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Flow cytometer evaluation guide

Informed purchasing decisions through understanding experimental design and instrument capabilities

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Power in knowledge

Now more than ever and across vast areas of study, researchers are developing innovative applications for new areas of research, improving data quality, and expanding the boundaries of experimental limitations. These advances are enabled by increasingly sophisticated instruments and software—including in flow cytometry. Researcher demand is driving the evolution of instrumentation throughout the market, resulting in flow cytometers designed to fulfill the needs of different applications, sample types and volumes, cells of interest, and research goals.

Perhaps you have identified the need for a new instrument in your lab, want to increase day-to-day efficiency, or have become aware of new technology and advances in system design. Whatever the reasons motivating you to consider purchasing a flow cytometer, this guide will help you along the way with advice on comparing technical specifications, evaluating system hardware and software, and asking key questions.

This guide was developed with input from system engineers, flow cytometry core directors, and researchers with important perspectives for comparing instrument options. Whether you are a principal investigator or a core facility manager, an expert or new user, or comparing five different brands or two, this guide contains key insights to help you with your decision.

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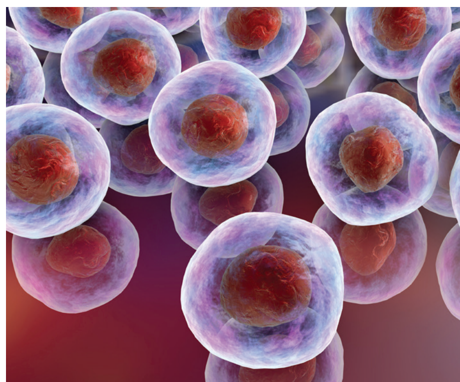

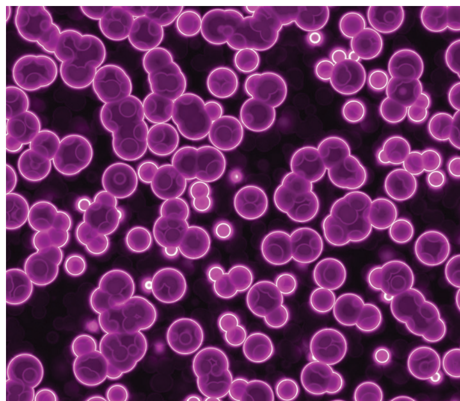

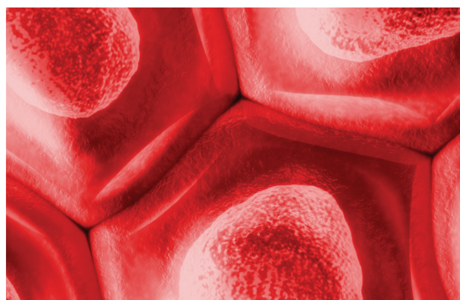

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Evaluate user needs when considering a flow cytometer

A one-size-fits-all approach is not the way to tackle science's big questions. Personalize the flow cytometer purchase by thinking about the needs of the laboratory. The first step is to identify users of the instrument and their preferences. The next step is to evaluate current experiments and determine what hardware features are required. Finally, plan for future needs by considering a flow cytometer that is configured to upgraded specifications. It is critical to understand the needs of the laboratory in order to select the best instrument to enable specific research projects.

Key evaluation steps

1

Flow cytometers are important instruments to researchers of all skill levels. Before purchasing an instrument, identify users and consider who will be maintaining the instrument. Determine if the instrument can guide basic users and fulfill the complex needs of advanced researchers.

Identify users

Evaluate applications

To fulfill the needs of the laboratory, carefully examine the hardware components and options available for purchase. Ask lab and core users about features and components they use on a flow cytometer. Consider the amount of time each user needs and the complexity of the experiments performed.

2

3

Think about the future. Consider the expansion of research areas and the growth in user expertise. Understand if the instrument can support your research as it progresses. Some flow cytometers are upgradable from a basic model. Understand the future options or upgrades that are available to add experimental value to your instrument.

Plan for future needs

Finding the fit

Finding the fit

Flow cytometers are becoming a more common piece of laboratory equipment. These instruments operate using similar processes (Figure 1), but no two models are alike, as each has different features and functions. There is a range of flow cytometers spanning different price points and needs of a laboratory, while covering a variety of research applications. While reading this guide, think about instrument capabilities and components that best address and satisfy the needs of users.

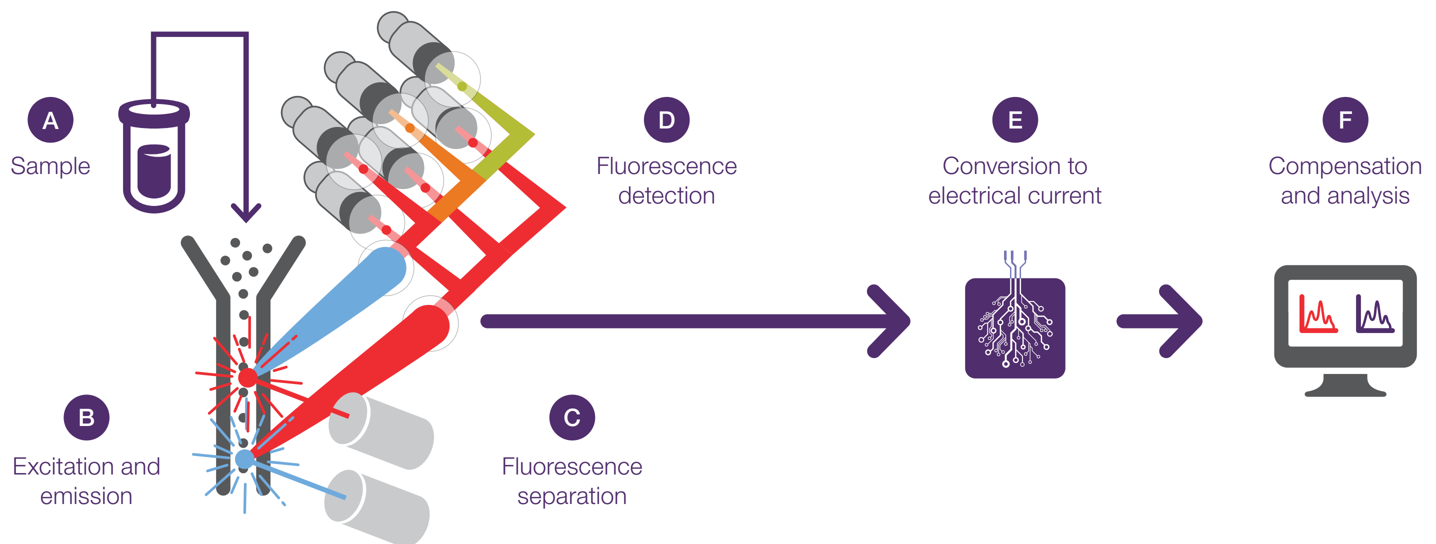


Figure 1. Flow cytometer overview. (A) Sample enters into the flow cytometer. A process called hydrodynamic focusing directs the sample into a narrow stream with the purpose of moving one cell at a time into the optical system. **(B)** An individual cell passes through the interrogation point in the optical system. A fluorophore-labeled cell will be excited by a laser and emit a fluorescence signal. **(C)** The emitted signal will be guided by fluorescence separation. **(D)** Emitted light is captured by detectors. **(E)** The light signal is converted to an electrical current by the detector. **(F)** Electrical signals can then be processed as data.

Fluidics

Fluidics

Flow cytometry analyzes cells or other particles suspended in fluid for physical properties. The flow of cells or particles gives a distinct advantage over other methods, such as imaging and PCR, by collecting information from single cells at a statistical relevance in a heterogeneous sample. Sophisticated technology is required for a sample to enter the instrument and be delivered to the optical system.

Fluidics delivery systems

The power of flow cytometry is the ability to quantitate the physical characteristics of individual cells in a heterogeneous population. This process requires the fluidics system to deliver large numbers of cells into the flow cytometer in order to produce a statistically significant amount of data. The flow of cells into and through the instrument must be consistent for accurate counting and measurements. Pressure-based and volumetrics-based fluidics are two common mechanisms for cells to enter into the flow cytometer.

Samples with large volumes of cells can quickly enter the instrument with pressure-based fluidics. A pressurized system is created by a tube forming a seal with the instrument. The sealed tube initiates a difference between the pressure of the sample and that of the instrument sheath fluid to move cells into the optical system (Figure 2A). Pressure-based systems allow for smooth sample delivery, larger sample volume, and less risk of running out of sample.

Volumetrics-based fluidics enable more flexible cell delivery. The mechanism for this system measures a precise volume of sample and injects it into the instrument (Figure 2B). Benefits include the ability to determine absolute cell count and accommodate difficult samples, including sticky cells or large cells, by being less sensitive to clogging from back pressure. This system is flexible, being able to work with many tubes or plates since it is a nonpressurized system.

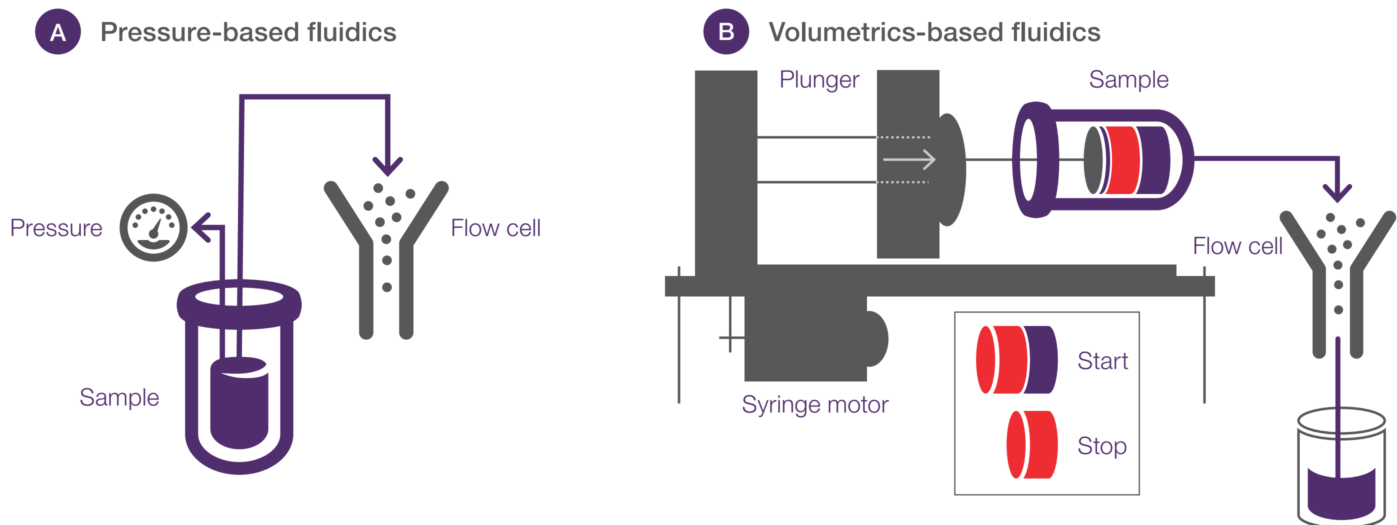


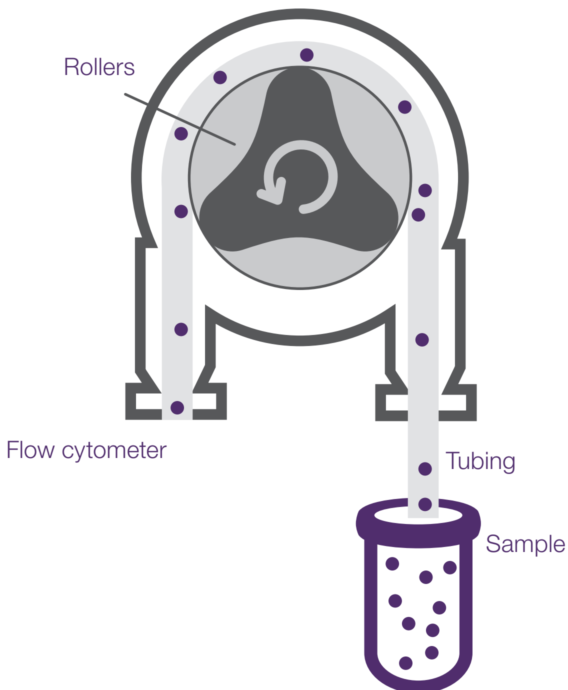
Figure 2. Systems for sample entry into the flow cytometer. (A) Pressure-based fluidics rely on the pressure difference between the sample fluid stream and the sheath fluid surrounding the sample stream. **(B)** Volumetrics-based fluidics provide the ability to obtain concentration and count measurements, since the exact sample volume can be measured.

Sample delivery in pressure- and volumetrics-based systems is accomplished using either peristaltic or syringe pumps.

Peristaltic pumps are a versatile, low-cost alternative for less expensive instruments (Figure 3A). Fluidics systems equipped with these pumps employ a series of rotating rollers to press on the tubing. This type of system can quickly move large sample volumes.

Volumetric delivery systems require a syringe pump in order to precisely control and measure sample volume (Figure 3B). Syringe pumps provide the mechanism for absolute cell counting and sample recovery. The returned sample can be used for other experiments such as PCR and imaging, or be stored to run again.

A Peristaltic pump



B Syringe pump

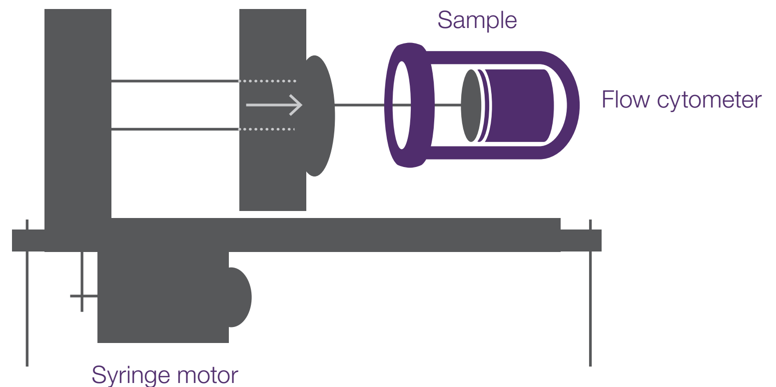


Figure 3. Two types of pumps. (A) A peristaltic pump moves fluids using a rotating-head system. The rollers in the head depress the tubing and drive the fluid forward. **(B)** A syringe pump draws up a sample and injects it into the fluidics system.

Solve flow cytometry problems

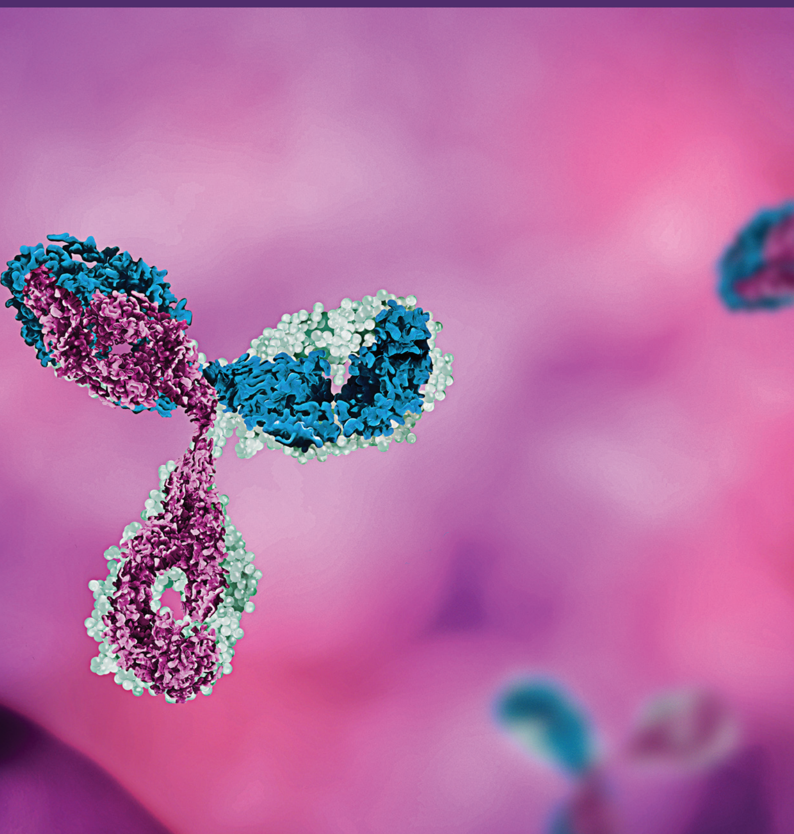
Problem:

Tumor samples are characterized for cancer cells, B cells, T cells, and other immune cells with flow cytometry. Tumor samples have a greater probability to clog tubing and other components. These samples require tissue dissociation into single cells. This process can lead to dead cells releasing DNA and creating cell clumps.

How can you run tumor samples without clogging a flow cytometer?

Solution:

- Use an instrument with syringe-driven delivery, which minimizes back pressure issues that can lead to clogging
- Dilute the sample
- Limit the time spent preparing cells for flow cytometry. Optimize experiments by:
  - Minimizing incubation times
  - Adding EDTA to the sample
  - Adding DNase and RNase to the sample
  - Passing all samples through a 40 µm cell strainer prior to the run
- Mechanically dissociate cells by pipetting up and down, and vortex samples before placing the tube in the flow cytometer



Sample handling

The number of samples processed is heavily influenced by the type of fluidics system in the flow cytometer.

Pressure-based systems use tubes and mostly process one sample at a time. For high-throughput applications, these flow cytometers can be equipped with a separate multiwell sampler. Multiwell plate sampling may not be available in some models, as specialized tubes are required to create the pressure differential for samples to enter into the flow cytometer.

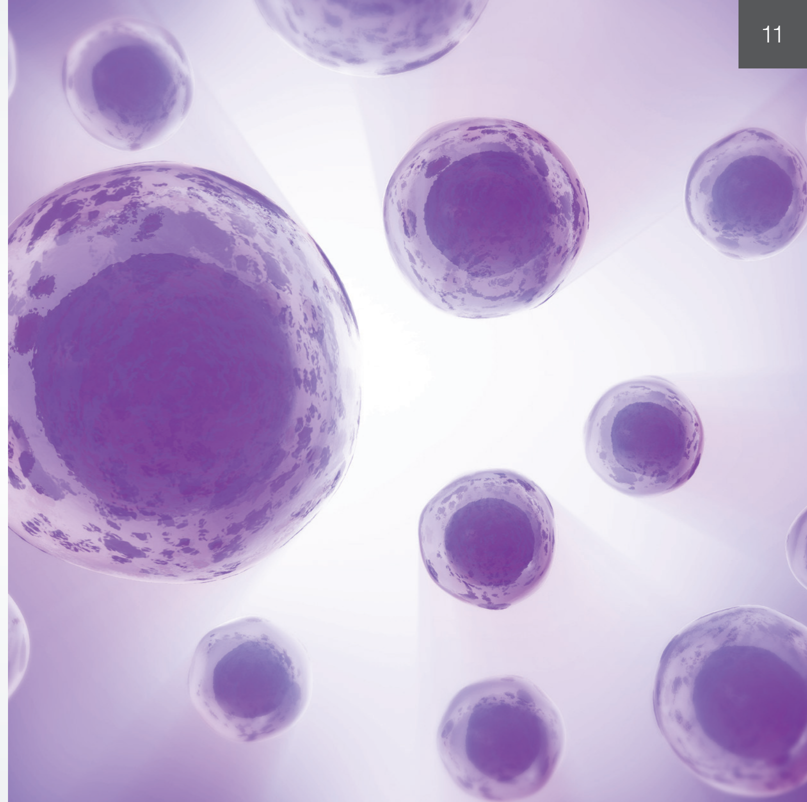
Some volumetrics-based flow cytometers have the capability to sample from both tubes and multiwell plates. The pipette-like syringe both mixes and injects the sample into the instrument.

Plate samplers can be attached to robotic handlers for high-throughput processing of many multiwell plates. These systems manage high sample volume through the integration of an external fluid supply to extend continuous run time for multiple plates.

Cell focusing systems

Cells enter into the flow cytometer in a misaligned, erratic fashion. Cells ideally flow past the interrogation laser in single file for individual detection, but there are some instances where the cells flow through together (as governed by Poisson distribution or samples with cell clumps). When there is more than one cell in the laser path at the same time, this is called coincidence.

Two cells simultaneously passing through the laser can lead to difficulty in distinguishing the cells of interest. If one cell is positive and the other is negative, and they both pass together through the laser, the instrument will read the event as a positive signal (Figure 4). When this occurs, the data obtained from this event is a doublet event and not usable. The technology behind the focusing system provides the mechanism for single-cell interrogation in the optical system.



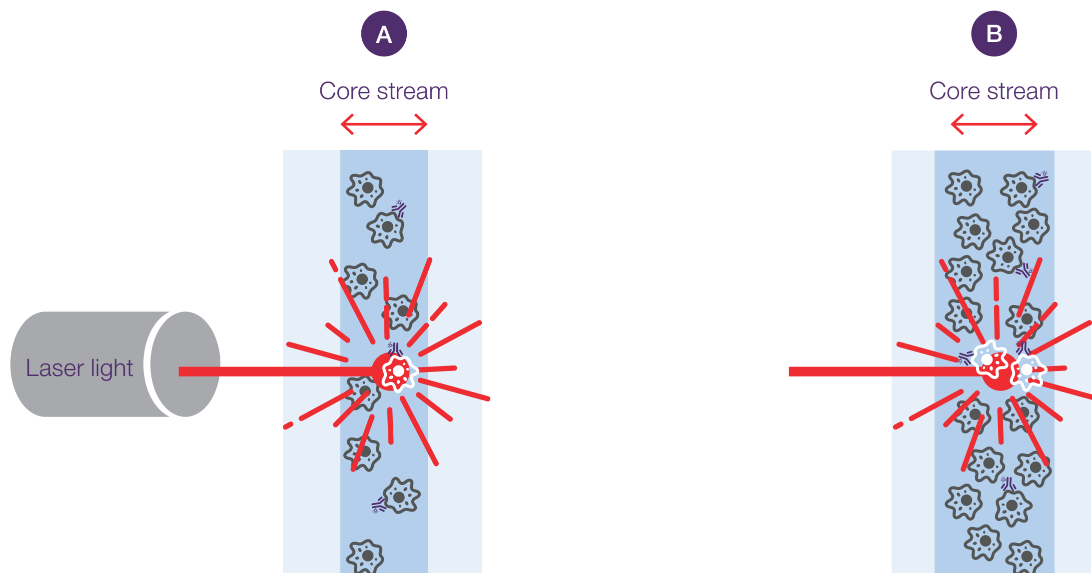


Figure 4. Coincidence rate. (A) One cell is excited by the laser beam. **(B)** Two cells are excited by the laser beam producing a doublet signal. Cells in doublets and triplets can mask the true biological nature of a sample.

Single cells flowing through the optical system can be obtained using different fluidics-based methods. Most flow cytometers use traditional fluidic focusing technology such as hydrodynamic focusing (Figure 5A). This technology uses the difference in pressure and speed between the sheath fluid and cell sample to move a cell forward.

Other fluidic systems use acoustics-assisted hydrodynamic focusing. This focusing method is a modification of the traditional system, using sound waves to focus cells into a core and traditional hydrodynamics to deliver the cells to the optical system (Figure 5B). Acoustics-assisted hydrodynamic focusing reduces fluid usage while maintaining the benefits of hydrodynamic focusing. The combined use of acoustic focusing and sheath fluid aligns cells into a single file, resulting in some experiments running at quicker speeds with lower coincidence rates. The small amount of sheath fluid has an added benefit of keeping the flow cell clean and less prone to clogging.

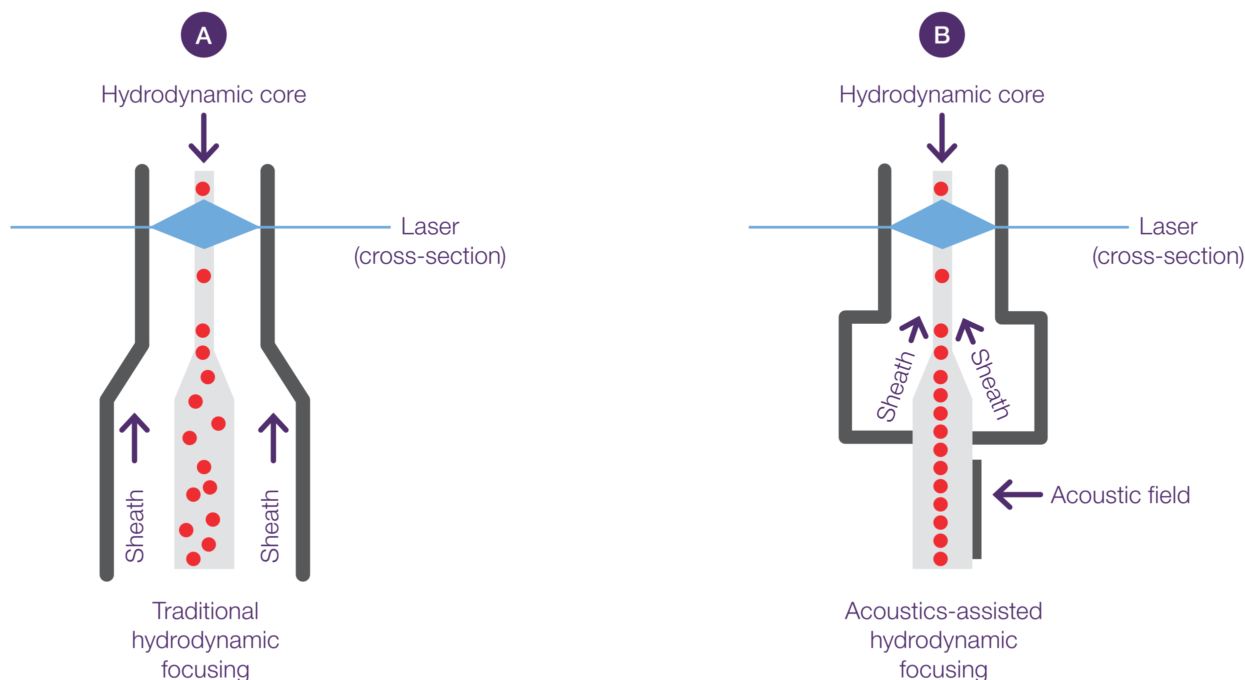


Figure 5. Cell focusing systems. (A) Hydrodynamic systems use the pressure of the sheath fluid and sample to guide the mixture of cells into a single stream. **(B)** Acoustics-assisted hydrodynamic systems use ultrasonic sound waves to gently prefocus the cells, followed by traditional hydrodynamic focusing.

Solve flow cytometry problems

Problem:

Platelets play a large role in homeostasis. These cells are sensitive to their environment, and their secretion factors will change upon manipulation. Traditional ways to detect platelets include lysing whole blood and then analyzing cells on a flow cytometer. This process is time-consuming and can compromise the platelet function.

What can be experimentally changed to understand the unmanipulated secretion factors produced by platelets?

Solution:

- Minimize platelet handling with no-wash, no-lyse sample preparation protocols and kits
- Dilute whole-blood samples to reduce coincidence and clogging
- Reduce run time and increase sample acquisition speed with acoustics-assisted hydrodynamic focusing; acoustic technology uses a 2.5 MHz frequency to align cells while protecting from cell degradation

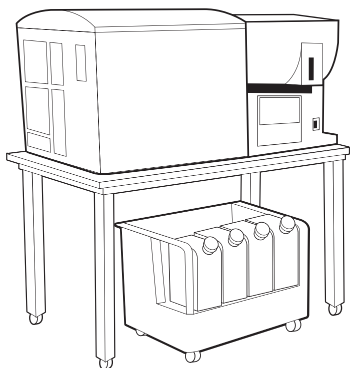
Fluid storage

A typical flow cytometer requires a series of tanks to hold each kind of fluid including focusing solutions, cleaning fluid, and liquid waste. The size of each tank varies; some units are small and may be contained within the instrument itself, while others are large and must be external units placed on the floor or require additional bench space.

A typical external tank has a capacity of 4 L or more (Figure 6A). These tanks are required for optimal performance of instruments equipped with pressure-based fluidics. Flow cytometers with external tanks require additional space and may be kept next to the flow cytometer or beneath the bench.

Environmentally friendly fluid tanks are smaller in size and range from 8 mL to 1.8 L. Typically, instruments with volumetrics-based fluidics are equipped with smaller tanks, as they use less fluid and generate less waste. Flow cytometers with internal tanks have a smaller footprint and can be held within a biosafety cabinet (Figure 6B).

A



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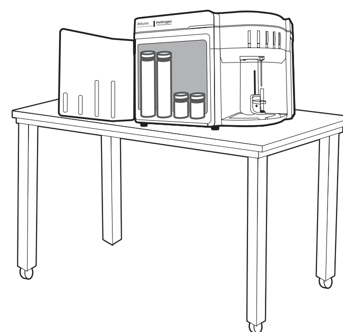


Figure 6. External and internal fluid storage. (A) External fluid storage presents a series of external tanks holding waste, wash, sheath, and shutdown fluids. **(B)** Environmentally friendly internal storage offers a series of smaller tanks held within the instrument.

Optics

Optics

The optical system requires the coordination of many components to generate fluorescence signals from a single cell. Understanding the options that create the optical system can help provide optimal data.

Lasers

Commercially available flow cytometers may include violet (405 nm), blue (488 nm), green (532 nm), yellow (561 nm), and red (637–640 nm) lasers (Figure 7). Base instruments typically include the blue 488 nm laser. Adding more lasers provides the option to increase the number of fluorophores that may be used in one experiment. Violet, blue, yellow-green, and red lasers are sufficient to excite most fluorophores used in multifluorophore experiments. Some flow cytometers can be equipped with a UV laser in the 350 nm range to spread out the excitation and emission wavelengths when designing multicolor experiments.

Laser tips

If funding is tight, consider upgrading the number of lasers after purchase of a flow cytometer. Some instruments are configurable, and you can add more lasers and color channels when funds are available.

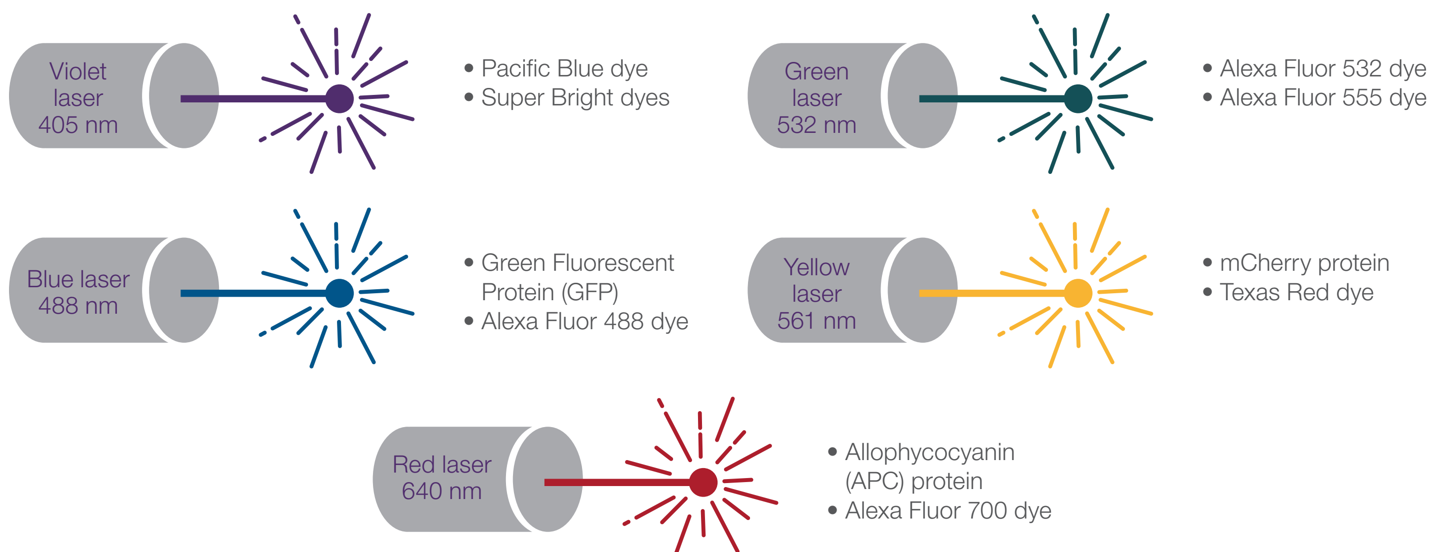


Figure 7. Laser wavelengths with common fluorescent proteins and selected dyes.

Each fluorophore has an excitation energy range and a specific light-emitting spectrum. Cells can be labeled with multiple fluorescently conjugated antibodies, fluorescent dyes, and fluorescent proteins. Lasers emit power at specific wavelengths to provide the different amounts of required excitation energy (Figure 8).

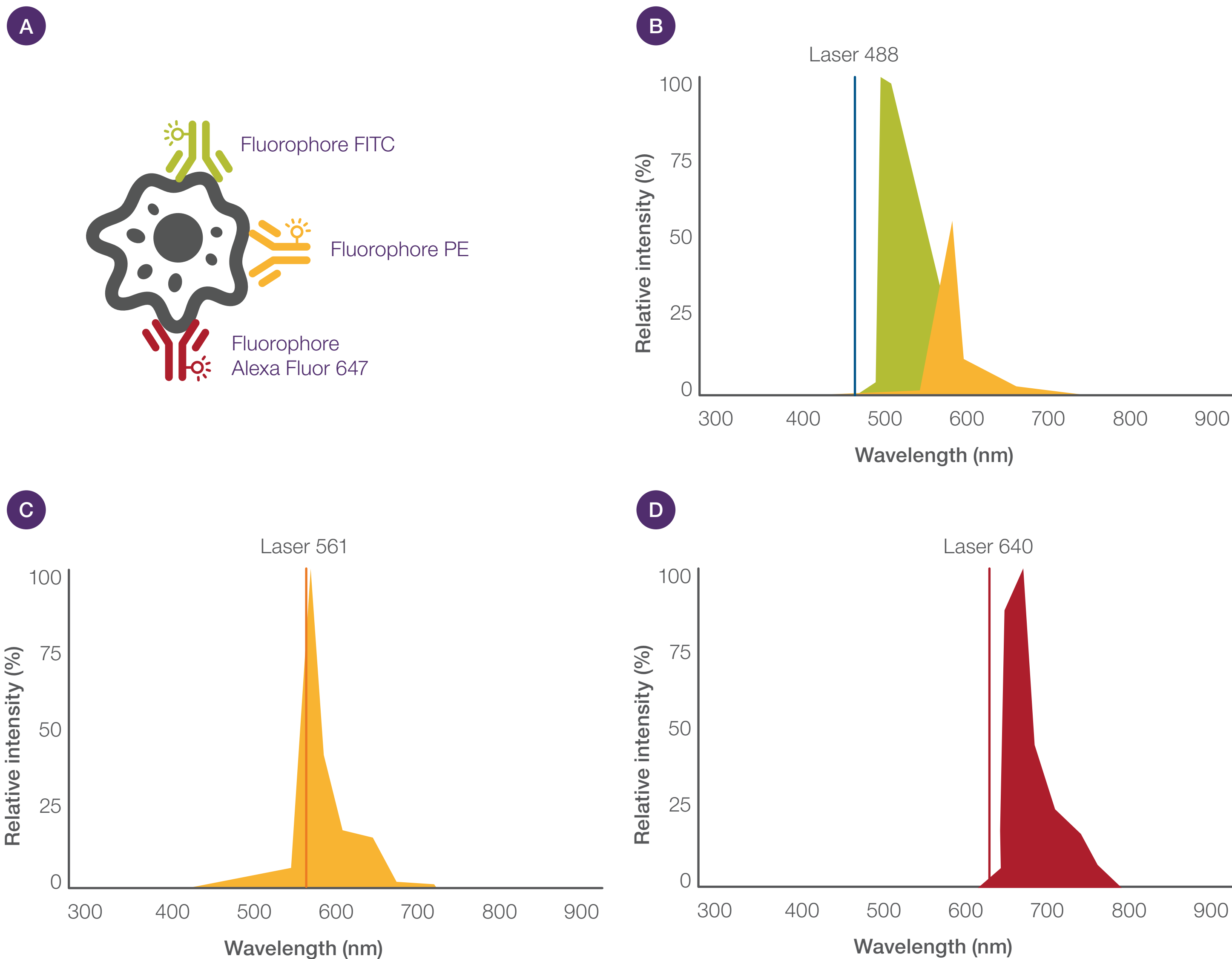


Figure 8. Laser excitation and emission wavelengths of fluorescent dyes. (A) Cell with multiple fluorescent labels. **(B)** PE and FITC are both excited by the blue 488 nm laser and have overlapping emission spectrums. **(C)** PE is excited by the yellow 561 nm laser, but FITC is not. **(D)** Alexa Fluor™ 647 dye is excited by the red 640 nm laser.

Some fluorophores have multiple excitation wavelengths of different efficiencies. For example, either a blue (488 nm) or yellow (561 nm) laser can excite the fluorophore PE. A blue 488 nm laser excites PE (Figure 9A) at a lower energy level. A yellow 561 nm laser provides more excitation to the fluorophore and results in a greater amount of emitted photons (Figure 9B). Stronger emitted light may resolve a small, dim population of cells that might not have been seen on an instrument without a 561 nm laser.

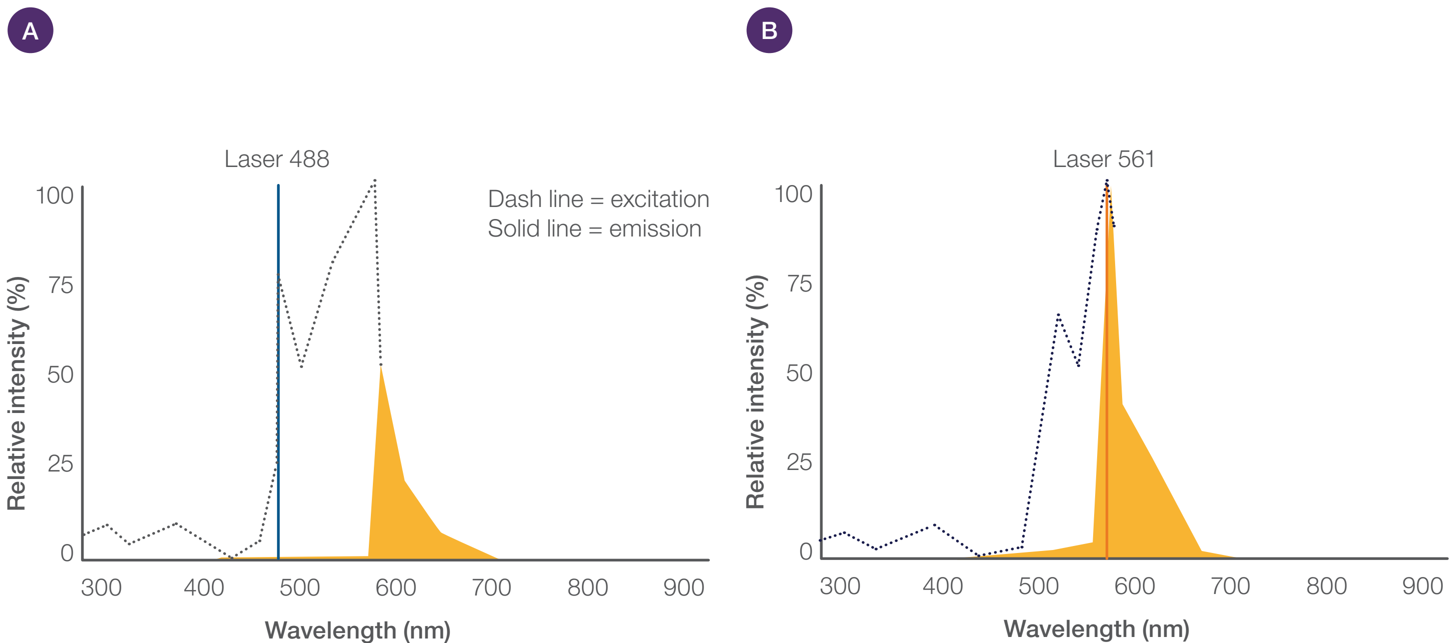


Figure 9. Double excitation maximums. (A) PE can be excited with the 488 nm laser. **(B)** PE has another excitation maximum at 561 nm. Excitation of PE by this laser more efficiently emits fluorescence.

Laser power

Laser beams produce energy required to excite fluorescent dyes, proteins, and conjugated antibodies used in multiparametric detection and phenotyping of cells.

The amount of laser power can affect the fluorescence detected from an experiment. If the laser power is too low, a fluorophore-labeled cell with low antigen expression may not emit all of the potential signal (Figure 10). Optimal power is required for full characterization of protein expression. This is important because some experiments performed to characterize certain cell types use the amount of fluorescence signal as a positive or negative indicator.

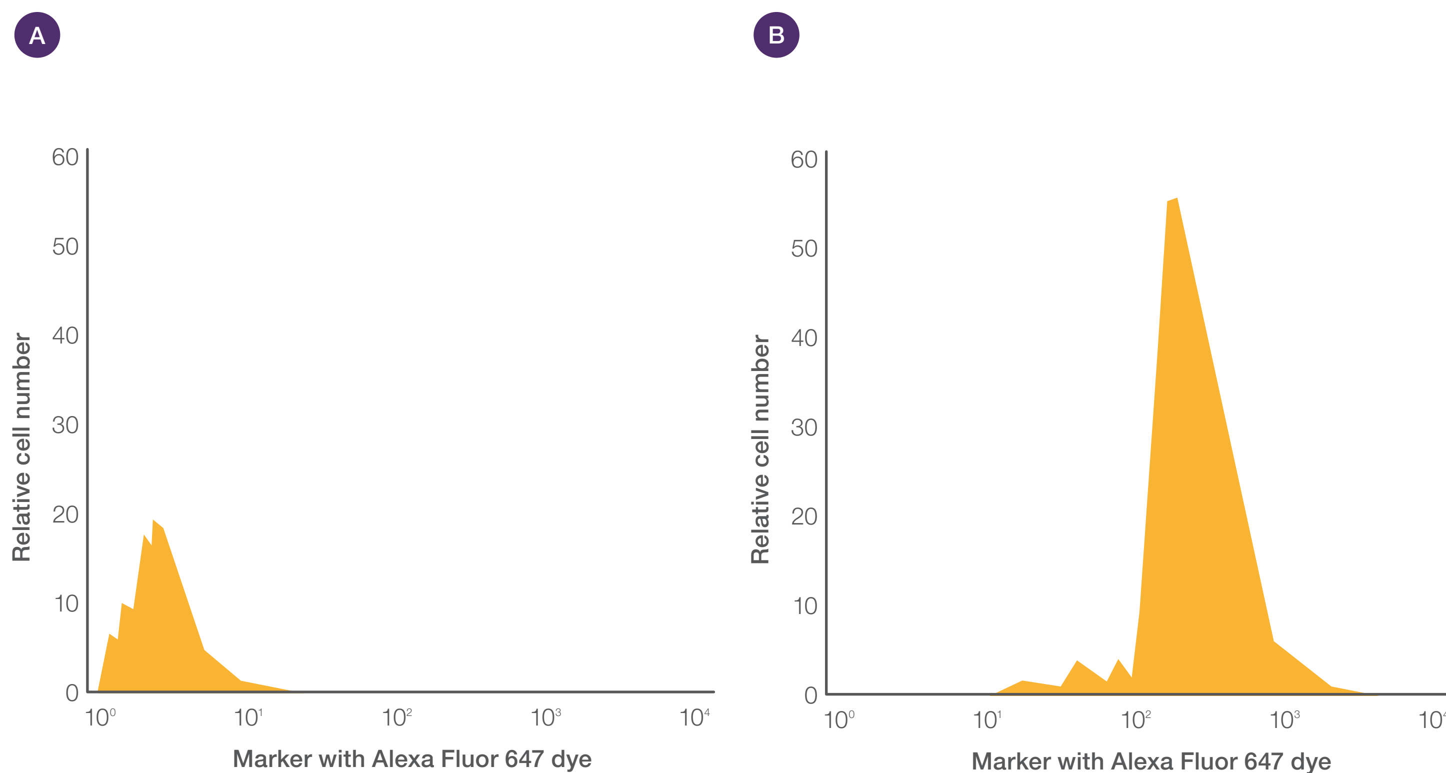


Figure 10. Laser power affects the results. (A) Suboptimal laser power can lead to inaccurately low fluorescence signal and a false result. **(B)** Stronger laser power results in a greater fluorescence signal, enabling detection of the labeled protein. This leads to a more accurate result.

Laser profile

Every cell should receive similar laser exposure in order to enable comparison of emitted fluorescence signals between cells in an experiment. Consistent laser beam output with correct laser alignment helps provide an optimal signal to every cell that passes through the interrogation point (Figure 11A). Well-aligned laser beams result in data produced by biological, not instrument, variation.

In samples with weakly labeled cells, suboptimal excitation energy can lead to ambiguous results. When laser power is not consistent, there will be more variation between collected emissions from cells, resulting in greater statistical error. Shifts in laser alignment may provide less or unequal excitation energy to each cell passing through the beam (Figure 11B).

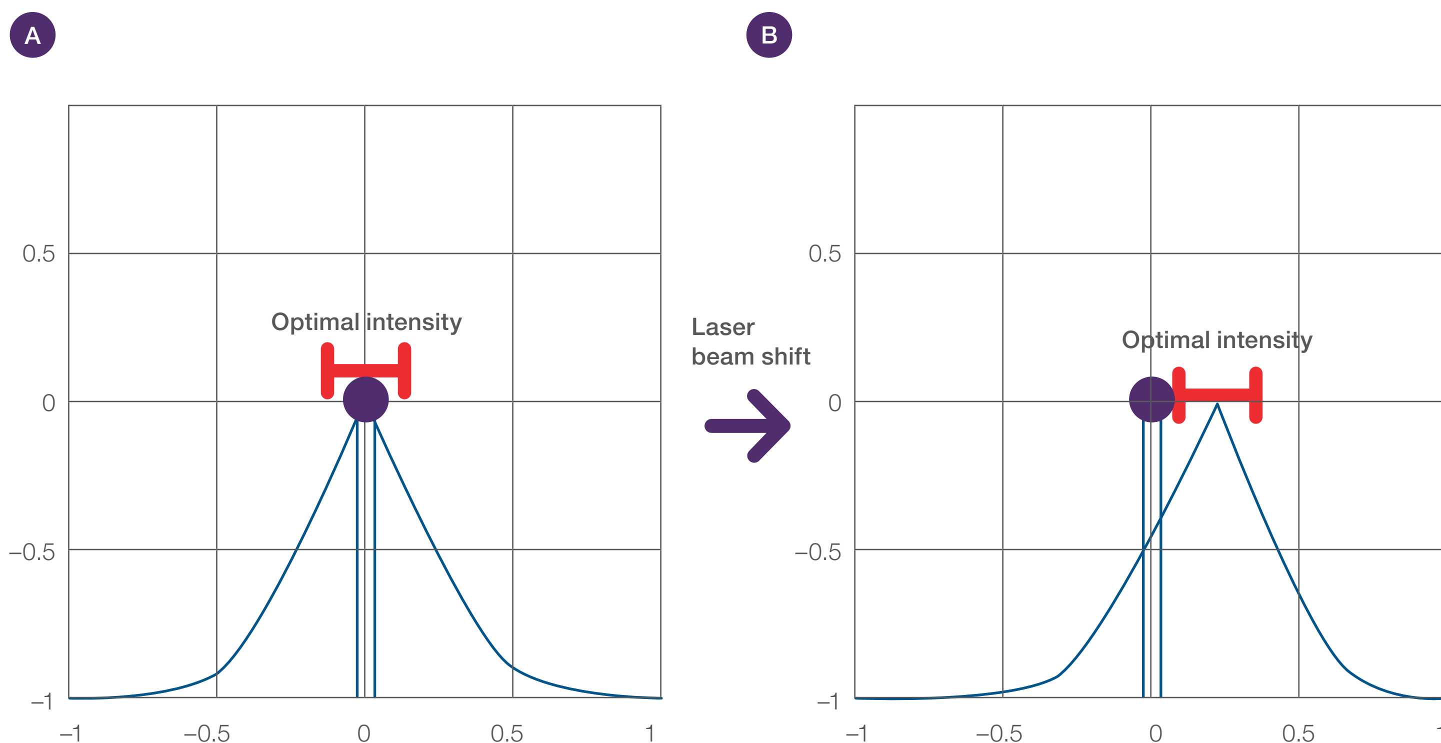


Figure 11. Laser alignment. (A) Cells receive full laser power with properly aligned lasers. This ensures each cell will have similar energy for fluorophore excitation, providing less data variation. **(B)** Laser misalignment may cause uneven distribution of energy for fluorophore excitation. This may increase the chances for data variation.

Gaussian laser beams are found in most flow cytometers (Figure 12A). Cells must pass through the center of the beam for optimal excitation energy.

Recently developed flat-top laser profiles cover more area to excite fluorophores (Figure 12B). The wider apex allows cells in a sample to have an even application of energy to help produce consistent results.

Solve flow cytometry problems

Problem:

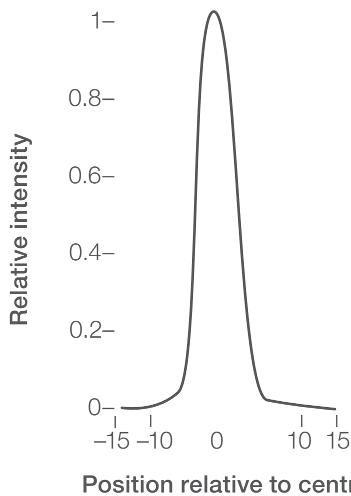
Lasers are a major factor in the total cost of an instrument. Shared equipment like flow cytometers often have multiple users; accidents such as leaving the lasers on overnight or longer are prone to occur. This will decrease the amount of usable time on a laser and add additional costs in requiring laser replacement.

What are the options to extend laser life?

Solution:

- Life span can be maintained by limiting full power of the laser when not in use; look for features such as simmer mode, which holds the laser in a standby state and only applies full power when analyzing a sample
- Quality lasers will have a long life span, determined by the number of hours of usable laser power

A Gaussian intensity profile at focus



B Flat-top intensity profile at focus

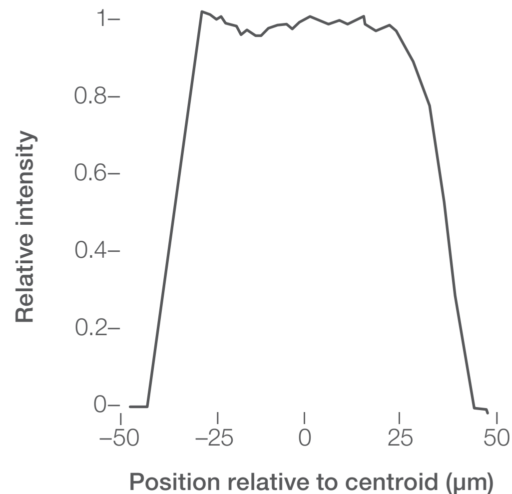


Figure 12. Laser profiles. (A) In a Gaussian emission profile, photons are dense and most intense in the center. **(B)** A flat-top beam intensity profile covers a larger and flatter area. Cells pass through a wider apex to emit fluorescence.

Laser arrangement

Multiple lasers in one instrument are most commonly arranged in two formations. The arrangement affects both the number of fluorophores used in an experiment and the detection of cell fluorescence.

Collinear spatial arrangement fires all lasers at the same time to focus laser light onto one area, resulting in the simultaneous excitation of many different fluorescent dyes (Figure 13A). This laser arrangement is less flexible in multicolor panel building, as multiple fluorescence signals are simultaneously emitted and require greater compensation. A collinear laser arrangement works well with lasers of two different wavelengths that excite fluorescent dyes with minimally overlapping or nonoverlapping emission spectra.

A spatially separated arrangement sequentially fires lasers on the cell stream (Figure 13B). Cells stained with multiple fluorophores pass through each laser beam one at a time for separate excitation and detection of emission spectra. This type of arrangement provides flexibility for the number of dyes used in one experiment. The individually emitted fluorescence provides higher sensitivity and requires less compensation. Large multicolor panels can be more readily built using this arrangement, as it generates significantly less fluorescence overlap in similar emission spectra.

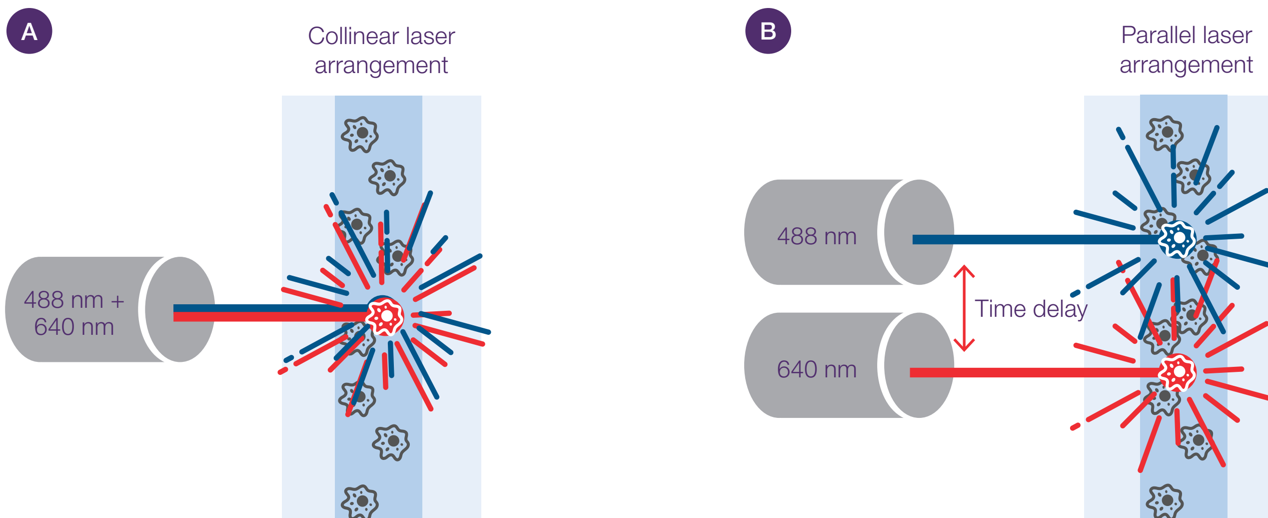


Figure 13. Laser arrangement. (A) Collinear laser arrangements have multiple lasers arranged to focus on a single spot of the interrogation point. **(B)** Spatially separated lasers are individually arranged and focused along the interrogation point.

Fluorescence separation

Excited fluorophores emit light in a limited spectrum, not at a specific wavelength (Figure 14A). Emitted fluorescence needs to be passed through optical filters before it is captured and analyzed. Without fluorescence separation, the unfiltered light from multiple fluorophores would overlap and be difficult to analyze (Figure 14B).

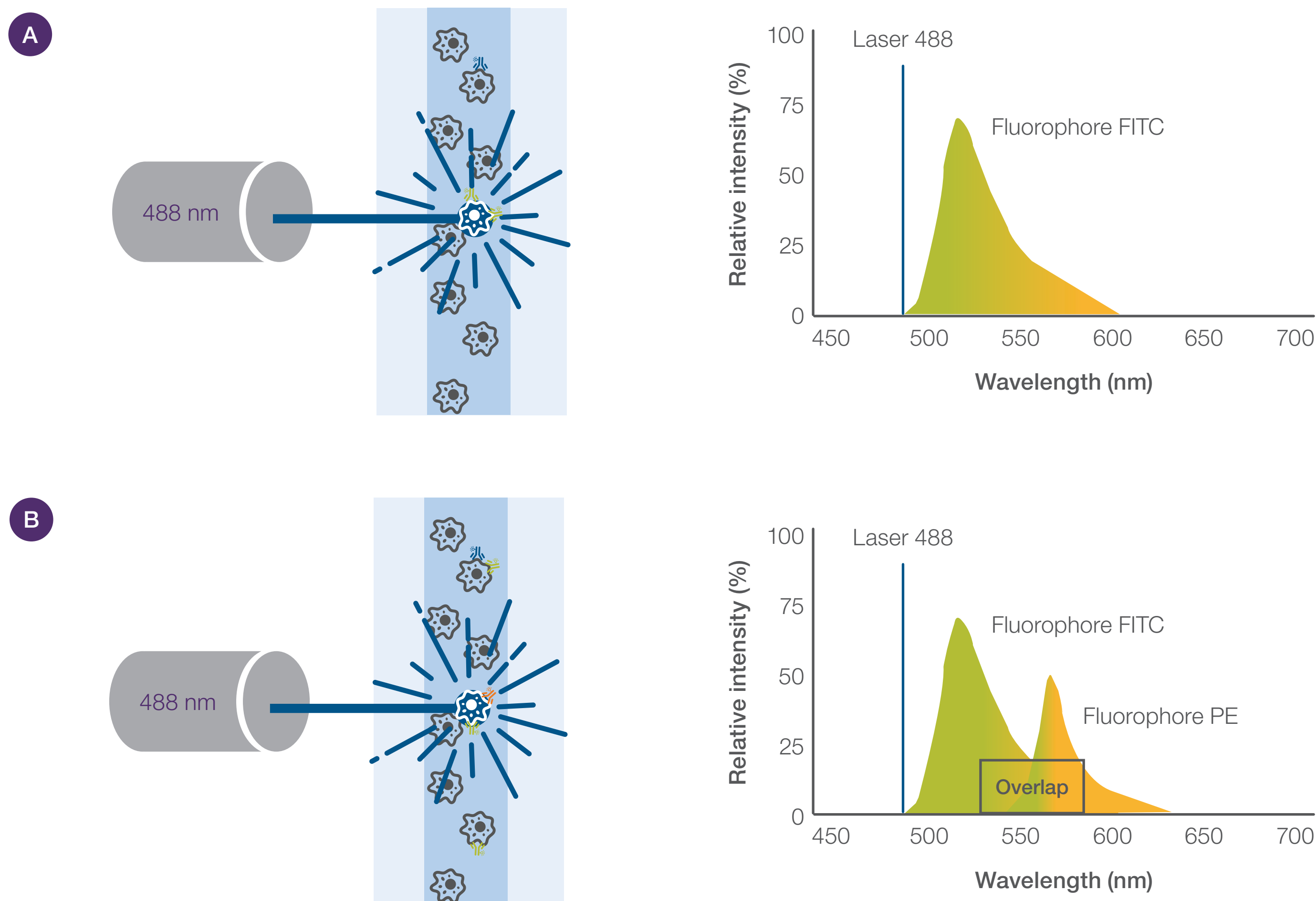


Figure 14. Fluorescence emission. (A) Excited fluorophores emit light in a limited range of wavelengths. **(B)** Some fluorophores can have overlapping emission spectra.

Analysis of cells labeled with multiple fluorophore-conjugated antibodies is enabled by optical filters (Figure 15A). These light separation components are the most commonly used fluorescence separation components in flow cytometers, as they can easily be accessed and changed by the user. Combining different filters allows varying color combinations to create both small and large multicolor panels. Look for a system with easily changeable filters.

Spectral flow cytometry is different from conventional flow cytometry. Instruments with this technology use prisms to focus emitted fluorescence in the visible light spectrum (Figure 15B). Light emitted from multiple fluorophores will overlap, and requires a second step with complex algorithms for separation. The benefit of this technology is that it expands the total number of color channels from fewer lasers.

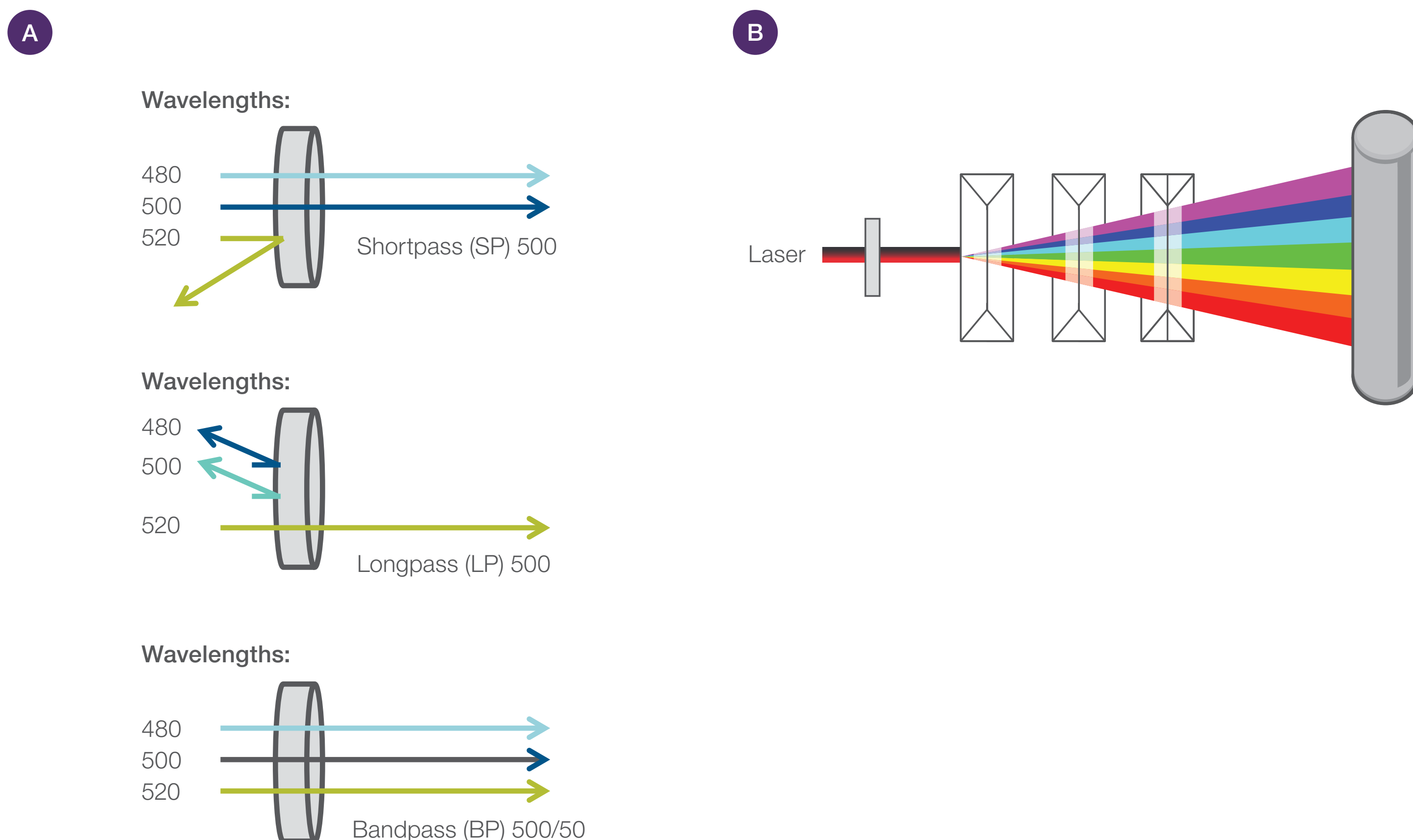


Figure 15. Components for fluorescence separation. (A) Optical filters divide the emitted signal from fluorophores based on the fluorescence wavelength range. **(B)** Prism monochromator arrays separate light into regions of different wavelengths and focus light from these regions onto different detectors.

Emission detectors

The emitted fluorescence from a single cell is a weak signal. The light needs to be converted into an adjustable form of data for analysis. An emission detector captures fluorescence and converts the light waves into an electronic signal. This step is important, as individual electronic signals can be amplified and the data can then be processed by the user.

Photomultiplier tubes (PMTs) are detectors found in the majority of flow cytometers (Figure 16A). Multiple PMTs are arranged at the end of the optical path and capture each filtered fluorescence signal. This type of detection device is useful with multicolor panel experiments because PMTs can capture a dynamic range of fluorescence, from very dim to very bright signals.

Alternative detection devices include the avalanche photodiode (APD) to conduct and multiply electrons generated from emitted fluorescence (Figure 16B). Experiments that heavily use fluorophores emitting in the red area of the spectrum benefit from the APD. These detectors efficiently capture signals emitted in the red and near-infrared spectral regions.

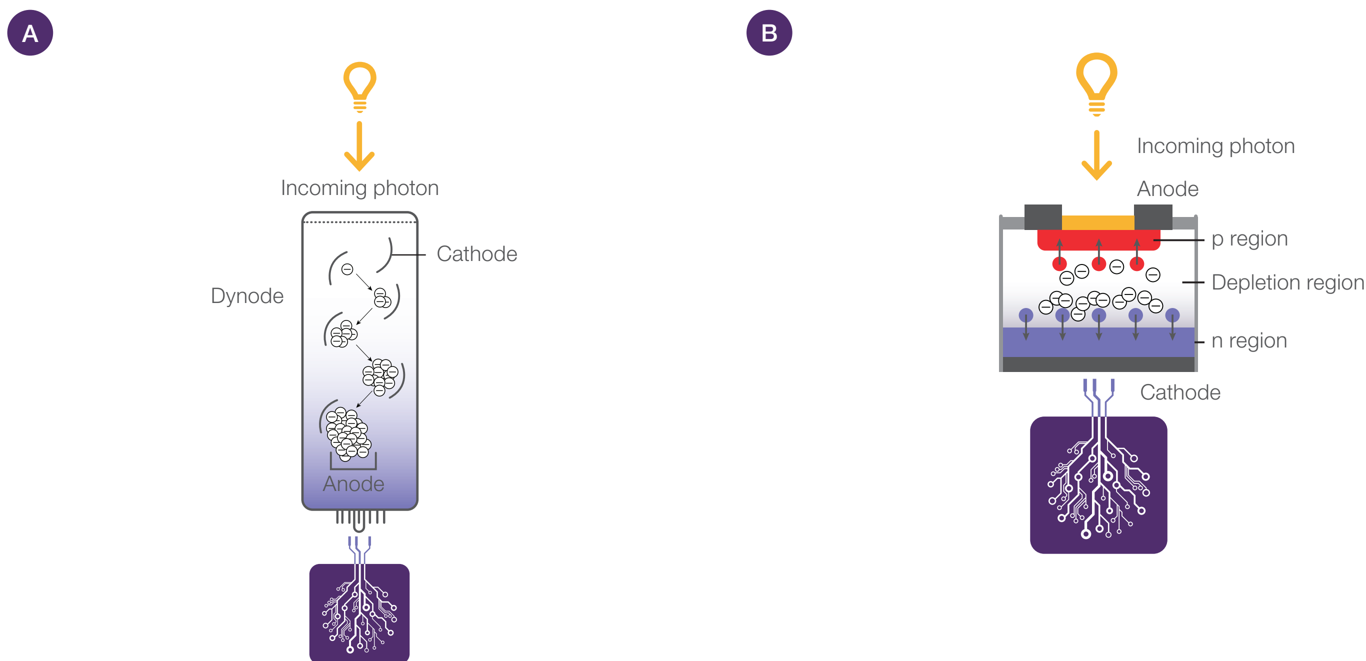


Figure 16. Components for emission detection. (A) PMTs capture and multiply the signal emitted by a fluorophore. **(B)** APDs capture photons on a semiconductor and then multiply the signal.

Solve flow cytometry problems

Problem:

Regulatory T cells (Tregs) distinguish and maintain the relationship between immunity and tolerance (self vs. nonself). Markers for Tregs include CD4, CD25, FOXP3, and other markers. While there are good antibodies that bind to these targets, Tregs are often difficult to identify because of the combination of low- and high-density antigens. A combination of antigen densities creates a sample with both dim and bright signals. Certain flow cytometers are better equipped with components to detect a range of emitted fluorescence signals.

What is required to design a good experiment for immunophenotyping immune cells?

Solution:

- Match fluorophores by brightness to antigen density:
  - Low-density antigens should be labeled with brighter dyes
  - High-density antigens should be labeled with dimmer dyes
- Tandem dyes are effective for mid- to high-density antigens
- Spatially separated lasers excite fluorophores at different distances; coexpressed markers benefit from this arrangement, as it minimizes spectral overlap and creates well-separated channels
- Dim and bright markers should not occupy adjacent channels
- Instruments equipped with PMTs have a greater dynamic range

Voltage settings

Sample background may be detected as autofluorescence from cells or other components of a sample running on a flow cytometer. Fully visualizing the physical characteristics of stained cells requires capturing the full range of signals, from negative (dim) to positive. In other words, the ideal PMT voltage must be adjusted to view the maximum separation between the stain's fluorescence and the cell's detectable (negative) autofluorescence.

Before running cell samples, PMT voltages of the flow cytometer should be optimized using a fluorescent standard to calculate the optimal voltages for each PMT.

A PMT voltage may be adjusted if necessary, especially if the positive signal is too bright. Users may adjust the voltage using unstained cells to determine background signal (Figure 17A). Lowering the voltage of the PMT allows proper measurement of the positive signal and decreases the intensity value of the negative population (Figure 17B).

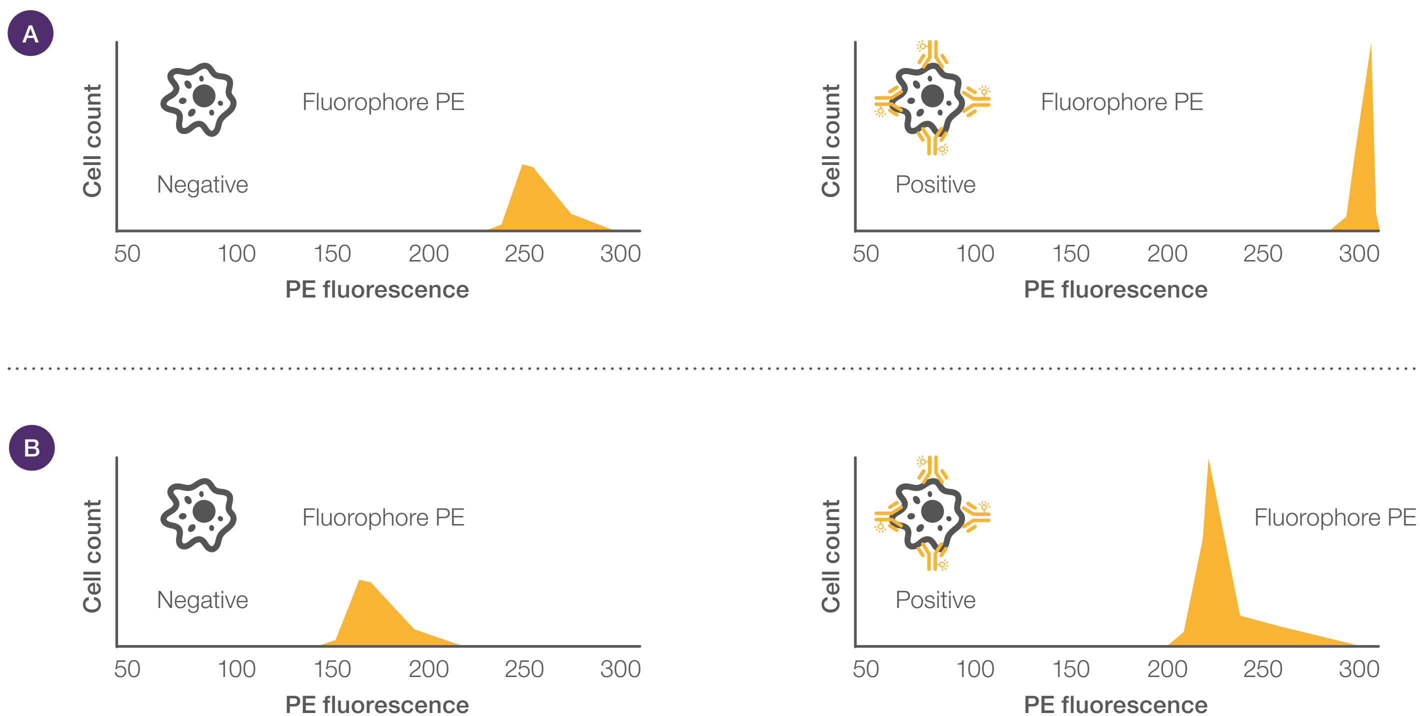
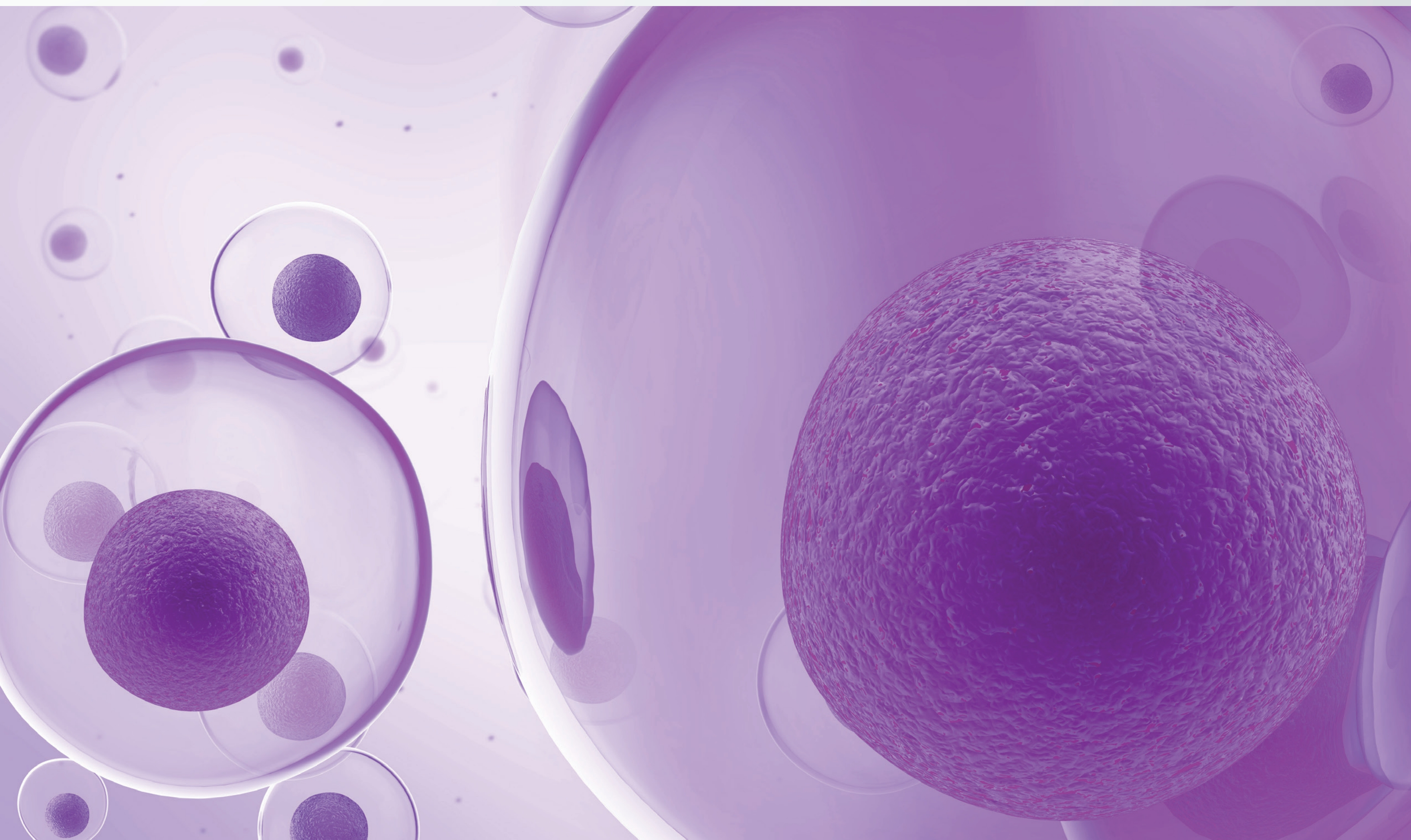


Figure 17. Voltage settings. (A) Positive signal emitted from a fluorophore-labeled cell is not in the detectable range and is off scale because the PMT voltage is too high. **(B)** The voltage is lowered and the positive signal is now detectable.

The amount of fluorescence signal detected by the flow cytometer is controlled by voltage settings. Setting a higher or lower voltage produces more or less gain on the PMT detector for proper fluorescence signal measurement.

Locked voltage settings are a feature of simple flow cytometers, so simple flow cytometers offer a limited range of detection of emitted fluorescence. Adjusting voltage settings can be challenging since the user needs to be able to distinguish positive cell populations from background. New users may over- or under-adjust voltage. To help these users, voltages are locked for a small panel of fluorescent dyes and their associated detectors. Instruments with locked settings simplify the process by limiting users' ability to change voltage settings.

Unlocked or adjustable voltage settings are preferable for users running multicolor panel experiments. Locked voltage settings are preset to detect fluorescence emitted from a limited panel of fluorescent dyes. Adjustable voltage settings mean that users are not restricted to a set panel of fluorescent dyes. This feature allows users to employ larger panels or include more types of dyes. Adjustable voltage settings also allow users to check their single-stained positive controls to place their PMT signals on scale. PMT voltages may also be adjusted for optimal signal compensation as part of multiparameter experiments.



Fluorescence detection and compensation

Emission from multiple fluorophores produces a range of light in the visible spectrum. Data collection from multicolor panels will produce overlapping fluorescence signals. This can be problematic, as positive single-labeled cells may appear as double-positive cells (Figure 18).

Figure 18. Compensation. (A) Overlapping emitted signals can produce false-positive data points. **(B)** Compensation removes the effect of the overlapping emitted fluorescence. The compensated data will produce more accurate results.

Compensation is the separation of signal from each fluorophore by a set of algorithms. Traditional compensation separates specific wavelengths. Each fluorophore is measured individually, and then algorithms are used to create a matrix of correction values. These corrections are applied to the spectral overlap to produce individual values (Figure 19A). This process straightforwardly and quickly computes signal compensation.

Spectral unmixing is different from traditional compensation in that it provides the spectrum of emitted light versus a single wavelength (Figure 19B). This method may be useful for cells with high levels of autofluorescence, as it is computed as a separate color.

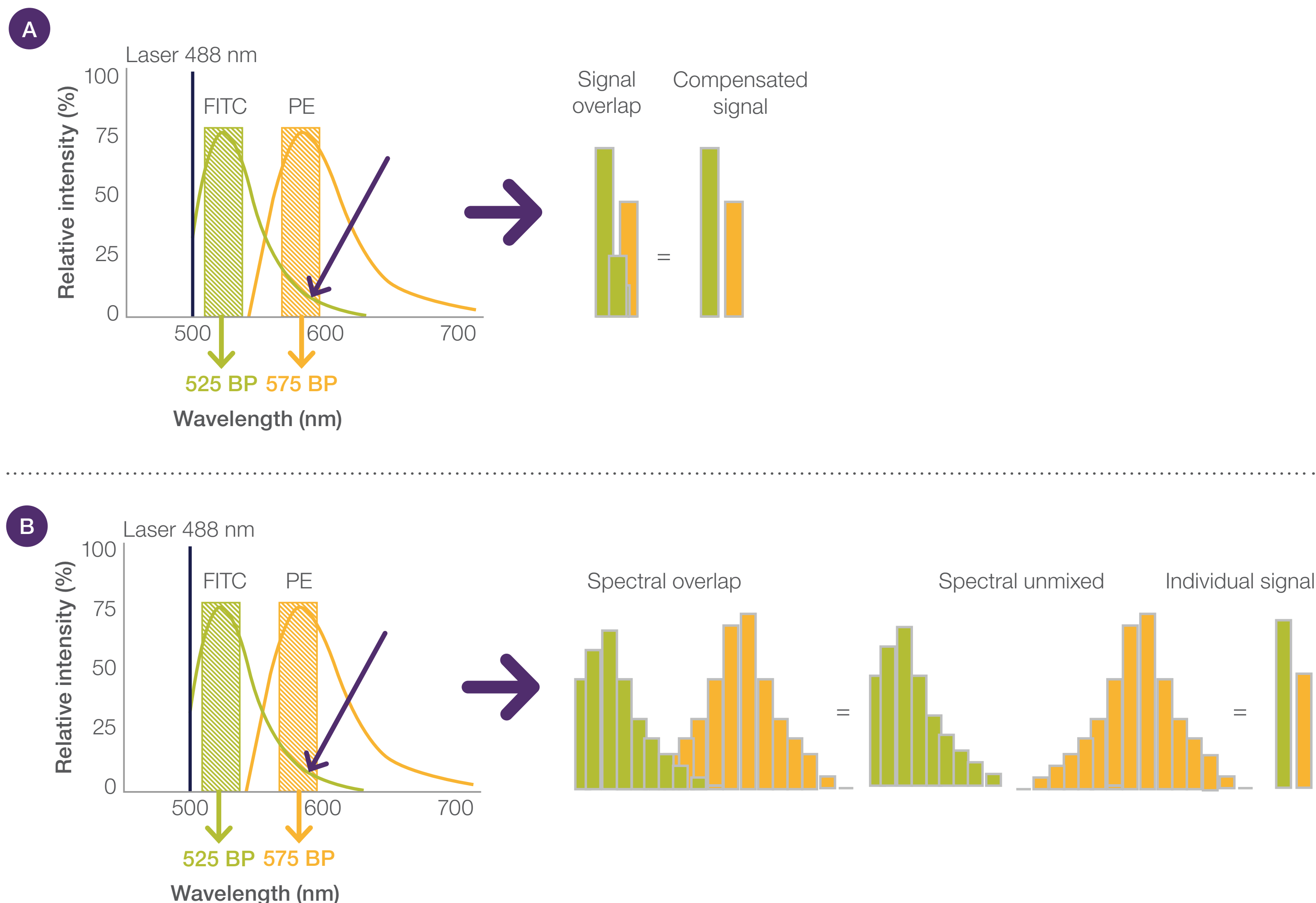


Figure 19. Compensation techniques. (A) Traditional compensation uses well-researched sets of algorithms to analyze data from fluorophores, negative gating, or unstained controls to process overlapping emission into a single color. **(B)** Spectral unmixing uses sets of algorithms such as constrained Poisson regression or nonnegative matrix factorization to process light into a single color.

Channels

Components to capture emitted light in both PMTs and APDs are called channels. The number of fluorophores detected by a flow cytometer will be determined by the number of available channels.

A one-laser system can have multiple channels (Figure 20A). This means a single laser can excite multiple fluorophores, and the detectors can capture emitted light at different wavelengths from a sample. This type of experiment requires complex compensation, as all fluorophores can emit light in overlapping wavelengths (Figure 20B).

Multiple lasers may be of benefit since they provide more channels (Figure 20C). Additional channels confer easier panel design, as there are more fluorophore choices and less compensation. This may allow for an experiment with better resolution of populations (Figure 20D).

A

Excitation laser	Channel (filter set)	Dye
Blue: 488 nm	BL1 (525/50)	FITC Alexa Fluor 488
	BL2 (590/40)	PE PE–Alexa Fluor 610 PE–Texas Red
	BL3 (695/40)	PE–Alexa Fluor 680 PE–Cy®5 PE–Cy®5.5 PerCP–Cy5.5 PerCP–eFluor 710
	BL4 (780/60)	PE–Alexa Fluor 710 PE–Cy®7

C

Excitation laser	Channel (filter set)	Dye
Violet: 405 nm	VL1 (440/50)	Super Bright 436 Alexa Fluor 405 eFluor 450 Pacific Blue
	VL2 (512/25)	eFluor 506 Pacific Green
	VL3 (603/48)	Super Bright 600 Pacific Orange
	VL4 (710/50)	Super Bright 645
Blue: 488 nm	BL1 (525/50)	FITC Alexa Fluor 488
	BL2 (590/40)	PE PE–Alexa Fluor 610 PE–Texas Red
	BL3 (695/40)	PE–Alexa Fluor 700 PE–Cy5 PE–Cy5.5 PerCP–Cy5.5 PerCP–eFluor 710
	BL4 (780/60)	PE–Alexa Fluor 700 PE–Cy7
Red: 640 nm	RL1 (670/14)	APC eFluor 660 Alexa Fluor 647
	RL2 (720/30)	Alexa Fluor 680 Alexa Fluor 700 APC–Alexa Fluor 700
	RL3 (780/60)	Alexa Fluor 700 Alexa Fluor 750 APC–eFluor 780 APC–Cy7

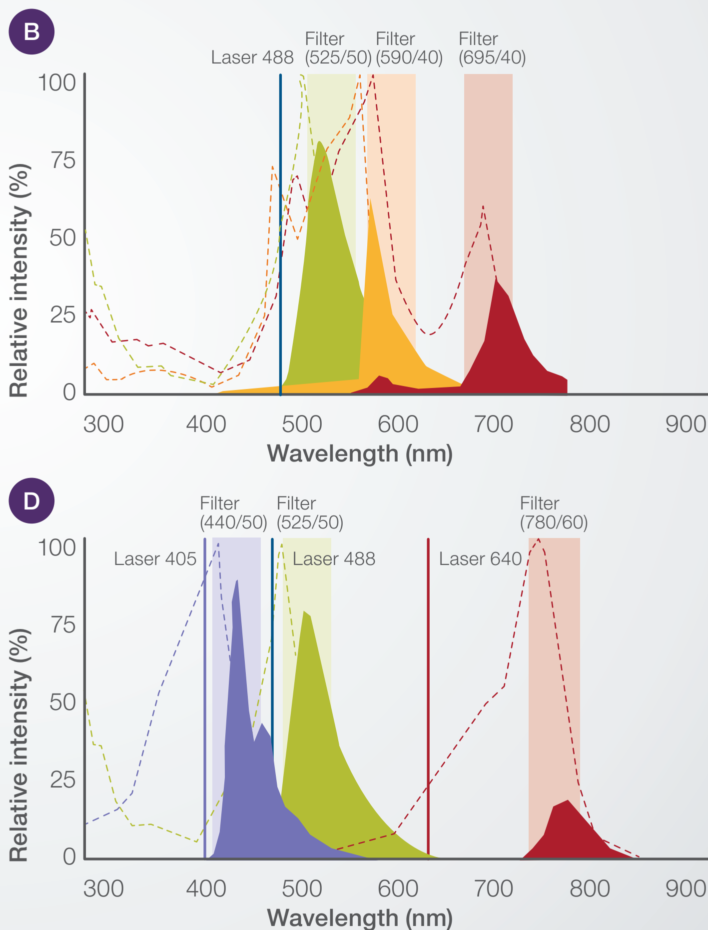


Figure 20. Channels. (A) A one-laser system can have multiple channels. **(B)** The emitted light overlaps and requires compensation. **(C)** A 3-laser system will have more channels because there are more detectors. **(D)** The channels are spread out, allowing for more fluorophore options and less compensation.

Instrument
maintenance

Instrument maintenance

Flow cytometers are sophisticated instruments designed to last many years. Most components are permanent fixtures. Periodic maintenance and routine cleaning will minimize replacement of parts and help provide high-quality data.

Routine cleaning

One type of problem from the lack of regular cleaning is the presence of material from previous samples (Figure 21). Carryover cells from previous samples are a problem for sensitive experiments such as measurement of rare cells (Figure 21A). The rate of false positives increases when carryover cells are present. Flushing between samples or after experiments can reduce the number of leftover cells or particles from a previous experiment.

Regular cleaning prevents buildup of fluorescent dyes. Certain dyes, such as nucleic acid–binding dyes, are more prone to sticking (Figure 21B). Collection of sticky dyes, cells, and debris create more opportunity for tubes to become clogged.

Instruments that run cell culture or tissue samples have a higher chance of bacterial contamination (Figure 21C). Signs of contamination include cloudy fluid in the waste bottle and a high number of event collections in blank samples. Running fluidics cleaning solution or bleach can discourage microbial growth.

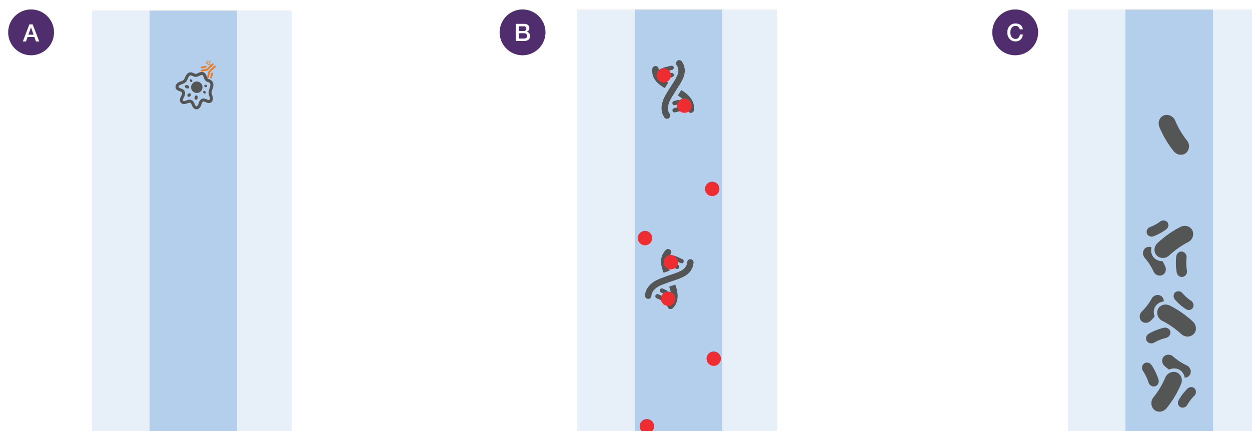


Figure 21. Common contaminants from improper maintenance. (A) Carryover is contamination with cells or particles from previous samples. **(B)** Dyes that bind to nucleic acids are sticky and can create background signal. **(C)** Microbes can grow in fluidics bottles and other parts of a flow cytometer.

Minimize contamination between samples with cleaning. Between each experiment, run 10% bleach and then DI water before running the next experiment (Figure 22A). Some instruments have automatic cleaning between samples.

Most systems require cleaning with distilled water and cleaning solution before use and at the end of the day to remove cells and dyes from flow cytometer tubes and flow cells (Figure 22B). Startup and shutdown cleaning protocols are detailed in most user manuals. Daily maintenance helps ensure that fluidics tubes and flow cells are free from contamination with microbes or old sample material.

Some flow cytometers have additional daily maintenance requirements, including fluid tank preparation, a check for air bubbles trapped in filters, and emptying of waste containers (Figure 22C). Other flow cytometers have automated protocols with software guidance. Automated systems reduce the user's burden of certain tasks such as air bubble checks or shutdown procedures. These protocols are in place to save time and provide thorough cleaning.

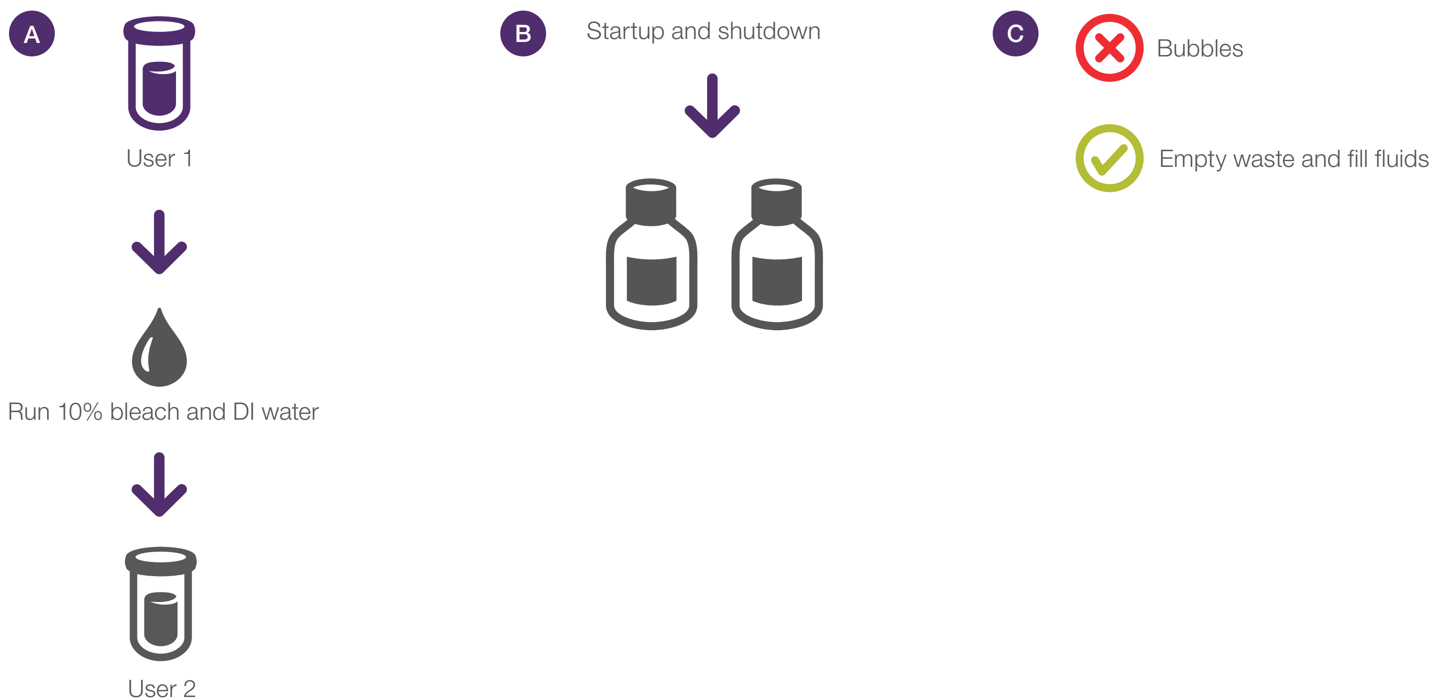


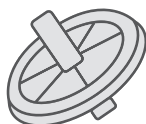
Figure 22. Daily cleaning. (A) Run bleach and DI water between samples. Some instruments can automatically clean. **(B)** Startup and daily shutdown cleaning procedures help prevent buildup of dyes and carryover of cells. **(C)** Additional daily maintenance checks may involve automated protocols with software guidance.

Fluidics decontamination

A combination of fluidics flushing, changing of sheath filters, and deep instrument cleaning can remove carryover, fluorescent dyes, and microbes. Most flow cytometers require a deep cleaning of sheath tubing, waste tubing, fluidics containers, and the flow cell every 2 weeks to one month. This prevents the buildup of debris and removes biohazardous material and microbes.

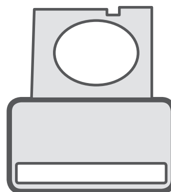
Depending on the type of fluidics system, components may have to be removed and replaced or cleaned (Figure 23). Peristaltic systems require that tubing be removed and replaced every 6 months to a year. Without replacement, contaminants or debris will appear during event collection. Pressure systems require regular maintenance of nozzle tips and associated parts for samples to quickly enter flow cytometers. Syringe-based systems also require cleaning of the syringe to reduce contaminating cells. Follow user manuals and log maintenance to help ensure data integrity.

A



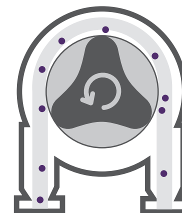
Replace filters to decrease carryover or contamination

B



Dust optical filters to minimize background signal

C



Replace tubing for normal wear and tear, and to decrease carryover or contamination

D

Replace syringe if there is not enough sample, or reduce the number of events collected

E

Replace o-rings above sample injection probes (SIP) for pressurized systems

F

All fluidics bottles, regardless of systems, should be cleaned

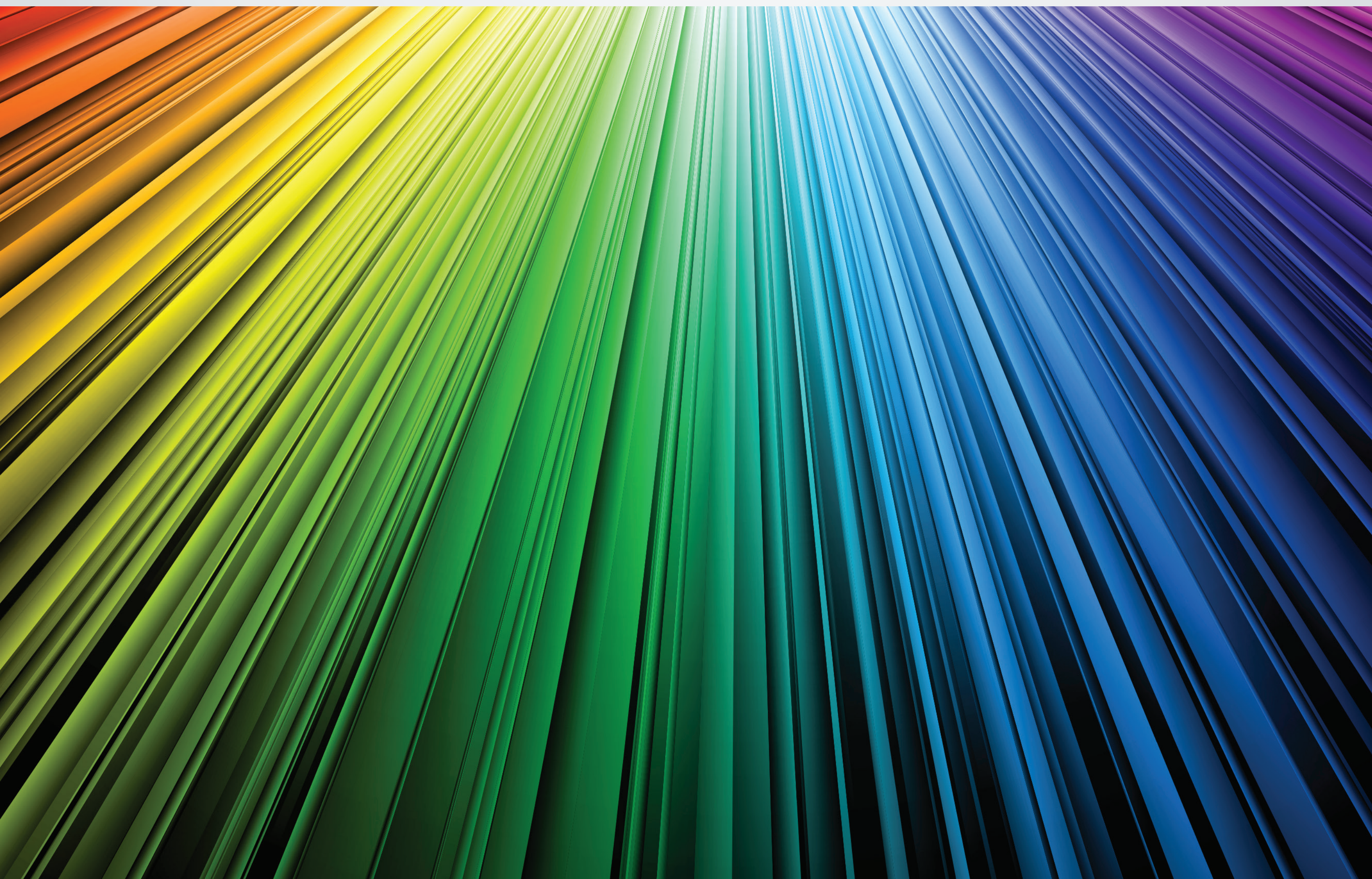
Figure 23. Fluidics maintenance. (A–F) Clean or replace flow cytometry instrument parts to maintain normal instrument performance and minimize carryover, background noise, and microbial contamination.

Performance tracking

Most instruments have daily QC or performance evaluations. Daily performance evaluations can be tracked over time.

Performance tracking is a comprehensive set of procedures for monitoring the daily performance of a flow cytometer. Fluorescence-tracking beads define baseline performance and enable daily measurements of the flow cytometer's performance. This process helps to maintain experimental reproducibility.

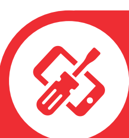
Calibration beads do have expiration dates. Certain flow cytometers with unautomated performance tracking will not calibrate to expired beads. Check the maintenance and performance requirements of each flow cytometer system for continuity of operation at the manufacturer's specifications.



Instrument support

Instrument support

When you are making a big decision, you often need support. This is where a company with service agreements and commitment to the researcher is most important. Here are a few programs you should look for before purchasing a flow cytometer.



Instrument and warranty service

This is separate from the warranty and paid service. Each contract comes with a variety of on-site services, including parts, technician travel, labor, phone support, preventive care, and maintenance.

Customer service and application support

Flow cytometry has a number of applications and specialty reagents for both simple and complex experiments. Find companies with expertise in both instruments and supporting products. Application specialists are separate from technical support and can be a good resource for support.

Education

Some manufacturers offer educational seminars and additional user training. There are significant differences in both basic and advanced educational materials offered by various instrument manufacturers, particularly for teaching complex topics such as panel design and compensation. Along with educational training, some manufacturers offer kit and reagent support. Explore manufacturers' websites or talk to your sales representative to see what is available.

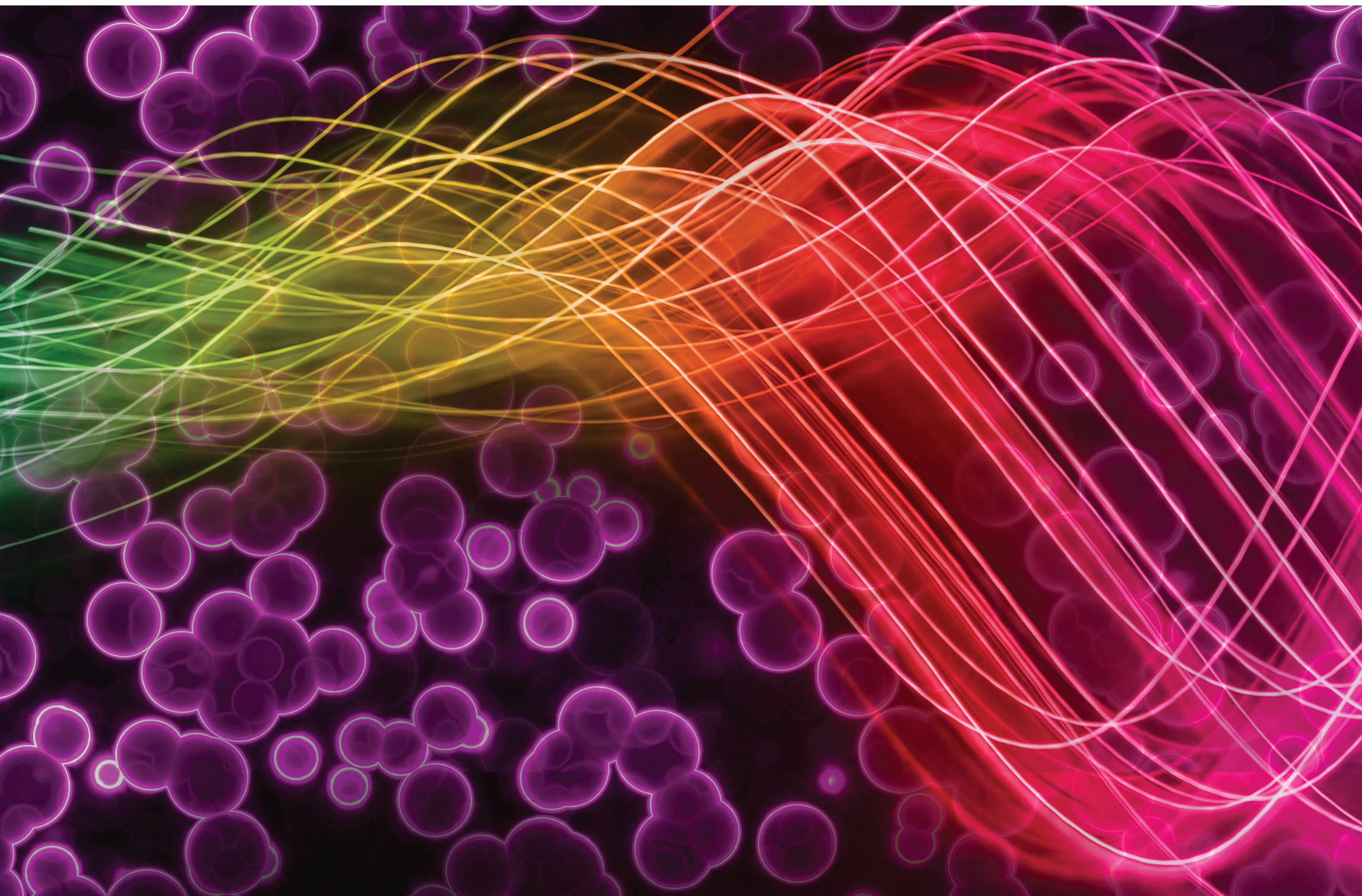
Training and events

Look for a well-defined and personalized onboarding process—a company that will help you set up your instrument, train your users, and follow up to make sure that everything is working as planned.

Choose an instrument that will support your goals

Discovery is at the heart of the research process. A flow cytometer can provide meaningful data on the physical characteristics of millions of individual cells. Combing these data sets allows researchers to gain insights and knowledge of vast biological systems. Advanced technology and instrument engineering are helping to address a wider range of biological questions with the availability of specialized features, streamlined protocols, diverse application capabilities, and coordinating antibody and reagent portfolios. There are many strong instrument options in the flow cytometry market. Identify the instrument that will best fit your needs without compromise.

invitrogen



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

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