may also be expert technicians within your organization who are adept at hands-on assessment of engineering.

Directions: Assign everyone who will be participating in the standardized evaluations across each of the candidate instruments. Each person should have a unique user number. The user numbers will be used throughout this workbook. User numbers facilitate equality of opinions within the group.

Assigned user number	Name	Title/Role	Email	Mobile
User 1				
User 2				
User 3				
User 4				
User 5				
User 6				
User 7				
User 8				
User 9				
User 10				
User 11				
User 12				
User 13				
User 14				
User 15				

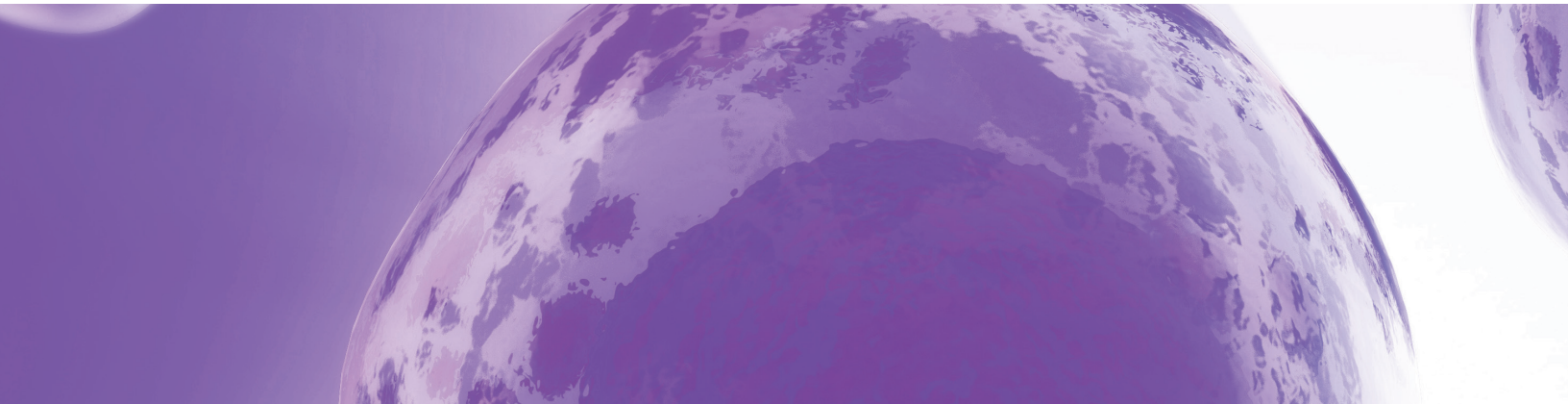
Define | Document | Determine | Decide

Exercise 2: Characterize your user base

Next, think of those who will actually be using the instrument. While having these users in attendance at demos, seminars, and meetings regarding each candidate instrument is ideal, these individuals may or may not be participating in instrument evaluations. Therefore, getting a representative user composite is important.

Directions: Populate the fields with information about potential users of this new instrument.

		User 1	User 2	User 3	User 4	User 5	User 6	User 7	User 8	User 9	User 10	User 11	User 12	User 13	User 14	User 15
Level of flow cytometry expertise	Proficient															
	Competent															
	Adv. beginner															
	Novice															
Frequency of use	Daily															
	Weekly															
	Monthly															
	Occasionally															
Number of colors each user will need	>14															
	10–14															
	5–9															
	1–4															



Define › Document › Determine › Decide

Exercises 3a–3d: Establish research area and focus planning

Plan for applications that will be run on the new instrument.
Define the work that this instrument will help achieve.

Exercise 3a: Research area

In the box below, fill in your primary research area.

What is your primary research area?	

Did you know?

Flow cytometry is used in many research areas, including:

- Aquaculture
- Bacteriology
- Cardiology
- Cytology
- Dermatology
- Dermopathy
- Diagnostics
- Gastroenterology
- Geosciences
- Hematology
- Histology
- Horticulture
- Immuno-oncology
- Immunobiology
- Immunology
- Microbiology
- Neurology
- Neuroscience
- Oncology
- Pathology
- Pediatrics
- Pharmacology
- Rheumatology
- Synthetic biology
- Toxicology
- Translational research
- Veterinary medicine
- Virology

Flow cytometry is being actively employed in an unprecedented variety of research topics. It can be advantageous for labs to discuss the performance of the instruments they are considering with a lab doing the same or similar work.

Exercise 3b: Topic of interest

In the box below, summarize your area of study.

What are you studying?	

Exercise 3c: Methods and models

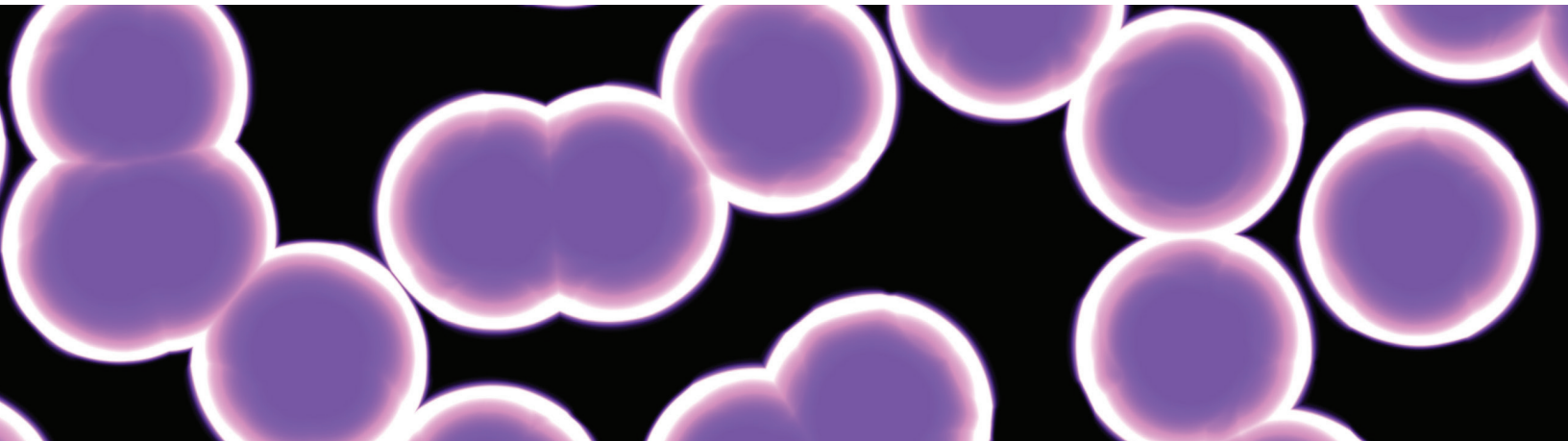
In the box below, write in the experimental setup, modeling, and approach you will be using in your research.

How are you trying to study your research questions?	

Exercise 3d: Samples type(s)

In the box below, write in the sample type(s) you will be working with on your new flow cytometer.

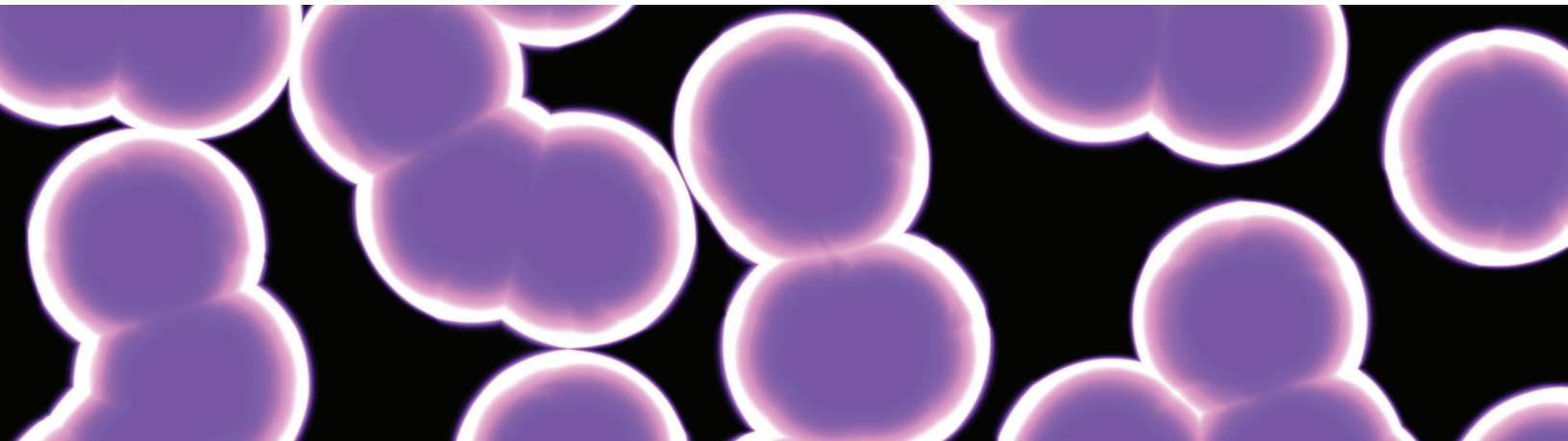
What types of samples are you planning to run?	



Did you know?

The sample types you will be running are an important factor within the decision process, as each type of sample has inherent processing implications. Flow cytometry is a powerful technology supporting deeper analysis across an array of samples, including:

- Adipose tissue
- Algae
- Autotrophic bacteria
- Bacteria
- Cancer stem cells
- Cytokines
- Dendritic cells
- Exosomes
- HEK cells
- HeLa cells
- Hemopoietic stem cells
- Human epidermal cells
- Human gut bacteria
- Joint fluid
- Kidney tissue
- Kinases
- Lung tissue
- Macrophage cells
- Mammalian cells
- Marine cyanobacteria (*Prochlorococcus)*
- Marine microalgae
- MDSC populations
- Micron-sized virus particles
- Milk
- Mouse blood
- Mouse bone marrow
- Mouse spleen
- Nanoparticles
- NK cells
- Platelets
- Primary cells
- Primary patient samples
- Proteins
- Radioactive samples
- Red blood cells
- Refined lactose
- Somatic cells
- Sperm
- Spermatogenic cells
- Stem cells
- Testicular stem cells
- Tissue
- Tumors
- Water
- Whole blood
- Yeast cells



Interrogation by flow cytometry requires single-cell suspension of intact cellular structures. However, not all samples are similar in characteristics; some pose challenges such as clogging, or long-indebted run times.

Exercise 3e: Sample characteristics

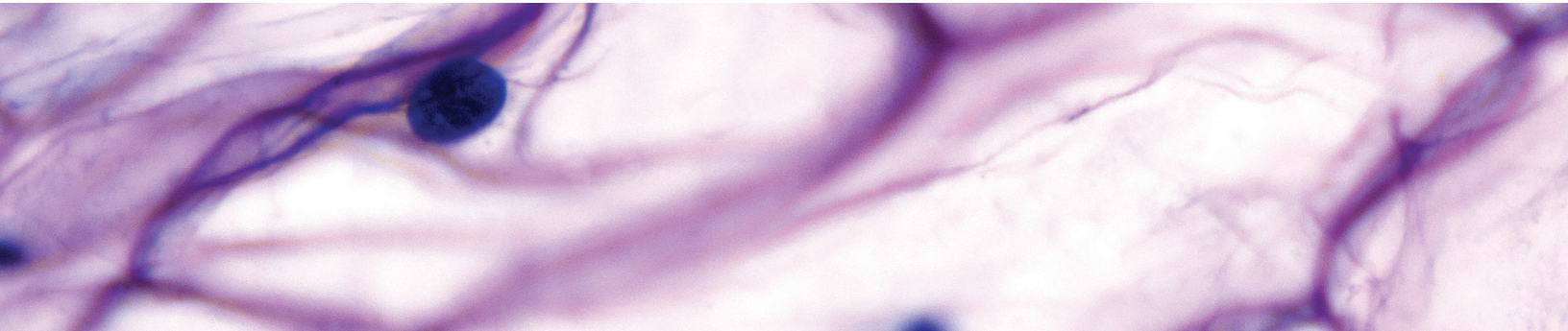
Describe characteristics of your samples.

	No	Maybe	Yes	Bonus—my samples have clogged an instrument
Clumpy				
High viscosity				
Large				
Sticky				
Difficult				
Precious/rare events				
Microscale				
Dilute				
Disaggregated				
Sensitive				
Patient-sourced				
Abnormal				
Engineered				
Other:				
Other:				

Exercise 3f: Applications

In the box below, write your anticipated applications.

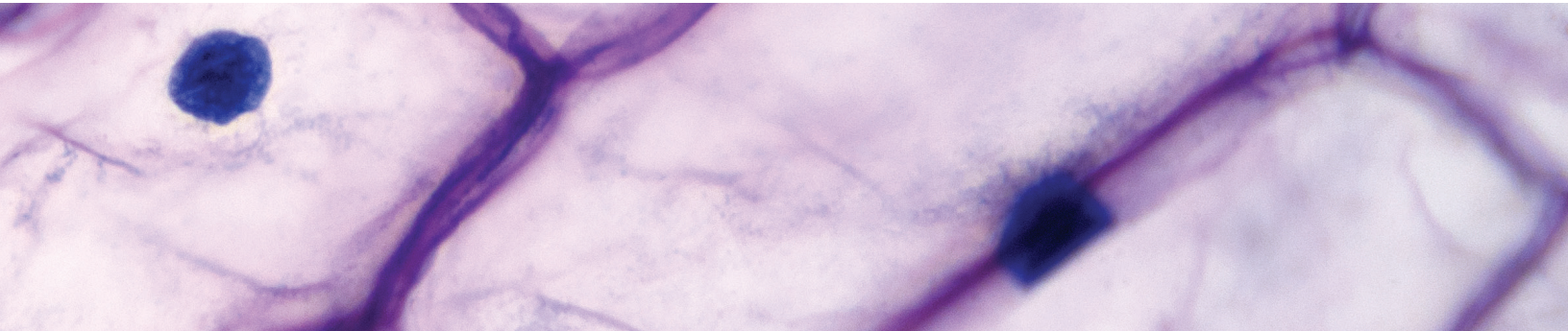
What applications will you be running?	



Did you know?

Flow cytometry enables many applications, techniques, and assays. Depending on how an instrument is engineered, there may be mechanical or software features that enable these uses particularly well. While a specific application such as immunophenotyping may come to mind first when you think of flow cytometry, many other types of studies are supported by flow cytometry, including:

- Cell counting
- Amino acid function
- Antibody screening
- Apoptosis assays
- Bacterial counts
- Basophil activation test (BAT)
- Bead-based cytokine expression
- Cell cycle analysis
- Cell isolation
- Cell line development
- Cell proliferation
- Cell signaling
- CRISPR
- Drug screening
- Fermentation monitoring
- Fluorescent protein expression
- Gene expression
- Intracellular phenotyping
- No wash, no lyse blood protocols
- Phagocytosis
- Phenotyping
- Proliferation
- Protein aggregation
- Protein degradation
- Protein expression
- Protein interactions
- Rare event analysis
- RNA
- SYTO9/PI detection
- Transduction
- Transfection
- Viability assays



Define | Document | Determine | Decide

Exercise 4: Set your priorities

Directions: For this prioritization exercise, identify three features and three capabilities to be factors in the comparisons of the candidate instruments.

Priority	Rationale	Feature		Capabilities	
Essential	Must-haves, make or break	1		1	
		2		2	
		3		3	
High priority	These matter significantly to this decision	1		1	
		2		2	
		3		3	
Medium priority	Would be nice to have these	1		1	
		2		2	
		3		3	
Low priority	Might be useful at times	1		1	
		2		2	
		3		3	
Not a priority	We wouldn't need this anyway	1		1	
		2		2	
		3		3	

Define | Document | Determine | Decide

Exercise 5: Plan out your modes of evaluation

Directions: Define the evaluations that will be completed for each candidate instrument.

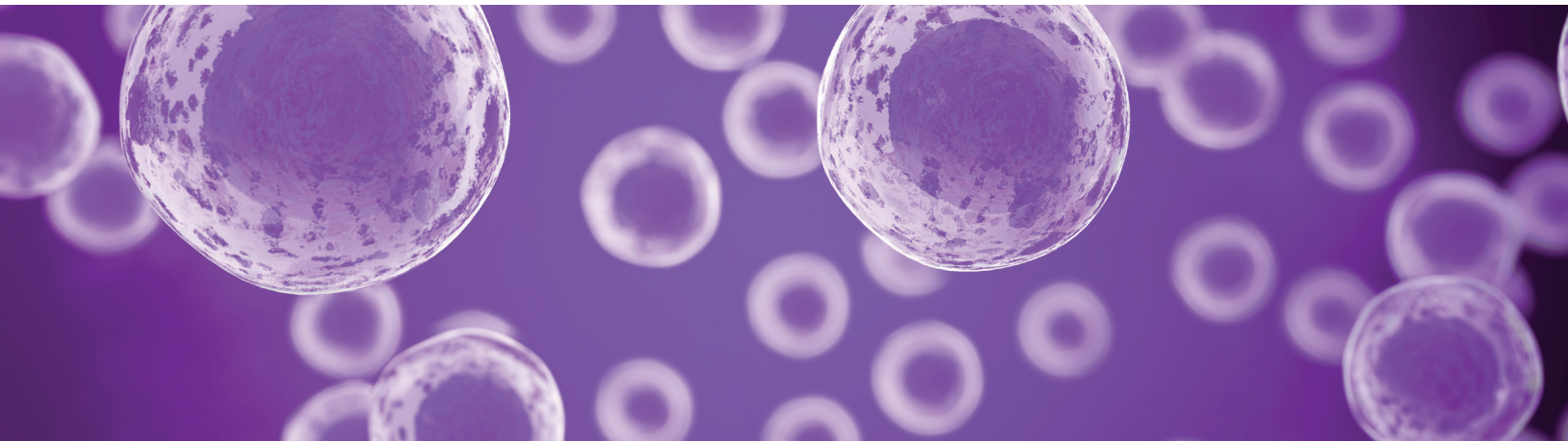
	Types of technical evaluation planned for each candidate instrument
Instrument A	☐ In-lab ☐ 3D demo ☐ Seminar ☐ WebEx ☐ Reference interview ☐ Conference ☐ Road show ☐ Other:
Instrument B	☐ In-lab ☐ 3D demo ☐ Seminar ☐ WebEx ☐ Reference interview ☐ Conference ☐ Road show ☐ Other:
Instrument C	☐ In-lab ☐ 3D demo ☐ Seminar ☐ WebEx ☐ Reference interview ☐ Conference ☐ Road show ☐ Other:
Instrument D	☐ In-lab q3D demo q Seminar q WebEx q Reference interview ☐ Conference q Road show q Other:

Define | Document | Determine | Decide

Exercise 6: Schedule demo and evaluation participants

Directions: Indicate with a check mark the availability of each person who will be participating in this decision. The more intact the group is for each evaluation, the stronger the input, points of observation, and contrast ability.

	Planning to participate in the evaluations			
	Instrument A	Instrument B	Instrument C	Instrument D
User 1				
User 2				
User 3				
User 4				
User 5				
User 6				
User 7				
User 8				
User 9				
User 10				
User 11				
User 12				
User 13				
User 14				
User 15				



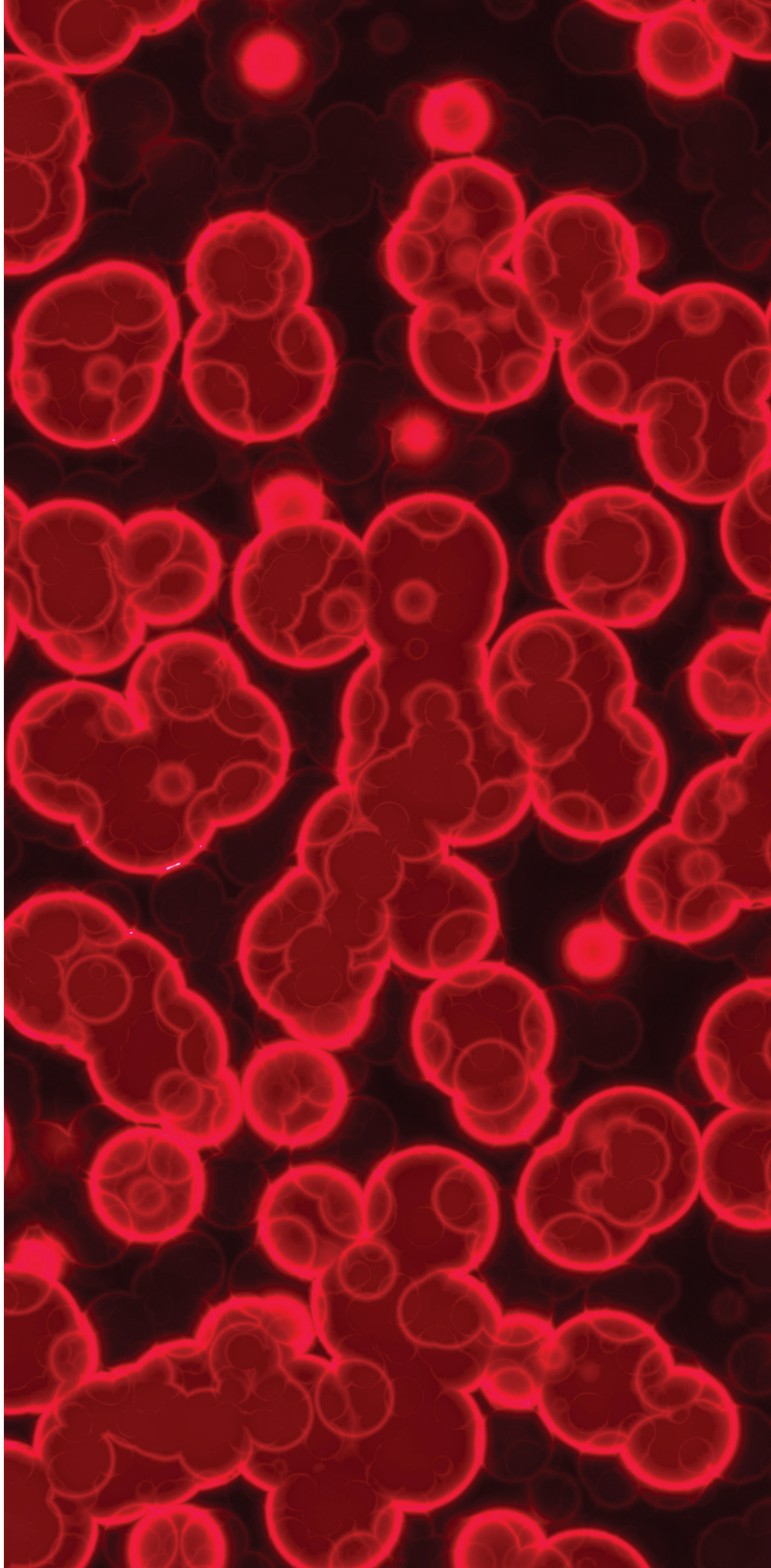
Define	Document	Determine	Decide
	Gather technical specifications, feedback from colleagues, and capabilities on multiple instruments.		

Seek out technical documentation for each instrument to be able to decipher features and capabilities between instruments. If you already own one or more of the instruments, gathering current materials on the instrument(s) is still advised. Many manufacturers regularly update their instruments and software. Gathering technical information on all instruments also supports a measured comparison across platforms.

An important step is to narrow down your list of instruments. At this point in the decision process, there exists a risk of eliminating an instrument too early. Be aware of this bias, especially this early in the decision process. Do not lose sight of the core objective, and deliberately plan for comparisons of multiple flow cytometers under conditions as equivalent as possible.

Treat the hunt for information as a cataloging of all instruments on the market, not so much as a means of eliminating some from the running. Consider this an exercise of divergent cataloging.

Identify which instruments your group will be comparing during this decision process. You may be comparing only two or more than four; but for the purposes of this workbook, there will be spots in each exercise for four instruments.



Define | Document | Determine | Decide

Exercise 7: Create your list of candidate instruments

Directions: Fill in information about the candidate instruments. Once filled in, each instrument will be referred to by the letter denoted in the first column of the table below.

Name	Make	Model	Contact	Notes
Instrument A			Name: Mobile: Email: Website:	
Instrument B			Name: Mobile: Email: Website:	
Instrument C			Name: Mobile: Email: Website:	
Instrument D			Name: Mobile: Email: Website:	

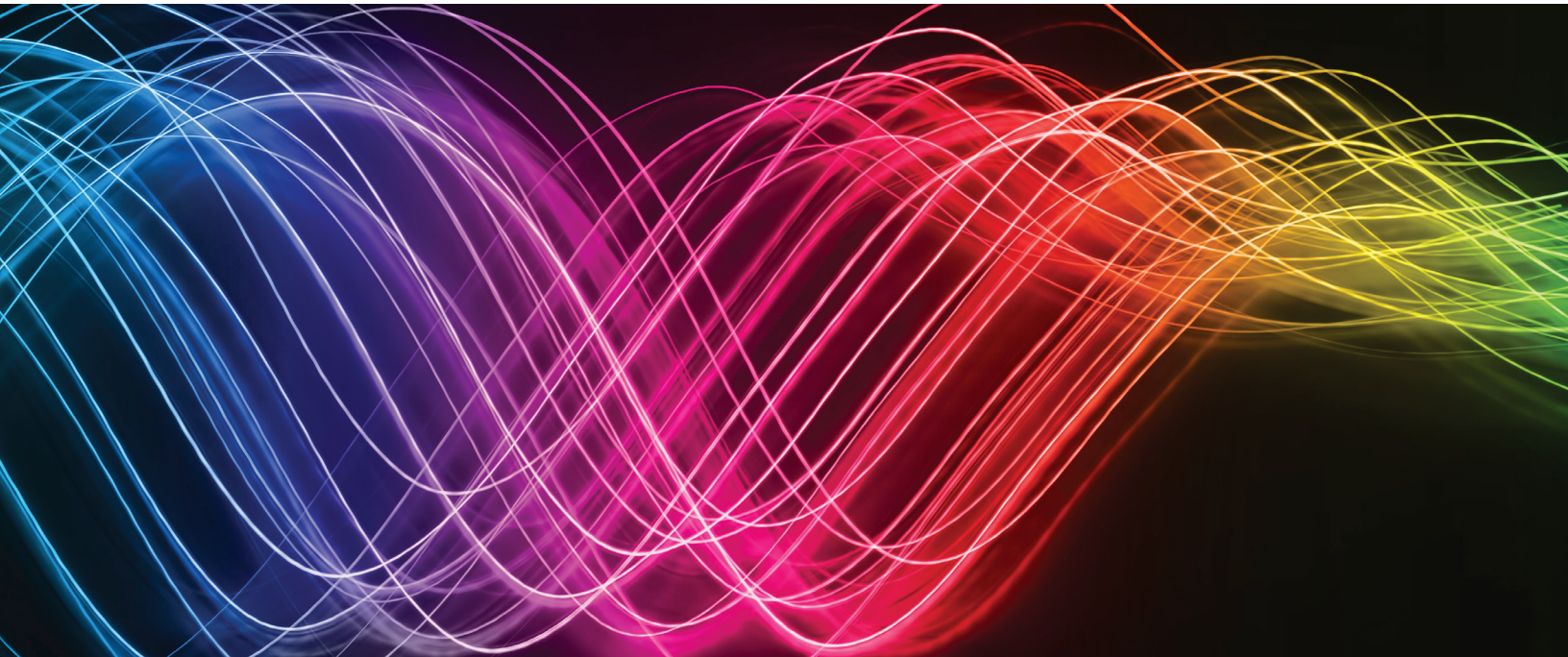
Define | Document | Determine | Decide

Exercises 8a–8d: Complete technical specification comparison table

Extract and document specifics on each candidate instrument. You will be comparing this information head to head from each instrument in the next step.

Exercise 8a: Optics specifications

			Instrument A	Instrument B	Instrument C	Instrument D
Optics	Lasers	Ultraviolet laser (355 nm)				
		Violet (405 nm)				
		Blue (488 nm)				
		Yellow (561 nm)				
		Green (532 nm)				
		Red (637 nm)				
	Laser profile	Gaussian or flat-top	☐ Gaussian ☐ Flat-top	☐ Gaussian ☐ Flat-top	☐ Gaussian ☐ Flat-top	☐ Gaussian ☐ Flat-top
	Laser power	Amount of measured usable laser power after light has gone through the beam optics and shaping filters (mW)				
		Vendor-specified theoretical maximum (mW)				
	Emission filters	Number of filters				
	Laser separation	Distance (µm)				
		Collinear or spatially separated				
	Fixed alignment (no user maintenance required)	Yes or no				
	Laser warm-up time	Time (min)				
	Field upgradable	Yes or no				



Exercise 8b: Fluidics specifications

			Instrument A	Instrument B	Instrument C	Instrument D
Fluidics	Flow cell size	Dimensions (µm)				
	Sample analysis volume	Minimum (µL) to maximum (mL) volume				
	Sample flow rates	Flow rate (µL/min)				
	Sample delivery system	Peristaltic pump or syringe pump				
	Fluid-level sensing	Yes or no				
	Expanded fluidic capacity	Volume of fluid (L)				
	Fluidic reservoir fluid capacity	Sheath (L)				
		Waste (L)				
		Wash (L)				
		Shutdown (L)				
	Sample tube sizes	Minimum to maximum dimensions (mm)				
	Automated maintenance cycles	Yes or no				
	Startup and shutdown time	Time (min)				

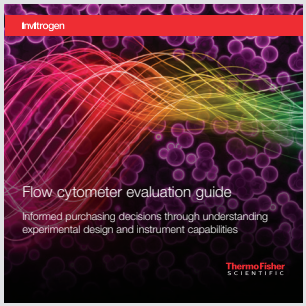
Exercise 8c: Performance specifications

			Instrument A	Instrument B	Instrument C	Instrument D
Performance	Fluorescence sensitivity	Molecules of equivalent soluble fluorochrome (MESF) for FITC				
		MESF for PE				
		MESF for APC				
	Fluorescence resolution	CV (%)				
	Data acquisition rate	Events/sec				
	Maximum electronic speed	Events/sec				
	Method of calculation of data acquisition	Poisson distribution or electronic speed				
	Carryover	Sample carryover (%)				
	Fluorescent detectors	No. of individual detectors				
	Minimum particle size	Particle size on side scatter (µm)				

Resource: Flow Cytometer Evaluation Guide

Flow cytometer evaluation guide
Download or request a printed copy of the guide.

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



Define | Document | Determine | Decide

Exercise 9: Gather subjective input

The first half of the "document" step was the process of gathering objective, quantitative variables for multiple instruments. The second half of this process is gathering subjective information from a variety of sources. Actively seeking out exposure to new solutions and subjective information from a wide variety of sources may help inform your decision process.

User base references—Ask the technical sales specialist for references from within the install base. These references are best if they are working on the same applications that you plan for the instrument. Getting input from labs that perform similar applications can provide insight on instrument features that you are considering.

Core labs—Speak to labs and core facilities for advice and experiences with instruments. Core lab managers are a particularly discerning source for information, since they are tasked with keeping up with instrument trends and technologies in order to deliver the best options for their users' applications. Ask about the straightforward nature of the software, the quality of data they are getting from an instrument, and the sense of urgency and competence displayed by service and support to help with your decision process.

Flow community—Contacting your colleagues and associates to ask about their experiences with various platforms will likely result in getting candid responses. Asking around is especially important during this information-gathering phase, as you may discover a new instrument or product feature. Many researchers that use flow cytometry will gladly discuss how they reasoned through their instrument decision, what they would have done differently, and what they like and dislike about their flow cytometer after having it up and running in their lab.

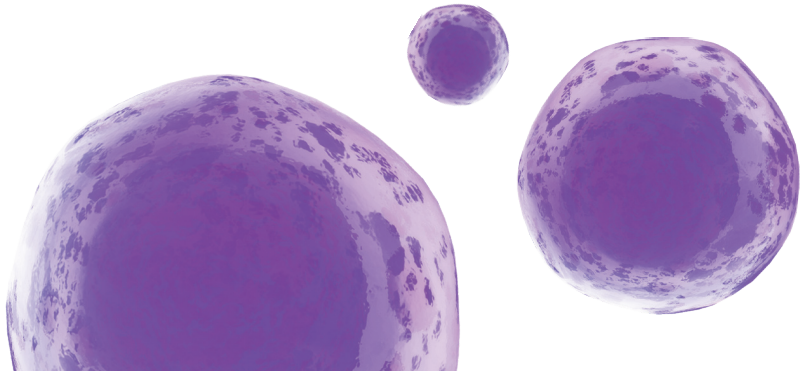
Conferences and regional meetings—Attending research conferences will offer an opportunity to review data quality, browse instruments on exhibit, and speak to the personnel in the booth. Some manufacturers staff their booth with a diverse array of engineers, R&D biologists, market researchers interested in your feedback, and PhD-level application specialists.

Manufacturer educational resources—The technical materials and educational resources from a variety of manufacturers may be of value during this period. Reviewing technical marketing materials is a method to identify the features and benefits of each instrument on the market, but more importantly, to offer evidence of aftermarket resources and educational support. Consider the volume, quality, recency, and generosity of each manufacturer. Also, gathering information on the comprehensiveness of the portfolio of reagents and antibodies will help inform your decision.

Publications and journals—Gather publications by people who are in your field, who are developing research avenues and questioning the boundaries. Aside from being a wealth of technical information, literature and publications help to verify how the data look on an instrument. This is particularly useful when reviewing research in a similar field of interest to yours.

Researcher comparisons—Seeking out articles and write-ups by researchers and core lab managers on the results of and data from quantitative, head-to-head, successive comparisons of multiple platforms will draw additional insights for comparing performances.

Blogs—Comparable to a timeline, blogs can be excellent sources of up-to-date, objective information about what's happening in flow cytometry. They can also be good sources of information about instruments.



Define	Document	Determine	Decide
		Determine performance of each candidate instrument by taking measurements of controlled variables across platforms.	

Now that you have gathered specifications and input from other sources, conduct the comparisons of the information across the candidate instruments.

Define | Document | Determine | Decide

Exercise 10: Plan in-lab demos

Site of the demo

A demo doesn't necessarily happen in your lab. Instead, these evaluation sessions can happen at a Customer Experience Center on a manufacturer's site, in the labs of colleagues, or as a combination of digital meetings and tools. Since the demo of each candidate instrument may not happen in your lab, take extra care in completing the preliminary work and compiling the data and user feedback from each demo to bring back to the table for discussions. If your evaluations include in-lab demos, note the following tips for hosting demos.

Needed space

The footprint of flow cytometers varies from instrument to instrument, so verify the minimum bench space of each instrument for which you will be hosting a demo. Also, factor in space for any accessory instruments such as a plate sampler, as well as additional fluidics tanks that will need to accompany the flow cytometer during everyday operation; and ask the vendor to show you these items' setup during the demonstration.

Power source

Connecting within the demo space is also important. Find out how many outlets are needed to power the system. The vendor should be able to provide an extension cord and power strip if needed.

Reservation duration

Most demos are scheduled to span 1–2 days, which is typically enough time to learn about the key features and competitive capabilities, test-drive the software for robustness, and in some cases run actual lab samples. If you would like more time for evaluation, request more days from the application scientist. This request is best made in advance of the scheduled demo. Also, include in your reservation the extra days each vendor will need to set up and dismantle the instrument.

Inbound shipments

Provide each vendor a receiving address. Some manufacturers ship 1–2 weeks prior to the demonstration, but most bring the instruments to the appointment. Reagents that have been ordered for a demonstration will arrive in the 1–2 weeks prior to the demonstration. Plan to store the reagents at the temperature listed on the packaging or inserts.

Define › Document › Determine › Decide

Exercise 11: Plan sample types and prep

Plan ahead

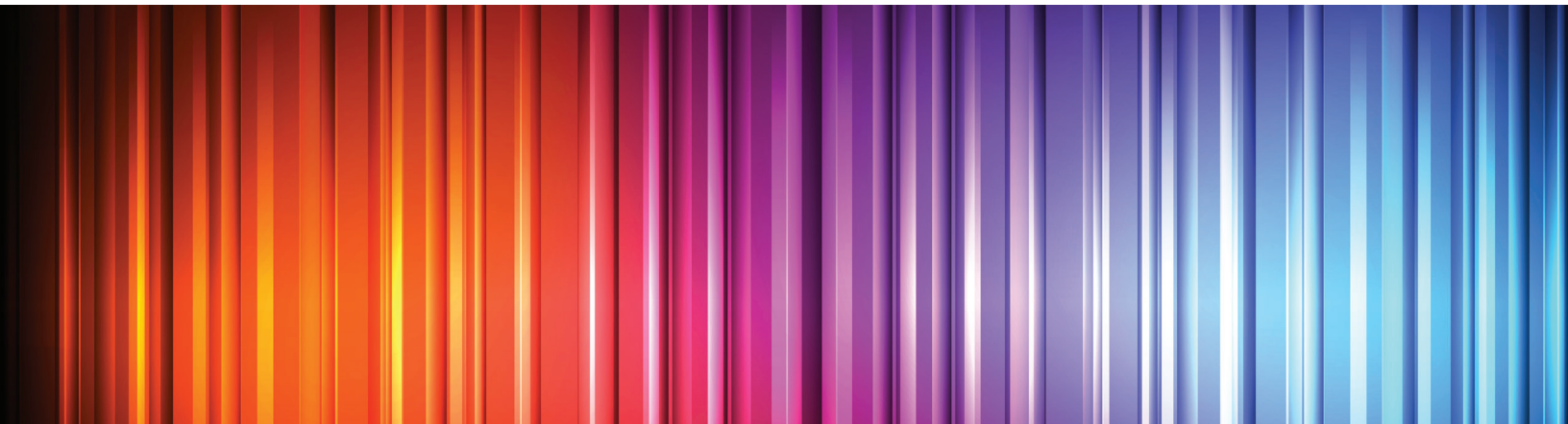
If you would like to run an experiment using the instrument, plan ahead and make sure that the field application specialist reviews the entire experimental plan with you prior to the demo. Running your own samples on multiple platforms is the most useful method of direct comparison due to your high familiarity with your samples.

Tubes and plates

Gather the various materials from around the lab that should be on hand for the demo. For example, make sure you try all of the types of tubes that anyone who will use the instrument might be using.

Demo experiments to test a flow cytometer

Running experiments that objectively evaluate the capabilities of each instrument helps facilitate this step in the selection process. We've provided some suggestions for experiments to run during a demo.



Using rainbow beads to evaluate a flow cytometer's fluorescence sensitivity

Rainbow calibration beads are particles with fluorophores that spectrally mimic fluorochromes commonly used in flow cytometry experiments. These beads span excitation wavelengths ranging from 365 to 650 nm (but do not cover UV wavelengths).

One purpose of the beads is to test the signal-to-noise resolution of a flow cytometer, or how well fluorescence signal from a cell can be detected over background. These beads are standardized, and thus, they offer an objective method to evaluate different flow cytometers.

Rainbow beads excited at a particular wavelength will emit eight different intensity peaks (Figure 1). One peak is unstained, while the other peaks have labels. Resolving each peak can provide the dynamic range of a specific photomultiplier tube (PMT) by showing the dimmest to brightest peaks in a histogram.

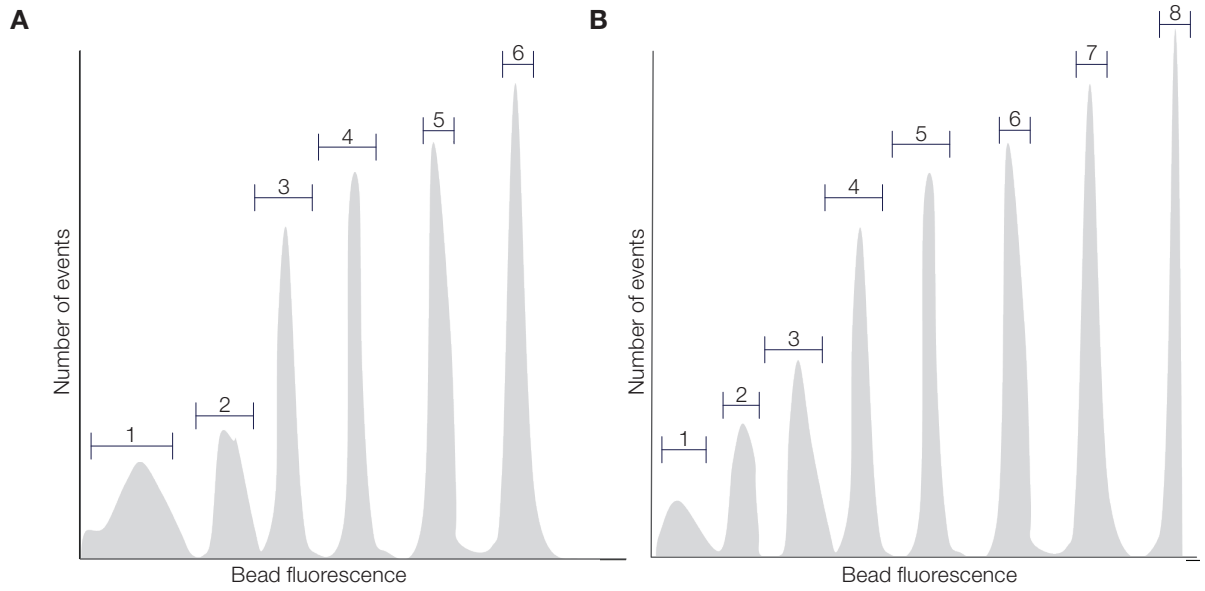


Figure 1. 8-peak fluorescence histogram. The individual peaks represent various fluorescence intensities. **(A)** 6 out of 8 possible peaks are detected. Peak 1 is from unstained beads, while peaks 2–6 are from beads emitting fluorescence. This type of data would show limited resolution of fluorescence detected in a channel. **(B)** All 8 peaks are detected, indicating full range of fluorescence resolution.

Exercise 12: Aquire fluorescence sensitivity plots

Directions: Collect fluorescence plots from each instrument.

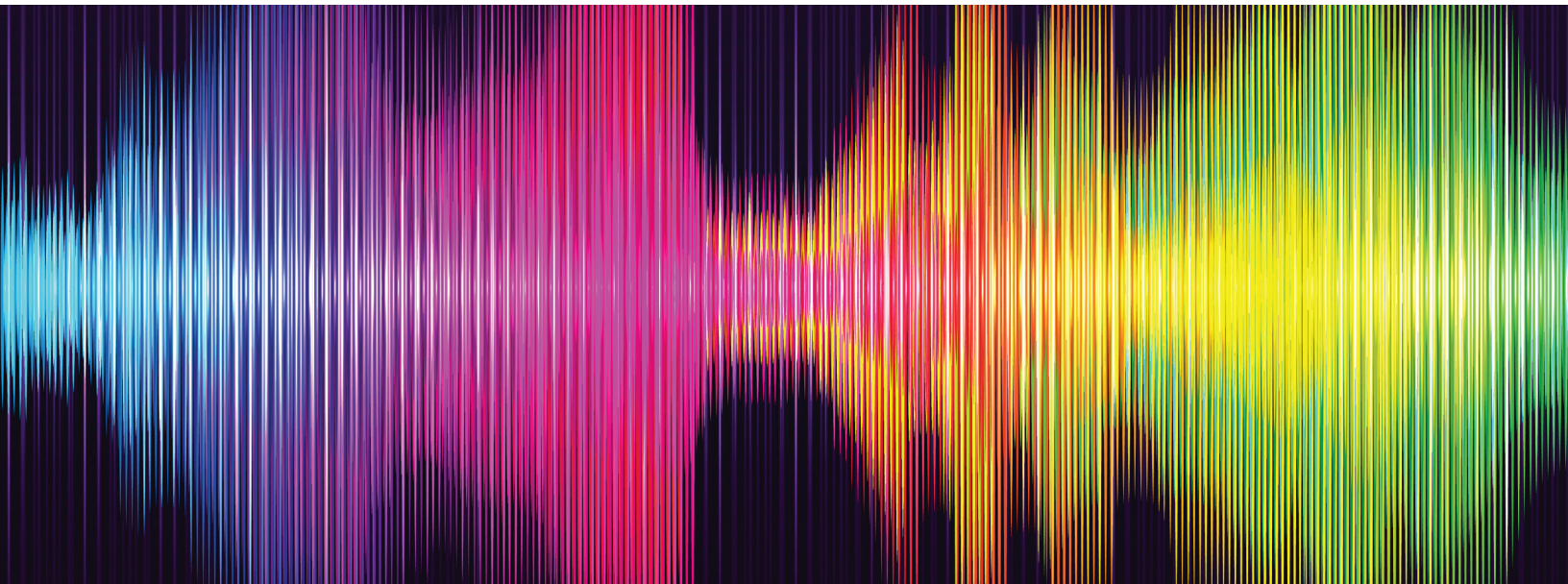
	Instrument A	Instrument B	Instrument C	Instrument D
Fluorescence plot 1				
Fluorescence plot 2				
Fluorescence plot 3				
Fluorescence plot 4				

Exercise 13: Determine number of peaks and resolution

Directions: Document the number of peaks and resolution quality from each channel on each instrument.

			Instrument A		Instrument B		Instrument C		Instrument D	
Fluorescence sensitivity	Channel	1	No. of peaks		No. of peaks		No. of peaks		No. of peaks	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
			Resolution quality		Resolution quality		Resolution quality		Resolution quality	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
		2	No. of peaks		No. of peaks		No. of peaks		No. of peaks	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
			Resolution quality		Resolution quality		Resolution quality		Resolution quality	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
		3	No. of peaks		No. of peaks		No. of peaks		No. of peaks	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
			Resolution quality		Resolution quality		Resolution quality		Resolution quality	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
		4	No. of peaks		No. of peaks		No. of peaks		No. of peaks	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
			Resolution quality		Resolution quality		Resolution quality		Resolution quality	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	

			Instrument A		Instrument B		Instrument C		Instrument D	
Fluorescence sensitivity	Channel	5	No. of peaks		No. of peaks		No. of peaks		No. of peaks	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
			Resolution quality		Resolution quality		Resolution quality		Resolution quality	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
		6	No. of peaks		No. of peaks		No. of peaks		No. of peaks	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	
			Resolution quality		Resolution quality		Resolution quality		Resolution quality	
			1		1		1		1	
			2		2		2		2	
			3		3		3		3	



Resource: Technical specification sheets

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.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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 thyrogen™ 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.

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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.

We have created a tip sheet on areas in which tech specs can differ, along with helpful hints about how to decipher the variations.

Download the tip sheet at **thermofisher.com/compareflow**

Testing a flow cytometer's ability to simultaneously detect multiple colors

Immunophenotyping is the analysis of a heterogeneous cell sample to detect the presence of a certain type of cell or physical characteristics of a cell population. These cells are labeled with antibodies conjugated to fluorophores in order to identify expressed antigens.

The purpose of a multicolor immunophenotyping experiment during a demo is to look for strong signal separation for data clarity in each channel.

Multicolor immunophenotyping is a more complicated experiment for a flow cytometer to process because it involves every component of the instrument. Data will look different depending on the instrument's components. Fluidic elements of the system generate statistical data by affecting the number of single cells and coincidences (non-single cells) that pass through the laser. Optic lasers and filters will define the number of fluorochromes used in one experiment to identify cell populations. Software will dictate the ease of compensating for fluorophore emission spillover. For these reasons, it is recommended that you verify a multicolor immunophenotyping panel before a demo of an instrument.

The five-step process for multicolor immunophenotyping verification

Optimize a multicolor panel before the demo in order to objectively compare instruments as follows:

Step 1: Assign lasers and filters

- Confirming which emitted fluorophores will be detected is based on lasers and filters
- Most flow cytometers will have violet (405 nm), blue (488 nm), green (532 nm), yellow (561 nm), and red (637-640 nm) lasers
- Filter combinations will determine emitted wavelengths

Step 2: Verify multicolor panel

- The objective of this step is to verify a panel prior to demo instead of testing the panel of a demo instrument
- Verification should include:
  - Percentage of negative and positive cell markers
  - Dynamic fluorescence range required to detect cell populations
- During this step, also tighten any fluorescent spread by titrating antibody concentrations for minimal nonspecific binding

Step 3: Provide an overview of experiment

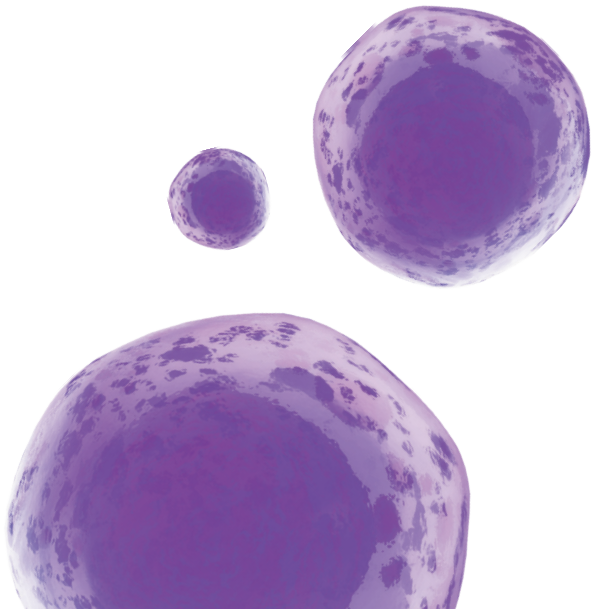
- Give a brief overview of:
  - Experimental protocol
  - Cell type(s)
  - Treatments
  - Cell concentration
- List fluorophores and identify markers with high, medium, and low expression
- Discuss anything problematic about the panel

Step 4: Prepare sample and controls

- Some fluorescent-labeled cells can be fixed the day before and stored at 4°C
- If preparing samples on the day of the demo, keep cells on ice or add DNase I/EDTA and filter to reduce aggregates
- Prepare both single-stained and fluorescence minus one (FMO) controls (the control staining should be less than the sample)
- Prepare enough sample for both tubes and plates
- If there is not enough sample for controls, run compensation beads

Step 5: Run experiment

- Collect experimental results as FCS files for comparison
- Document and compare the setup and shutdown procedures of each instrument (see Exercise 8b)
- Document processes for compensation setup, data collection, and file export for comparison



Recommendations for immunophenotyping experimental optimization and verification:

1. Clearly state what cell populations will be identified and what type of data needs to be collected.
2. List expressed markers to identify your cell population.
3. Identify which markers have high, medium, and low expression.
4. Select fluorophores based on available lasers and filter combinations (Table 1).
5. Assign highly expressed markers to dim fluorophores and markers with low expression to bright fluorophores.
6. Leave two channels open for viability dye and dump channel.
7. Titrate each antibody for the highest fluorescence signal with the lowest background in a sample population.
8. Run panel at least once on a flow cytometer with single-stained controls, negative control, and FMO control.

Table 1. Multicolor panel design. This example illustrates an 8-color panel designed for a 4-color laser system.

Laser	405		488		561		632	
Filter	**450/50**	**510/50**	**530/30**	**695/40**	**585/16**	**780/60**	**670/14**	**720/30**
Recommended dye	Super Bright 436 nm	eFluor 506 (or LIVE/DEAD Aqua)	FITC	PerCP-eFluor 710 or PerCP-5.5	PE	PE-Cy7 (streptavidin)	APC	Alexa Fluor 700
Emission	436 nm	506 nm (526 nm)	525 nm	710 nm	578 nm	785 nm	660 nm	719 nm
Compensation	None	FITC and PE	eFluor 506, PE, and PE-Cy7	APC and Alexa Fluor 700	eFluor 506, FITC, and PE-Cy7	eFluor 506, FITC, PE, and Alexa Fluor 700	PerCP-eFluor 710 or PerCP-5.5 and Alexa Fluor 700	APC and PerCP-eFluor 710 or PerCP-5.5

Exercise 14: Verify multicolor immunophenotype

Directions: Fill in details for each multicolor immunophenotyping experiment.

	Instrument A									
Multicolor immunophenotyping	Experiment name									
	Date									
	Notebook page									
	Overview									
	Cell information	Type								
		Size								
		Concentration								
	Analysis	Assay								
		Cell population								
		Modification								
	Sample	No. of tubes								
		No. of plate wells								
	Instrument	Lasers	405		488		561		632	
		Filters								
	Dye information	Recommended dye								
		Emission								
		Compensation								
	Sample information	Sample name in channel								
		Intracellular or surface staining								
		LIVE/DEAD channel								
		Dump channel								
	Controls	Single stain								
		FMO								

	Instrument B									
Multicolor immunophenotyping	Experiment name									
	Date									
	Notebook page									
	Overview									
	Cell information	Type								
		Size								
		Concentration								
	Analysis	Assay								
		Cell population								
		Modification								
	Sample	No. of tubes								
		No. of plate wells								
	Instrument	Lasers	405		488		561		632	
		Filters								
	Dye information	Recommended dye								
		Emission								
		Compensation								
	Sample information	Sample name in channel								
		Intracellular or surface staining								
		LIVE/DEAD channel								
		Dump channel								
	Controls	Single stain								
		FMO								

	Instrument C									
Multicolor immunophenotyping	Experiment name									
	Date									
	Notebook page									
	Overview									
	Cell information	Type								
		Size								
		Concentration								
	Analysis	Assay								
		Cell population								
		Modification								
	Sample	No. of tubes								
		No. of plate wells								
	Instrument	Lasers	405		488		561		632	
		Filters								
	Dye information	Recommended dye								
		Emission								
		Compensation								
	Sample information	Sample name in channel								
		Intracellular or surface staining								
		LIVE/DEAD channel								
		Dump channel								
	Controls	Single stain								
		FMO								

	Instrument D									
Multicolor immunophenotyping	Experiment name									
	Date									
	Notebook page									
	Overview									
	Cell information	Type								
		Size								
		Concentration								
	Analysis	Assay								
		Cell population								
		Modification								
	Sample	No. of tubes								
		No. of plate wells								
	Instrument	Lasers	405		488		561		632	
		Filters								
	Dye information	Recommended dye								
		Emission								
		Compensation								
	Sample information	Sample name in channel								
		Intracellular or surface staining								
		LIVE/DEAD channel								
		Dump channel								
	Controls	Single stain								
		FMO								

Define > Document > Determine > Decide

Exercise 15: Complete plot comparison grid

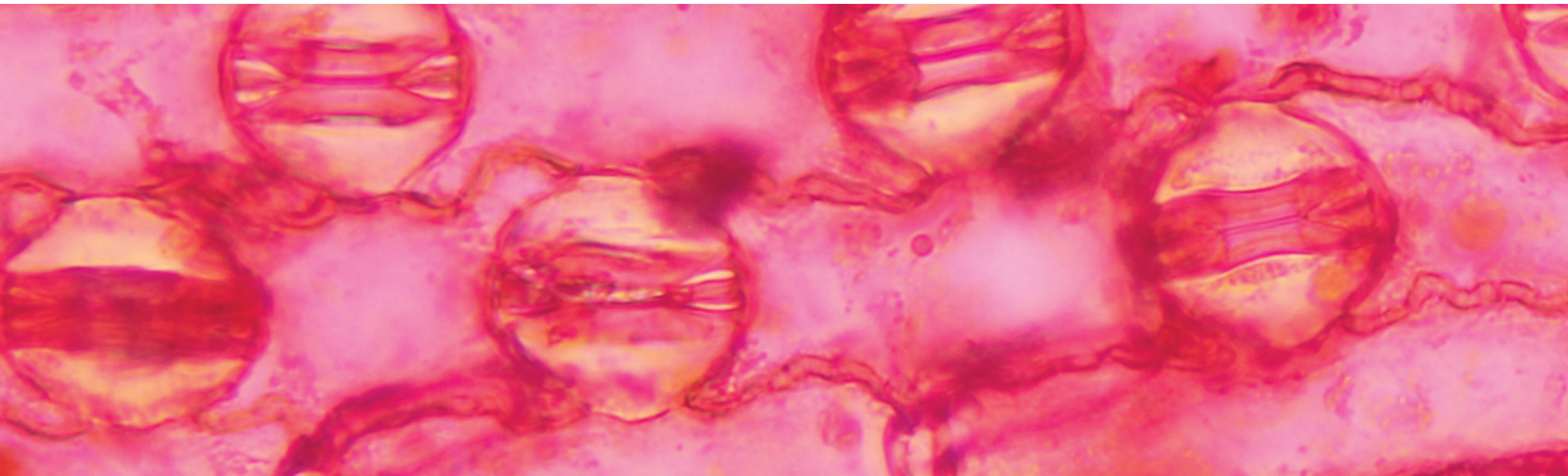
Directions: During each instrument demo, copy and paste plots from each instrument to facilitate side-by-side comparison. These clips may be placed within the exercise, or in a separate layout for direct comparison.

	Instrument A	Instrument B	Instrument C	Instrument D
Single gating plot				
Fluorescence plot 1				
Fluorescence plot 2				
Fluorescence plot 3				
Fluorescence plot 4				
Fluorescence plot 5				
Fluorescence plot 6				
Fluorescence plot 7				
Fluorescence plot 8				
Number of fluorophores per experiment				
Number of collected events				
Time required to process sample				

Determine the ability of a flow cytometer to process samples

Users of flow cytometers analyze not just mammalian cells, but also particles, small vesicles, plant cells, and microbes. Sample preparation is an important step, as the material to be analyzed should be in a solution. Wastewater, pond water, blood, beverages, and cell suspension may need minimal manipulation before analysis in some flow cytometers. Solid material such as tumors, food, or microbes on agar plates will need to be dissociated and filtered before being run in a flow cytometer.

The purpose of testing non-cell and microbial samples during a demo is to understand what types of samples can be processed by a particular flow cytometer. In addition, the fluidics system should be tested with different types of samples at different speeds.



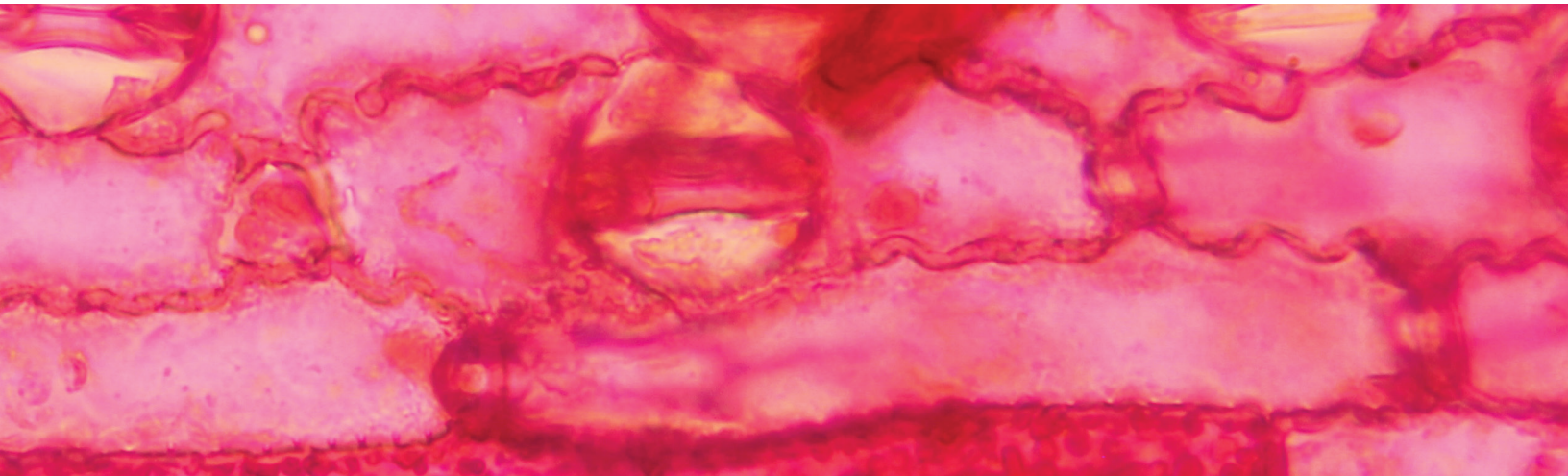
Define | Document | Determine | Decide

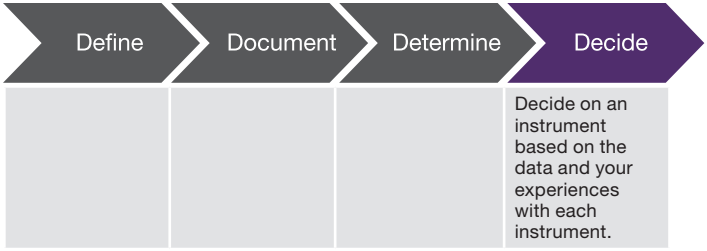
Exercise 16: Perform acquisition of dilute samples

Experiment:

1. Collect sample.
2. Prepare sample in both concentrated and dilute concentration.
3. Filter sample.
4. Prepare sample for both tubes and plates.
5. Collect and acquire events at three different speeds from slow to fast.

	Instrument A			Instrument B			Instrument C			Instrument D		
Sample type												
Condition of sample												
Acquisition speeds used	Rate 1	Rate 2	Rate 3	Rate 1	Rate 2	Rate 3	Rate 1	Rate 2	Rate 3	Rate 1	Rate 2	Rate 3
Number of collected events												
Time required to process sample												





Conclude: Make your decision based on the data and your experiences with each instrument

In the first step of the decision process, you defined your needs as researchers. Next, during the information-gathering phase, you documented information, specifications, and performance indications of each flow cytometer candidate. From among the gathered information, you then conducted your technical assessments of the candidate instruments. The final step of the decision process is to reach a consensus as a team and select an instrument.

This stage is about leveraging the work you've done thus far in this decision process:

- Performance data acquired
- Technical specifications gathered and comparison of calculations for specifications
- Mechanical assessments
- Insights and recommendations gathered from discussions with colleagues
- Application-specific considerations pertaining to addressing your biological focus

Now is the time to express your opinions, perceptions, and preferences as you arrive at a decision.

Exercise 17: Debrief

Meet as a decision team to debrief about the experience and share thoughts, opinions, and observations. Document quantitative and qualitative feedback from individuals who attended the demo. Along with the technical aspects of the demo, also collect assessments of the overall quality and organization of the demo itself, as these can indirectly reflect a company's intention and ability to support you for the life of the instrument.

Exercise 18: Determine subjective level of support vs. opposition rating scale

Directions: Based on your experiences with each candidate instrument, please cast your rating of each on a scale of 1–5, with one representing strong opposition to the instrument and five representing strong favor toward the instrument.

1 Strongly oppose — 2 Somewhat oppose — 3 Neutral — 4 Somewhat favor — 5 Strongly favor

	Instrument				Comments
	A	B	C	D	
User 1					
User 2					
User 3					
User 4					
User 5					
User 6					
User 7					
User 8					
User 9					
User 10					
User 11					
User 12					
User 13					
User 14					
User 15					

Exercise 19: Tally subjective scale scores

Directions: Tally the ratings from the subjective level of support vs. opposition rating scale (Exercise 18). First, count the number of marks for each response of the scale for each instrument. Next, add up all of the scores for each instrument.

In the example below, ten people were on the decision team, so there are ten scores of 1–5 for each instrument.

(Example)	Instrument A					Instrument B					Instrument C					Instrument D				
Response anchor count (how many people in the group gave the instrument a 1, 2, etc.)	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	2	1	1	4	2	2	3	3	1	1	0	1	2	3	4	1	6	3	0	0
Total for each anchor (anchor count times the number of votes)	2	2	3	16	10	2	6	9	4	5	0	2	6	12	20	1	12	9	0	0
Add together all scores for each instrument	33					26					40					22				
Average score (total score divided by the number of people on decision team who voted)	3.3					2.6					4.0					2.2				

Here is the blank form for your decision team to complete.

	Instrument A					Instrument B					Instrument C					Instrument D				
Response anchor count (how many people in the group gave the instrument a 1, 2, etc.)	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Total for each anchor (anchor count times the number of votes)																				
Add together all scores for each instrument																				
Average score (total score divided by the number of people on decision team who voted)																				

Define > Document > Determine > Decide

Exercise 20: Finalize decision

After all comparisons have concluded and all data have been gathered and organized, the decision team will go over the data and scores from the various candidate instruments and make a final choice. Reviewing the notes from various debriefing sessions, along with the other information, recommendations, and interactions with the instruments, will help your group to reach a decision.

Experiment and instrument help—for after your purchase and beyond

At Thermo Fisher Scientific, we understand that scientific advances happen in collaboration with others. No matter which instrument you ultimately select, we want you to succeed in your flow cytometry research; so we're here to provide the resources and tools to further your discoveries.

We provide not only aftermarket support and training for the instrument itself, but a suite of educational materials to further your science. We can help design workflows or panels, explain how to run samples through a flow cytometer, or troubleshoot experiments. The back pages of this workbook are filled with flow cytometry resources for you to access.

Flow Cytometry Resource Library

Expand your understanding with our curated collection of technical application notes, publications, videos, webinars, and scientific posters for flow cytometry.

- **Flow cytometry application notes, scientific posters, and *BioProbes* articles**—For various applications, detailing the conditions and reagents that were used to achieve the results; scientific posters presented by our R&D scientists at key conferences; and articles from *BioProbes Journal*
- **T Cell Stimulation and Proliferation eLearning Course**—A modular, animated, and narrated eLearning course on T cell activation and the methods used to measure T cell function; includes knowledge checks and a practical application session
- **Flow cytometry educational videos and webinars**—Media and learning tools for researchers interested in flow cytometry
- **Flow cytometry research tools**—Fluorescence SpectraViewer app, flow cytometry panel design tool, antibodies search tool, mobile apps, and more
- **Flow cytometry protocols**—Step-by-step instructions for successful fluorescence-based assays to measure cell proliferation, viability, and vitality using your flow cytometer
- ***The Molecular Probes Handbook: A Guide to Fluorescent Probes and Labeling Technologies***—Extensive references and technical notes; contains 3,000 technology solutions representing a wide range of biomolecular labeling and detection reagents

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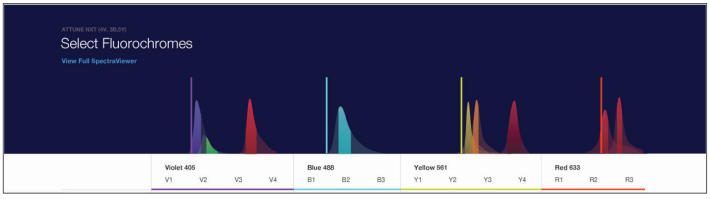

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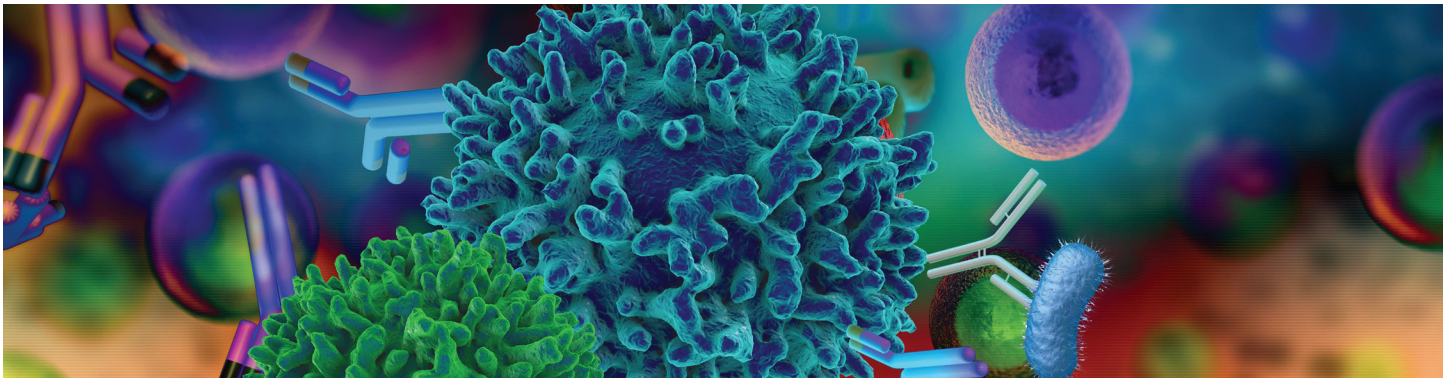
Molecular Probes School of Fluorescence

Flow cytometry basics

Learn how a flow cytometer works, including the fluidics, optics, and electronics. This is a free resource to help you get started with flow cytometry, which can be a complex and challenging application.

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



Flow Cytometry Learning Center

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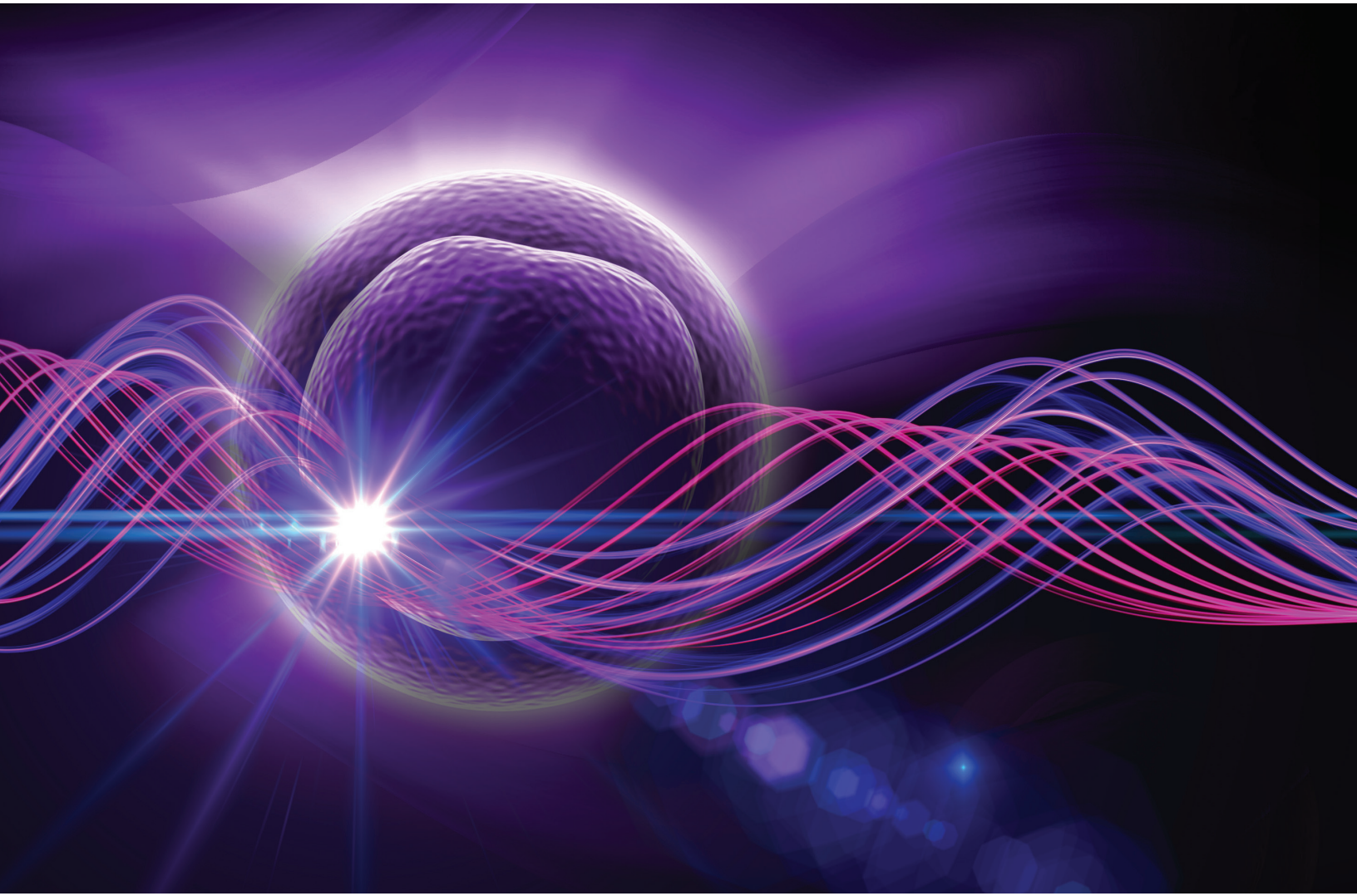

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