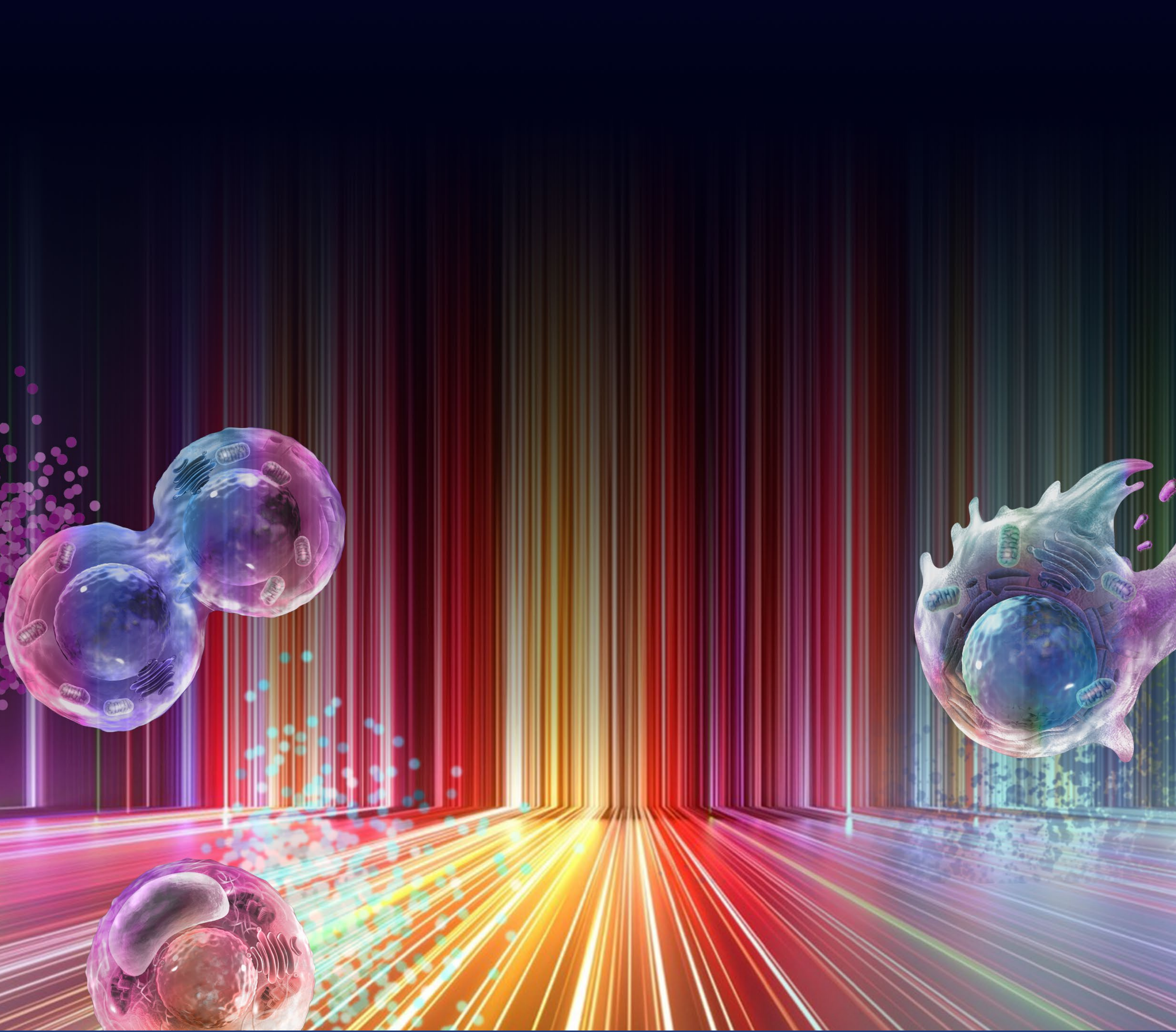

Spectral Flow Fundamentals

Expert Insights

WILEY Analytical Science

BD

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Introduction

As scientists navigate the intricate landscape of cellular differentiation and function, flow cytometry emerges as an indispensable tool, enabling the analysis of millions of cells in a single experiment. Central to this process is the art of panel design and the strategic selection of fluorochrome-conjugated antibodies, which illuminate specific cellular markers through a series of sophisticated transformations. Recent advancements have introduced a paradigm shift with the advent of spectral flow cytometry, complementing conventional methods and offering unprecedented flexibility and resolution. This eBook examines the comparative strengths of these approaches, highlighting the importance of thoughtful experimental design and robust controls in achieving high-quality data. By mastering these techniques, researchers can convert complex biological phenomena into quantifiable data, paving the way for groundbreaking discoveries in cellular biology.


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Key Learnings from this Expert Insight

Cytometry fundamentals: Gaining biological insights through a series of transformations

- Cytometry provides in-depth information on cellular populations, states and interactions, making it essential for single-cell studies of complex biological systems. It enables scientists to analyze millions of cells in a single experiment, revealing how they differentiate, function, and respond to environmental cues.
- Cytometry relies on labeling cells with fluorochrome-conjugated antibodies that bind to specific markers. Cell populations can be identified and characterized through a series of transformations, including the detection and conversion of these light signals—which are emitted when fluorochromes are excited—into useful biological information.
- Maintaining data quality while balancing the analysis of numerous parameters depends on a good experimental design. Thoughtful panel design and marker selection play a crucial role in achieving high-quality resolution of biological data, particularly as panel size increases to the high parameter space.

Paradigm shift: Complementary approaches of conventional and spectral flow cytometry

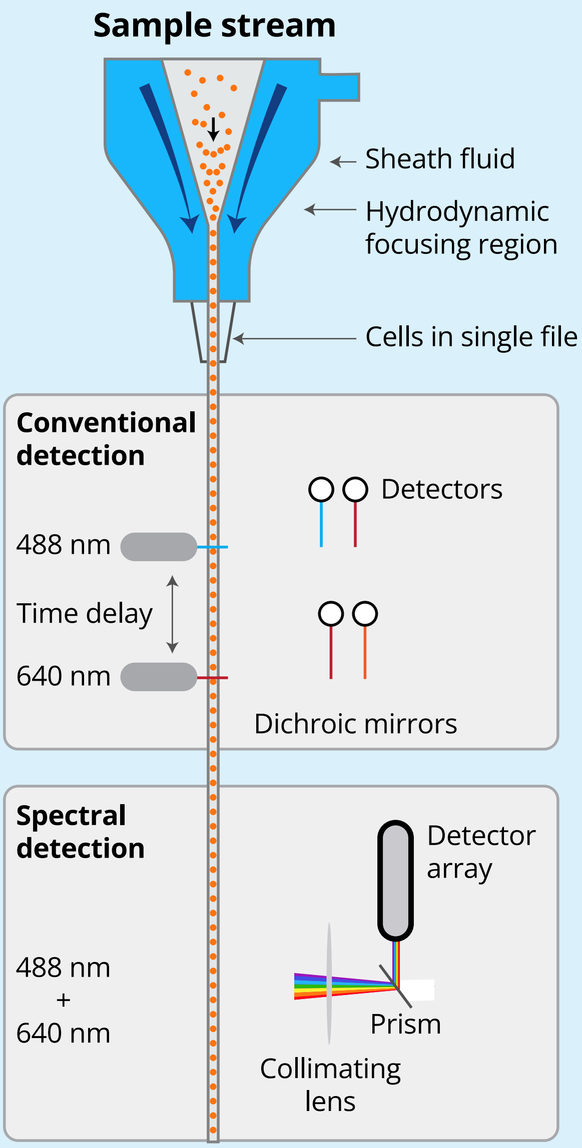
Recent developments in flow cytometry technology have introduced both conventional and spectral methods, which can be used independently or in combination depending on research needs. Both approaches have their own set of advantages and disadvantages and should be selected for the best fit to the experimental needs. The table below provides a concise comparison of these two approaches:

Aspect	Conventional flow cytometry	Spectral flow cytometry
Signal detection	Assigns one detector per fluorochrome	Uses all detectors to capture the full emission profile of each dye
Signal utilization	Relies mainly on the peak emission of a dye for signal detection	Analyzes the full emission profile of a dye for signal detection
Panel size limitation	Limited by detector crowding due to the finite number of available detectors	Limited by dye crowding from overlapping emission spectra
Dye overlap management	Signal spillover into other detectors	Signal overlap across the spectrum
Correction methods	Compensation algorithms subtract overlapping signals based on fluorochrome emission properties	Spectral unmixing algorithms separate signals by analyzing full emission spectra

Panel design and controls: Why they still matter in spectral flow cytometry

- To obtain high-quality data, careful panel design is essential. Researchers can reduce spectral overlap and increase resolution by carefully choosing markers, matching them with appropriate fluorochromes, and being aware of the instrument's capacities.
- Spectral flow cytometry requires proper controls. Single-stain controls ensure accurate compensation or unmixing, while autofluorescence controls improve clarity by removing background noise.

The principle of spectral flow cytometry



Spectral flow cytometry is a technique that allows researchers to analyze multiple characteristics of individual cells by measuring the light they emit after being tagged with specific fluorescent dyes. Unlike the conventional approach, which assigns each fluorochrome to a single detector, the spectral approach uses all detectors to capture and analyze the complete emission spectra of each dye. This approach allows for greater fluorochrome options, filter-independent detection, larger panels with highly overlapping fluorochromes, and autofluorescence extraction, enhancing flexibility and resolution in complex experiments.

Choosing between conventional and spectral flow cytometry

Both conventional and spectral flow cytometry offer valuable approaches for cell analysis, each with distinct advantages. Conventional cytometry remains a widely used and cost-effective method, while spectral cytometry provides greater flexibility and higher resolution in complex experiments. The key differences between these approaches are outlined below:

Conventional flow cytometry

Advantages

- A well-established, reliable, and cost-effective approach
- Workflows are familiar, with an extensive amount of previous data and applications
- Panel design and data analysis are simpler for less complex studies

Considerations

- Panel size expansion necessitates the addition of more detectors, increasing system complexity.
- To avoid signal spillover, careful fluorochrome selection is essential.
- Autofluorescence extraction capacity is limited, which can have an impact on resolution in certain samples.

Spectral flow cytometry

Advantages

- Overcomes panel-size limitations by using all detectors to capture entire emission spectra.
- Greater versatility in fluorochrome choices, allowing for highly multiplexed experiments.
- Autofluorescence extraction improves resolution in samples with high background fluorescence

Considerations

- Requires a shift in panel design strategies and data analysis workflows
- Advanced unmixing techniques are required for accurate signal separation.
- Higher initial instrument cost compared to conventional systems

PART 2

CHAPTER 1

Cytometry Fundamentals: Gaining Biological Insights Through a Series of Transformations

Why we do it

Cytometry is a powerful tool for studying complex biological processes at the single-cell level. By analyzing single cells one at a time, this method allows researchers to acquire detailed insights into cellular populations, states, and interactions. The ability to investigate millions of cells in a single experiment enables scientists to answer fundamental questions such as:

- What are these cells doing?
- How do they interact with their surroundings and other cells?
- What are their origins and fates?

The ultimate goal of cytometry is not just to study the profile of a vast number of cells, but to do so with precision and accuracy. Meaningful biological insights rely on the ability to distinguish between different cell types and states, often depending on subtle changes in the expression of markers or functional features.

As scientific questions become more complex, there is an increasing need to analyze numerous parameters in a single experiment. To achieve this, researchers aim to examine multiple markers at once, including cell surface proteins, internal compounds, and functional states. However, a fundamental challenge remains: balancing the number of parameters measured and the quality of data. While adding more parameters can result in a more comprehensive dataset, it also introduces challenges such as signal overlap, noise, and reduced resolution, which can hinder proper interpretation. Effective experimental design is thus essential to maximize both the breadth and depth of information while ensuring robust and reliable results.

How we do it

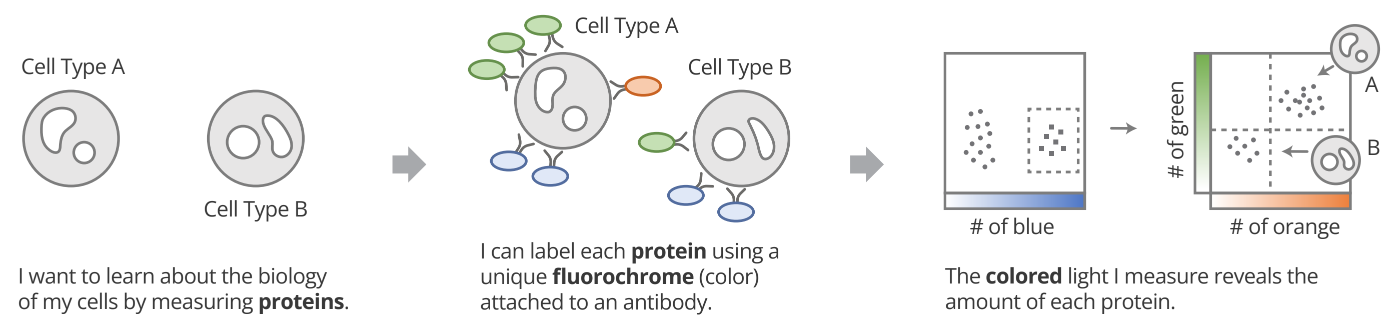
Webinar minute 11:30

Flow cytometry enables researchers to explore biological systems by converting light signals into meaningful biological data. It involves a series of transformations that begins with labeling cells using fluorochrome-conjugated antibodies that bind to specific surface proteins or intracellular markers. When excited by a laser, each fluorochrome (also referred to as a dye) emits light at different wavelengths, based on its chemical characteristics. This unique spectral signature allows for the precise identification of multiple markers within a sample.

Figure 1 illustrates this process step-by-step. Cells of different types, such as Cell Type A and Cell Type B, can be distinguished by employing antibodies conjugated to specific fluorochromes (colors). The intensity of the emitted light correlates with the number of tagged proteins present on each cell. By detecting this emitted light, cytometry transforms raw fluorescence data into quantitative information, allowing scientists to measure protein expression and differentiate cell types.

In any flow cytometer, detectors catch emitted light and assess fluorescence intensity at different wavelengths. These signals are then displayed to visualize population differences. For instance, in Figure 1, both Cell Type A and Cell Type B emit blue and green fluorescence, with Cell Type A additionally emitting orange fluorescence. This series of transformations, starting with antibody binding to antigens and extending to fluorescence detection and data visualization, enables the identification and separation of cell populations. This fundamental principle is applicable to both conventional and spectral flow cytometry.

Figure 1: Overview of cytometry. Labeled proteins on cells emit fluorescent light, which is measured to determine protein abundance on different cell types.

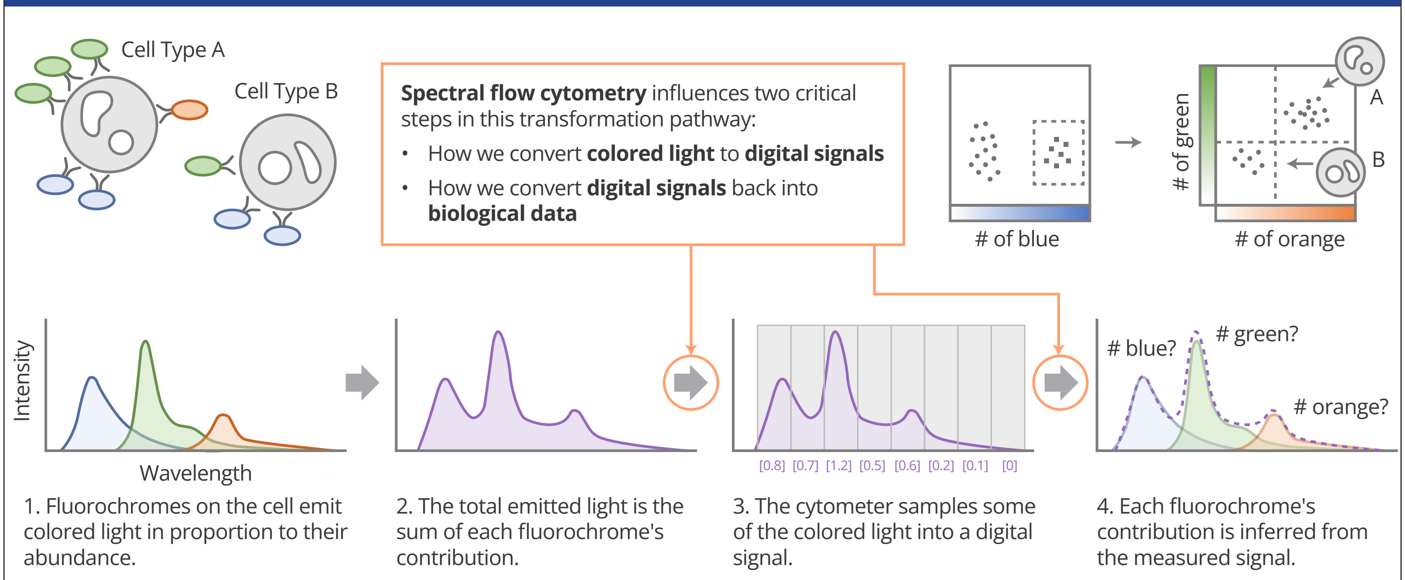


The light emitted by the fluorochromes is detected by the cytometer and converted into meaningful data, as shown in Figure 2. The emitted light from all fluorochromes combines to form the total emitted light, with each fluorochrome contributing proportionally to the overall signal. The cytometer's detectors capture and analyze this composite signal, recording light at particular wavelength intervals and converting it to digital data.

The final stage is computational processing, which consists of calculating the contribution of each fluorochrome to the total emitted light. In conventional flow cytometry, fluorescence spillover occurs when the emission spectrum of one fluorochrome overlaps with that of another, causing signals to bleed into neighboring detection channels.

Compensation algorithms are used to correct this overlap and ensure accurate data interpretation. In spectral flow cytometry, spillover is more accurately described as "spectral overlap," and it is resolved by unmixing algorithms that efficiently separate overlapping signals by analyzing the entire spectrum. This process relies on the unique spectral signature of each fluorochrome to distinguish them within the panel, transforming complex data into physiologically relevant information and enabling the identification of cell populations.

Figure 2: Spectral flow cytometry. Emitted light from multiple fluorochromes is detected, digitized, and mathematically unmixed to quantify individual protein signals.



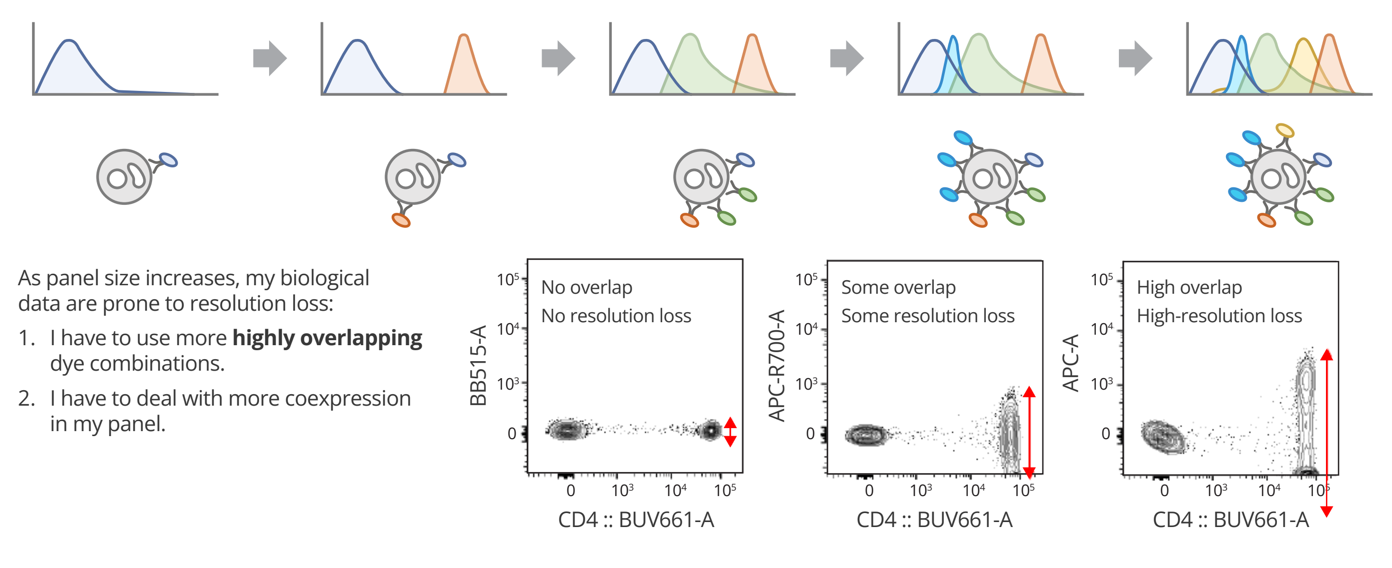
In cytometry, it is essential to strike the right balance between the number of parameters assessed and the quality of the data produced. Simply measuring more markers or using more dyes does not guarantee deeper insights. Instead, data resolution—how clearly cell populations and markers can be identified—is a crucial factor in determining the biological information obtained from an experiment. Achieving this balance ensures that the data collected is both useful and reliable.

As more dyes are added to a cytometry panel, the likelihood of emission spectra overlapping increases. This overlap hinders signal separation, resulting in lower resolution.

For instance, as shown in Figure 3, using dyes with minimal overlap yields high-resolution data. However, as overlap increases, the distinction between populations becomes blurred, leading to reduced resolution.

Therefore, building a cytometry panel involves more than simply adding dyes or increasing the number of detectors. Careful fluorochrome selection is essential to minimize overlap while maintaining data quality. This approach enables researchers to obtain the most useful, high-resolution information on the cells in their samples, even in complex studies with highly multiplexed panels.

Figure 3: Impact of crowded spectral space. Increasing panel size leads to greater spectral overlap (top). Examples show no overlap, some overlap, and high overlap with corresponding resolution loss (bottom).



CHAPTER 2

Paradigm Shift: Complementary Approaches of Conventional and Spectral Flow Cytometry

Why spectral flow cytometry?

The field of cytometry has grown to incorporate spectral flow cytometry as an alternative approach to conventional methods. In conventional cytometry, each fluorochrome is captured in a designated detector, so adding more colors requires identifying areas of the spectrum that are not already in use. The process is constrained by both the availability of compatible dyes and the instrument's capacity to measure them. Over time, expanding the cytometry toolkit involved introducing additional detectors or lasers to cover new regions of the excitation spectrum. This iterative approach resulted in high-parameter panels, such as the panel shown in Figure 4, capable of analyzing 28 colors across five lasers. However, this approach is limited by the availability of detectors and the potential spectral overlap, which can complicate data interpretation. Rather than replacing conventional systems, spectral flow cytometry provides an alternative for tackling specific issues, notably in studies using highly multiplexed panels.

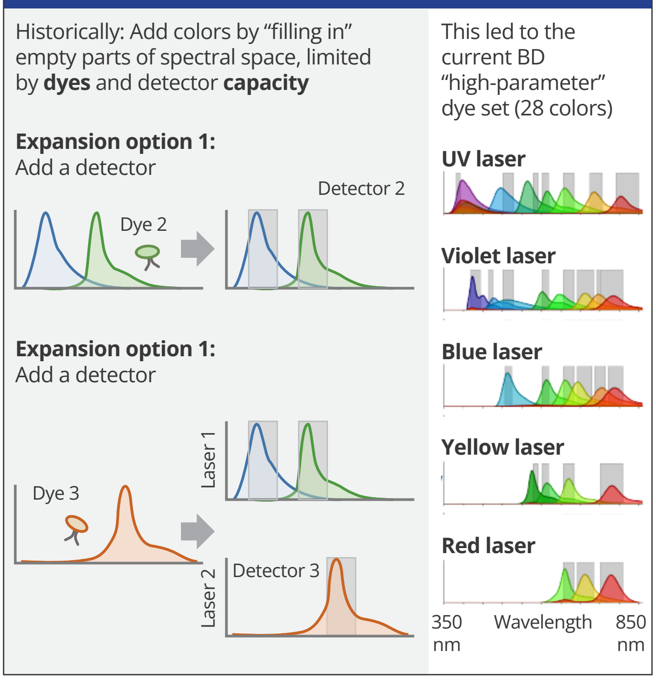
From detector crowding to dye crowding

Webinar minute 8:38

With the introduction of spectral flow cytometry, the limitation in panel size has shifted from the number of detectors in conventional systems to the availability of dyes with minimal spectral overlap. Unlike conventional cytometry, which isolates specific segments of the emission spectrum, spectral flow cytometry captures the entire emission profile of each fluorochrome. However, while spectral systems allow for the use of more dyes, they require rigorous controls and advanced computational tools to ensure accurate data interpretation.

Advances in dye chemistry have provided researchers with an expanded array of options for building panels, allowing for greater flexibility in experimental design. However, the increasing availability of fluorochromes has also crowded the spectral space, creating new challenges for panel design. Careful panel design remains essential for both conventional and spectral flow cytometry to ensure high-quality data. Tools such as those available in BD Research Cloud (BDRC) can assist researchers in avoiding highly overlapping dyes, simplifying the design process. Today, researchers must balance these challenges, by leveraging the complementary strengths of conventional and spectral flow cytometry, choosing the most suitable approach based on the study setting.

Figure 4: Addressing detector limitations in conventional cytometry. Adding detectors (option 1) or lasers (option 2) expanded panel size, enabling high-parameter cytometry capable of detecting up to 28 colors.



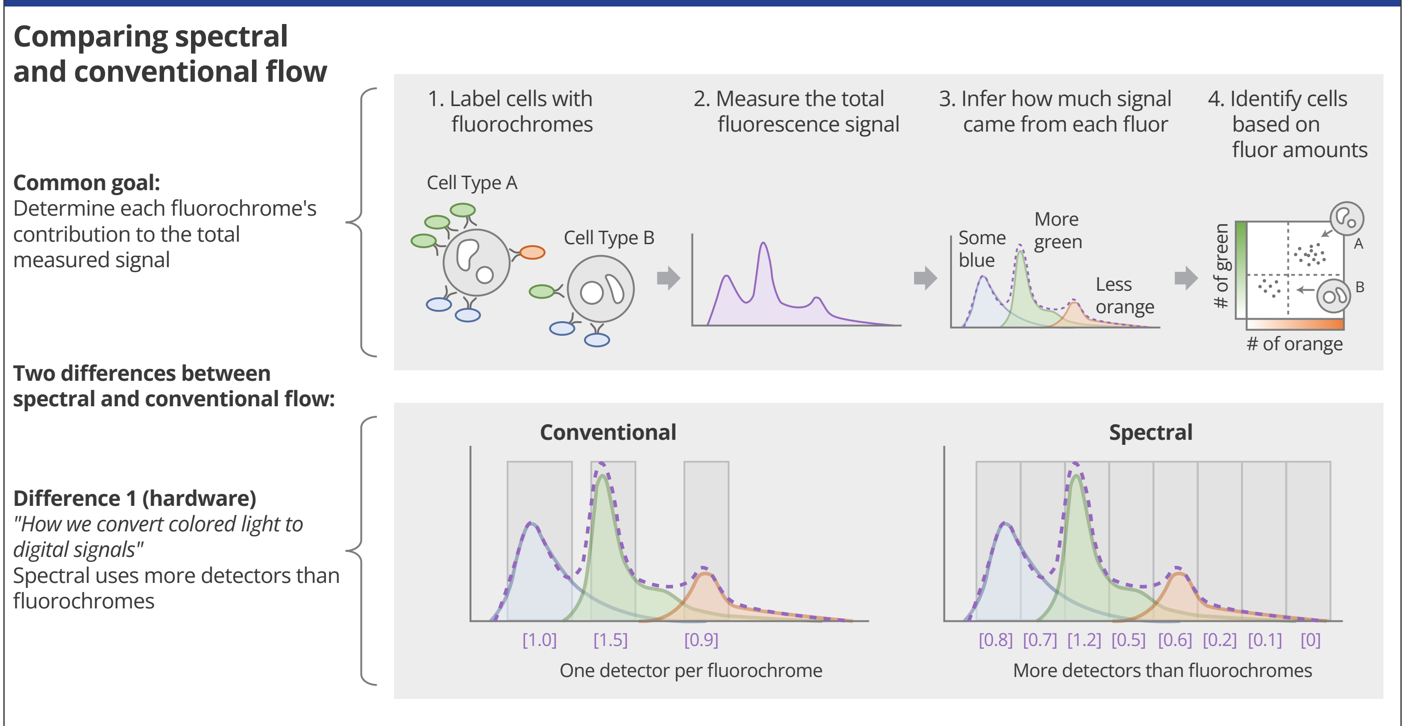
How spectral flow cytometry works

Webinar minute 14:02

While both conventional and spectral flow cytometry aim to quantify fluorescence emissions and extract biological insights, their methods differ in hardware and software approaches. The major distinction consists of how light signals are collected and how software converts these signals into meaningful biological information.

In conventional cytometry, each fluorochrome is assigned to a distinct detector, corresponding to the peak emission of the fluorochrome. Spectral cytometers, on the other hand, use more detectors than fluorochromes, allowing them to catch the entire emission profile of each dye and provide a more comprehensive picture of the emitted signals in the sample. As illustrated in Figure 5, spectral flow cytometry relies on computational unmixing algorithms to differentiate overlapping fluorescence signals. Despite their differences, both systems share the same goal: to precisely quantify the signal emitted by each fluorochrome in a sample and extract relevant biological information.

Figure 5: Comparison of signal detection in conventional and spectral flow cytometry. Conventional flow cytometry assigns one detector per fluorochrome, while spectral flow cytometry uses more detectors than fluorochromes to capture entire emission profiles, enabling advanced signal differentiation and analysis.



While spectral flow cytometry represents a cutting-edge innovation, conventional cytometry remains a trusted standard for many applications. The addition of detectors in conventional systems has been a reliable method for expanding panel sizes, though it requires careful design to avoid signal overlap. Spectral flow cytometry, on the other hand, minimizes these limitations by analyzing entire emission profiles of fluorochromes, allowing for more flexible dye combinations and expanded panel sizes. While this approach introduces changes in panel design and data analysis workflows, it is supported by advanced software that simplifies these processes for researchers. Both methods have distinct strengths: spectral flow cytometry offers flexibility in dye choice and excels in high-parameter and multiplexed experiments, while flow conventional cytometry remains a well-established and widely used method, valued for its simpler setup, lower cost, and familiarity among researchers.

Hardware: Laser and detector configurations

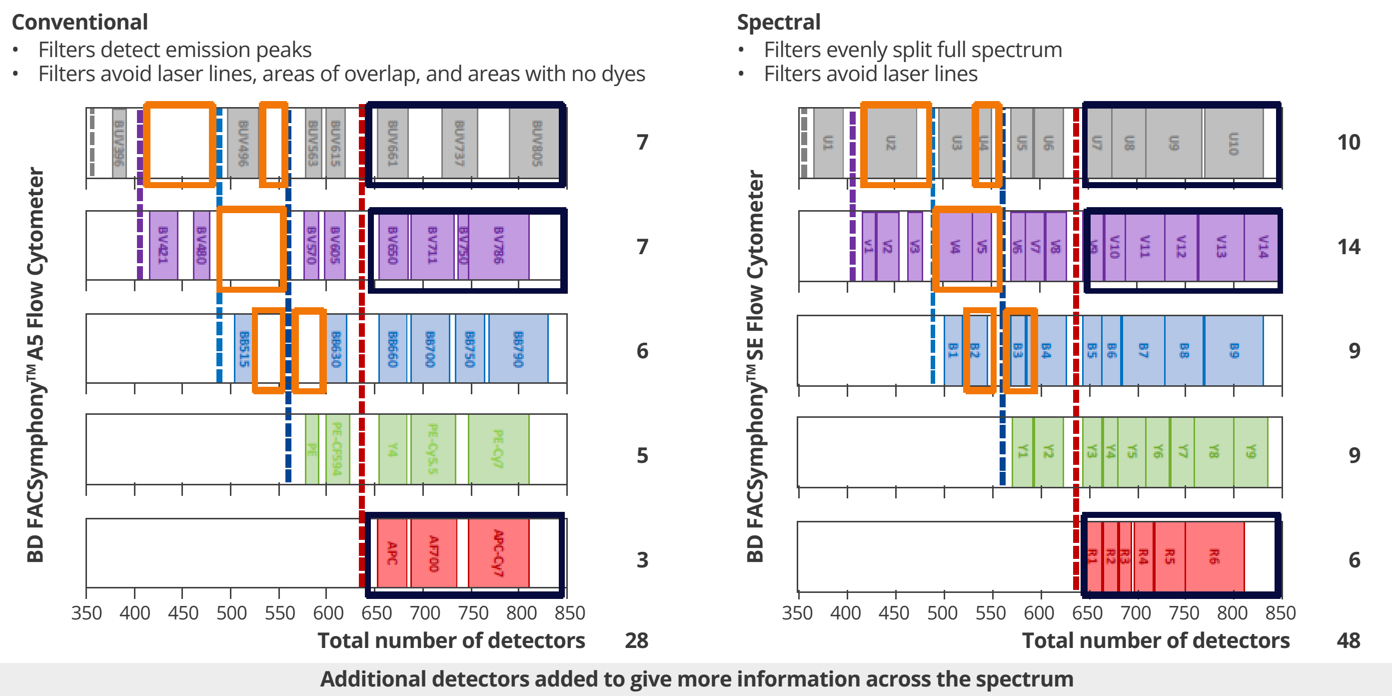
Webinar minute 14:55

A significant difference between conventional and spectral flow cytometry is how light signals are collected and processed by their respective hardware systems. Figure 6 compares the laser and detector configurations of the conventional BD FACSymphony A5™ cytometer and the spectral BD FACSymphony A5 SE™ cytometer. The colored vertical dashed lines in the figure indicate wavelengths at which each laser excites fluorochromes. The colored horizontal boxes represent detector bands, which indicate the range of emission wavelengths collected by each detector. In both configurations, the detector bands are designed to align with the emission peaks of commonly used dyes, ensuring optimal signal detection.

In the conventional configuration (Figure 6, left panel), bandpass filters direct specific parts of the spectrum to the designated detector. Filters are designed to maximize the detection of emission peaks while minimizing overlap and interference from laser lines. Each fluorochrome is allocated to a single detector, with bandpass filters directing specific wavelengths to each detector. Increasing the number of dyes requires adding more detectors. This design may include intentional gaps to avoid regions with high spectral overlap, ensuring optimal signal clarity. Such a setup demands careful panel design to minimize fluorescence spillover and improve resolution.

In the spectral configuration (Figure 6, right panel), detectors are distributed across the whole spectrum, capturing the full emission profile of all fluorochromes. While laser line avoidance is still necessary, this system eliminates the conventional constraint of assigning individual dyes to specific detectors. Instead of being limited by the number of detectors, as in conventional flow cytometry, spectral systems are limited by the availability of fluorochromes with distinct spectral signatures. This approach enables spectral systems to accommodate larger panels by leveraging the distinct emission profiles of fluorochromes rather than relying on an increase in detector numbers.

Figure 6: Comparison of detector configurations in conventional and spectral flow cytometry. Conventional systems (left) use bandpass filters to target specific emission peaks, avoiding laser lines and spectral overlap, with intentional gaps in coverage. Each fluorochrome is assigned to a single detector, requiring additional detectors to expand panel size. Spectral systems (right) distribute detectors across the full spectrum, capturing entire emission profiles while avoiding laser lines.



Software: Converting digital signals into meaningful data

A key distinction between conventional and spectral flow cytometry lies in how raw digital signals representing fluorescence intensity are converted into biologically meaningful data. In other words, if we have signals coming from multiple fluorochromes in a panel, some of which may have overlapping emission profiles, how do we distinguish them?

In conventional flow cytometry, compensation is applied to account for spectral overlap between fluorochromes. Where spectra overlap, fluorescence from one fluorochrome may be picked up in detectors assigned to another fluorochrome, leading to potential false positives. Compensation accounts for fluorescence spillover by removing signal contributions from surrounding channels, resulting in an accurate assignment of fluorescence to its originating fluorochrome.

Spectral flow cytometry utilizes spectral unmixing, a similar approach to compensation but applied across the full emission profiles of all fluorochromes. As some degree of spectral overlap unavoidably occurs, advanced algorithms are required to determine which signal corresponds to which fluorochrome. Spectral unmixing relies on mathematical models to deconvolute the full emission profiles of fluorochromes across multiple detectors, enabling the separation of overlapping signals.

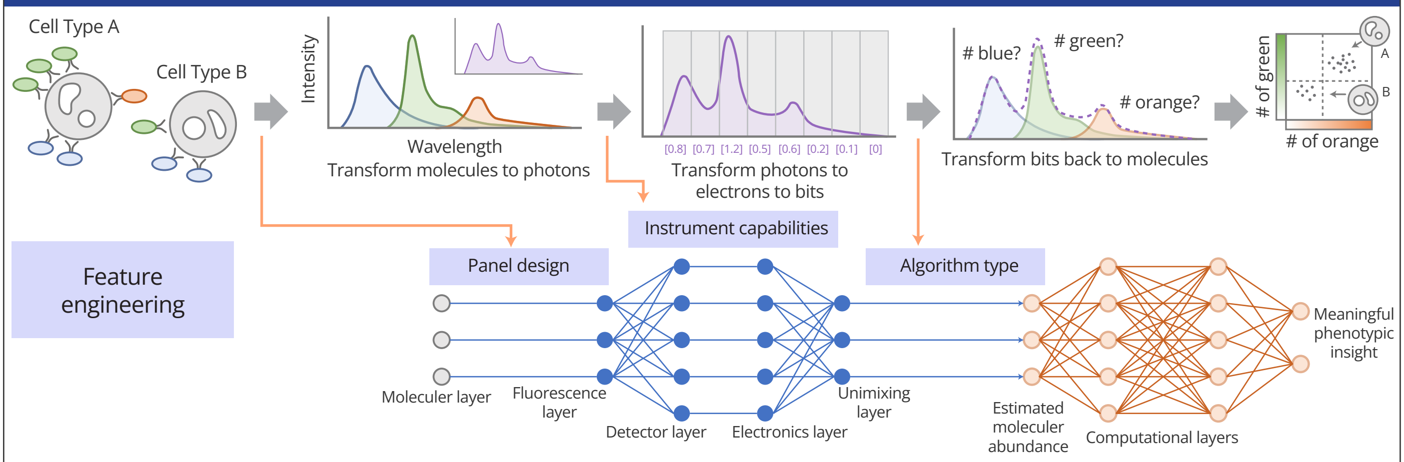
Both compensation and spectral unmixing aim to resolve signal overlap by accurately assigning fluorescence to the fluorochromes in the panel. Ultimately, these steps are critical for identifying which markers are present on specific cells in a sample. A more detailed explanation of spectral unmixing is provided in the Appendix.

Understanding the technology

Webinar minute 18:30

The quality of biological data in cytometry depends on the transformation process, which spans from detection to final interpretation. As shown in Figure 7, this process begins at the “molecular layer,” where researchers carefully select antibodies conjugated to fluorochromes to target specific biological molecules, an integral part of panel design that will be explored in greater detail in Chapter 3. When lasers excite these fluorochromes, they emit light signals. The emitted light then enters the “fluorescence layer,” where it is collected and separated into fluorochrome-specific wavelengths. Photodetectors in the “detector layer” convert the separated light into electrical signals, which are subsequently processed in the “electronics layer” into digital formats. Finally, in the “unmixing layer,” advanced mathematical algorithms assign portions of the total signal to specific fluorochromes, quantifying their contributions. This process allows for the accurate separation of overlapping signals, transforming digital signals into biologically interpretable data.

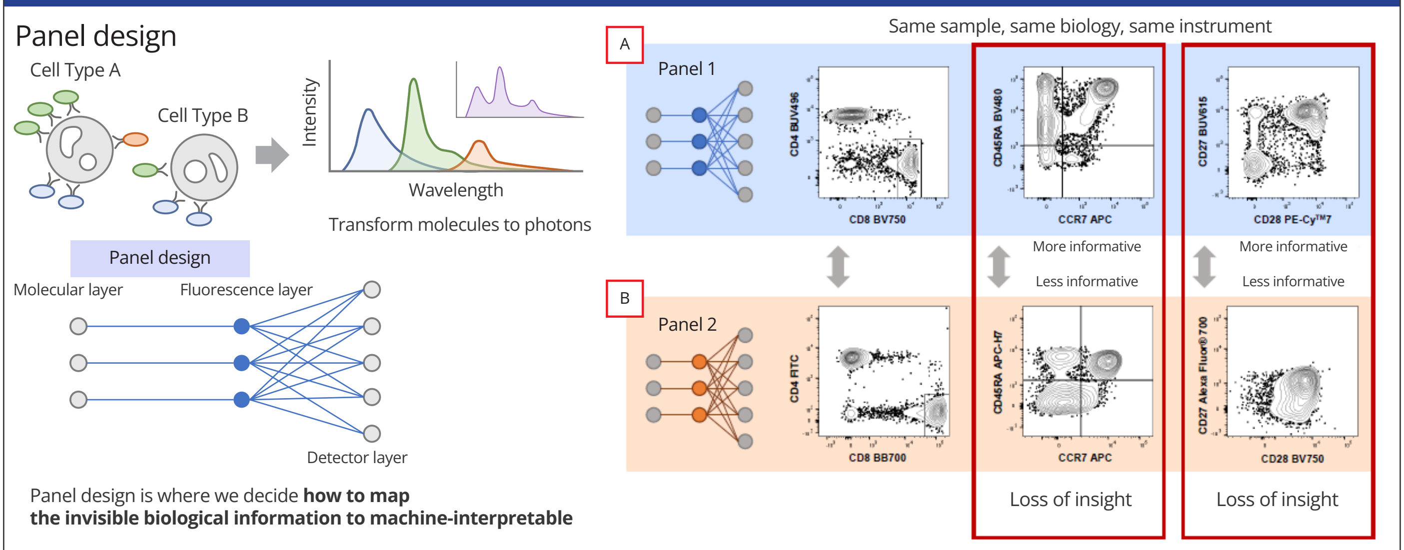
Figure 7: The signal transformation pathway in flow cytometry. Biological signals are converted into digital data through molecular, fluorescence, detector, electronics, and unmixing layers. Fluorochrome-labeled molecules emit photons, which are detected, digitized, and processed by algorithms to resolve spectral contributions.



The quality of cytometric data is influenced by panel design, instrumentation, and algorithms. Among these, panel design is critical, as it impacts the accuracy and depth of phenotypic data obtained from a sample, as illustrated by the example in Figure 8. Importantly, panel design is also the one aspect that users can control, making it a key area for optimizing experimental outcomes.

In this example, human whole blood from a single donor was stained using two different fluorochrome panels, Panel 1 (A) and Panel 2 (B). Both samples were analyzed using the same cytometer, but the results, shown as contour plots in the figure, reveal significant discrepancies. The plots in Panel 1 demonstrate clear distinctions between cell subtypes, while those in Panel 2, particularly the bottom-right panel, show significant information loss and reduced separation of cell populations. Notably, the fluorochromes selected for the panel were the only variable in this experiment; all other factors, including the sample and analyzer, were kept constant. This example highlights the importance of proper panel design in achieving robust and reliable cytometric results.

Figure 8: The impact of panel design on experimental outcomes. Two different fluorochrome panels (Panel 1, A; Panel 2, B) applied to identical samples using the same set of markers but with different fluorochrome assignments, yielding significantly different results.



Painting models

Webinar minute 24:24

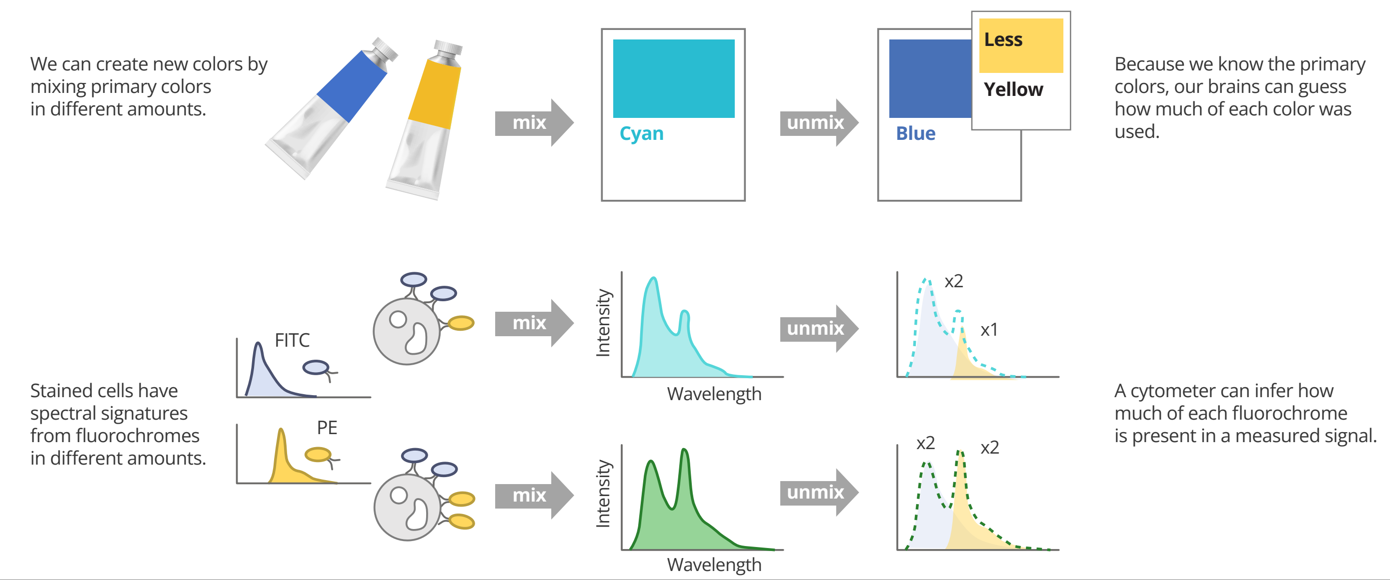
In conventional flow cytometry, each fluorochrome is designed to emit light at specific wavelengths when stimulated by a laser. Detectors are calibrated to capture these emissions, but since many fluorochromes have overlapping spectra, part of their signal spills into other channels. This overlap is addressed through a process called compensation, a process where the system mathematically adjusts for spillover by determining how much of a fluorochrome's signal appears in secondary detectors and subtracting it, ensuring the fluorochrome is only measured in its primary channel.

Spectral flow cytometry uses the same approach with a different implementation. Instead of relying on discrete detectors assigned to specific fluorochromes, it captures the full fluorescence emission profile of each fluorochrome across all detectors. This is more akin to how the human eye perceives color: our cone cells (red, green, and blue photodetectors) receive blended inputs from the light spectrum, and the brain interprets these signals to reconstruct the original color. For example, when looking at cyan, the human eye combines signals from blue and green light receptors to perceive it as a single color, with green cones acting as a proxy for yellow detection. This interpretation, however, is only possible because the brain already "knows" the initial colors and how they interact, allowing for an accurate decoding of the composite color.

Similarly, spectral flow cytometry reconstructs fluorescence signals by utilizing single-color controls to understand each fluorochrome's own spectral signature. Unlike compensation, which applies corrections only to certain spillover channels, spectral unmixing deconvolutes each fluorochrome's spectral signature from a complex mixture of signals detected across all channels. This makes unmixing more difficult to visualize than compensation because each detector contributes to the final signal reconstruction rather than merely subtracting spillage. Because of this, high-quality single-color controls are critical in spectral flow cytometry as they act as the system's reference for precisely deconstructing composite signals and assigning them to the correct fluorochromes.

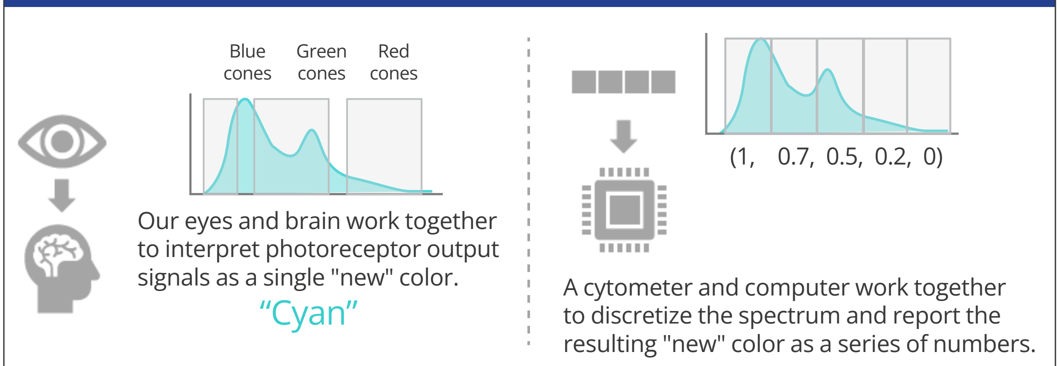
As illustrated in Figure 9, fluorochromes such as FITC (fluorescein isothiocyanate), which emits primarily green light, and PE (phycoerythrin), which emits orange-yellow light, may fluoresce simultaneously when bound to proteins expressed on the same cell. Their emission profiles partially overlap, resulting in a composite signal that includes both fluorochromes. Spectral cytometers use complex algorithms to "unmix" these signals by examining the full emission profile. This allows researchers to determine the specific contributions of FITC and PE to the composite fluorescence.

Figure 9: Spectral unmixing in cytometry. Just as primary colors mix to create new colors, fluorochromes combine to form unique spectral signatures. Spectral flow cytometers use unmixing algorithms to determine the contribution of each fluorochrome to the measured signal.



As illustrated in Figure 10, spectral cytometer detectors act similarly to cone cells, sensing light at multiple wavelengths. Specialized software then converts the data into a numerical value, allowing researchers to precisely resolve complex fluorescent signals.

Figure 10: An analogy between human vision and cytometer photodetectors. Like the cone cells in human eyes, cytometers use detectors to translate spectral signals into meaningful data.



Both compensation in conventional flow cytometry and unmixing in spectral flow cytometry rely on mathematical models to resolve overlapping signals. However, these strategies differ in their approach to managing challenges posed by signal overlap. Conventional flow cytometry has a one-detector-per-fluorochrome ratio, and the key issue is the control of signal spillover, which occurs when fluorescence emission from one dye is partially detected in a nearby detector. Compensation adjusts for these spillover artifacts in multicolor panels by working within two-dimensional emission patterns and assigning signals to the appropriate fluorochrome using spillover matrices, which are established through single-color controls prepared alongside the sample on the day of analysis.

In contrast, spectral flow cytometry employs a many-detectorper-fluorochrome ratio, leveraging the full spectral profile of each dye across multiple detectors. This means that the challenge shifts to managing signal similarity, where closely overlapping spectral signatures must be accurately separated. Spectral unmixing interprets the multidimensional spectral data collected by these detectors and converts it into a two-dimensional representation, allocating each signal to the appropriate fluorochrome. Both approaches require appropriate controls and careful panel design to minimize errors and ensure reliable data processing. For a deeper understanding of the mathematical principles underlying unmixing, refer to the Appendix.

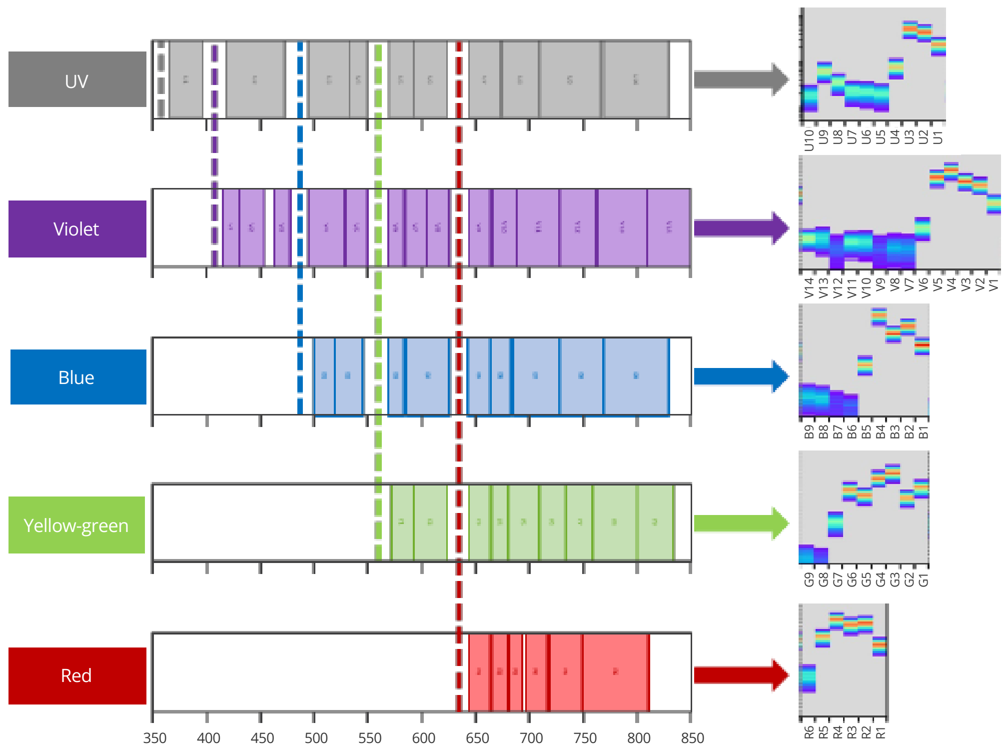
Spectral output: Visualizing and interpreting data

Webinar minute 16:14

One of the distinctive features of spectral flow cytometry is its data visualization method, which combines signals from all lasers and detectors into spectral plots (Figures 11 and 12). This approach allows researchers to examine the cumulative emission profile of fluorochromes, also known as their spectral signature.

Figure 11 displays the spectrum output of all five laser lines. Each dye's fluorescence emission is captured through various detectors, resulting in a single, continuous spectral signature. This visualization provides a comprehensive overview of how each fluorochrome fluoresces across the entire wavelength spectrum.

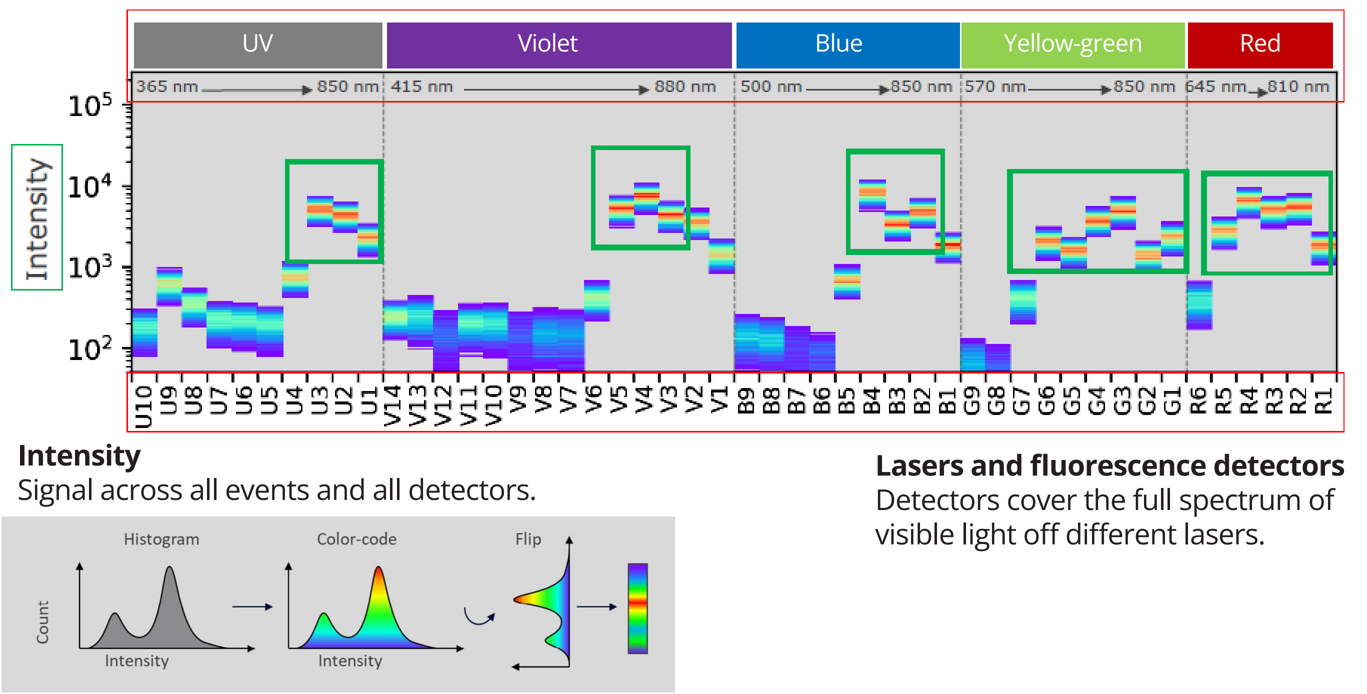
Figure 11: Integrated spectral output from multiple lasers. Full emission profiles of fluorochromes are captured across all lasers and detectors. This cumulative representation integrates fluorescence signals to create a single spectral profile for each dye.



The emission of each fluorochrome is displayed across the full spectrum.

In Figure 12, the detectors are arranged sequentially along the x-axis, organized by increasing wavelength and grouped by their corresponding lasers. The y-axis displays the signal intensity detected at each wavelength. The colored bands in the plot represent the intensity distribution across detectors, functioning as a heatmap. Regions with higher fluorescence intensity are depicted with “warmer” colors, such as red, while lower-intensity areas are shown with “colder” colors, such as blue. This display method simplifies complex data, enabling the precise detection of dye-specific spectral patterns.

Figure 12: Interpreting spectral plots. Detectors are arranged along the x-axis by increasing wavelength and grouped by laser. The y-axis represents fluorescence intensity, with color-coded bands indicating intensity distributions across detectors. Warmer colors (e.g., red) indicate higher fluorescence intensity, while colder colors (e.g., blue) represent lower fluorescence intensity.



PART 3

CHAPTER 3

Panel Design and Controls: Why They Still Matter in Spectral Flow Cytometry

Panel design

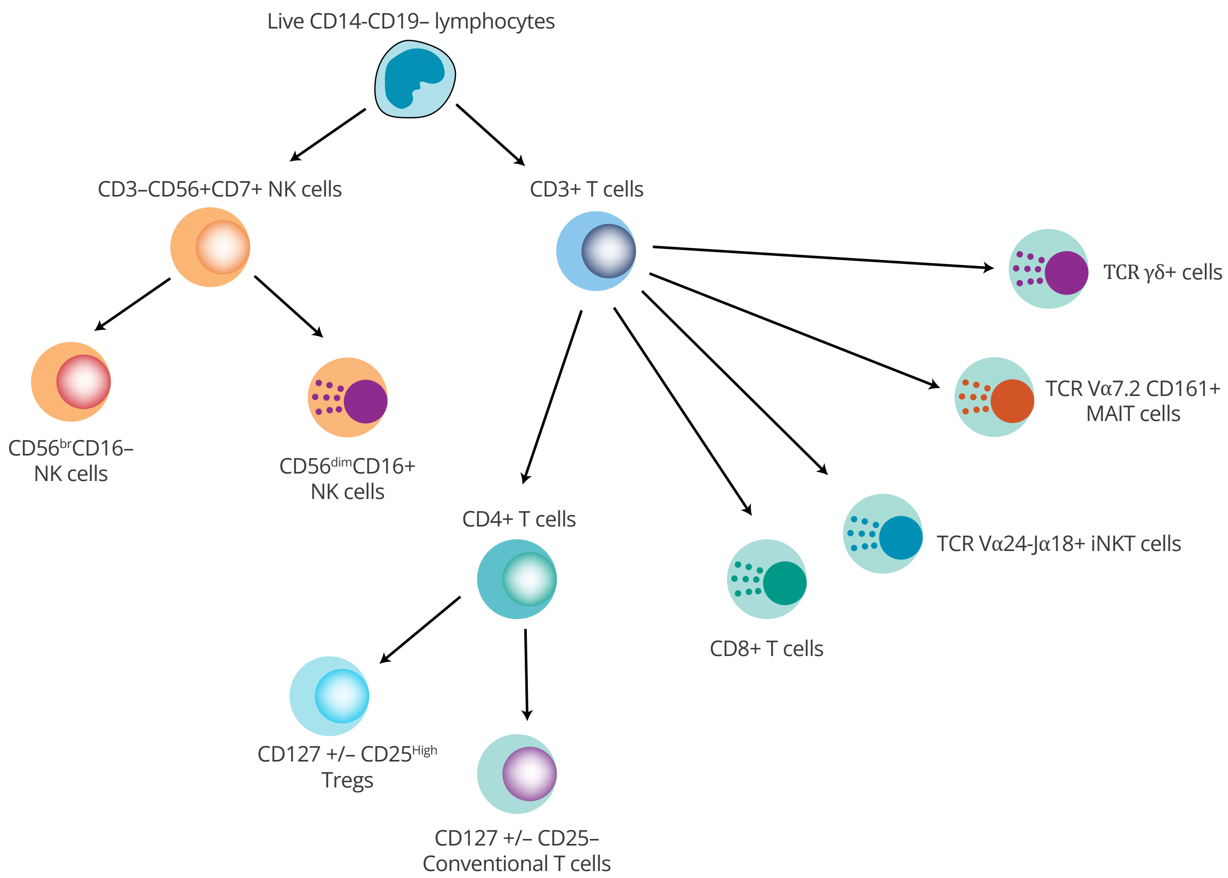
While the previous chapters described the similarities and differences between conventional and spectral flow cytometry technology, much remains the same with regard to panel design. Thoughtful panel design is still the key to quality data (Figure 7). This process requires careful experimental design to balance the quantity of data obtained with its quality. Panel design is one of the most significant steps in this process, as it maximizes data quality and resolution while minimizing spectral overlap. Effective panel design takes into consideration the previously described principles of fluorescence detection and spectral unmixing. It integrates three important factors: the biology of the target population, the properties of fluorochromes, and the capabilities of the instrument.

Know the biology of the target population

Panel design depends on a solid understanding of the biology behind the experiment. This comprises determining the target population or populations and choosing phenotypic markers that are both relevant to the scientific question and reflective of the functional characteristics of the cells being studied. To achieve this, the process requires validation, as expression levels may vary or be unknown in different experimental conditions. This includes understanding marker density, variations within the population, and co-expression patterns across several cell subsets. At least one marker is needed to distinguish one lineage of cells from another. In some cases, such as for natural killer (NK) cells, markers that exclude other lineages may be required to identify the population within a given sample. Most lineages contain specialized sub-populations distinguished by proteins that relate to their unique functional capacities, which must be included for deep cell profiling. Markers related to homing, activation, and exhaustion are usually evaluated for functional or phenotypic analysis and may be expressed on multiple lineages. Therefore, when designing complex panels, it is crucial to know how these markers are expressed on all cell subtypes within the given population, even if the marker is not used directly to characterize all of them. Resources like the BD Interactive Human Cell Map can offer useful insights and optimized panels of commonly studied lineages to use as a starting point for researchers who are studying a new cell type or who are looking for an overview of markers used to identify cell populations (Interactive Human Cell Map).

Figure 13 illustrates the importance of understanding antigen density and co-expression in panel design by showing essential markers used to detect and distinguish major immune cell subsets such as T cells, NK cells, and their respective subsets, many of which have shared markers. This underlines the complexity of marker expression across different cell types, emphasizing the importance of strategic marker selection to resolve populations effectively and achieve clear, interpretable data.

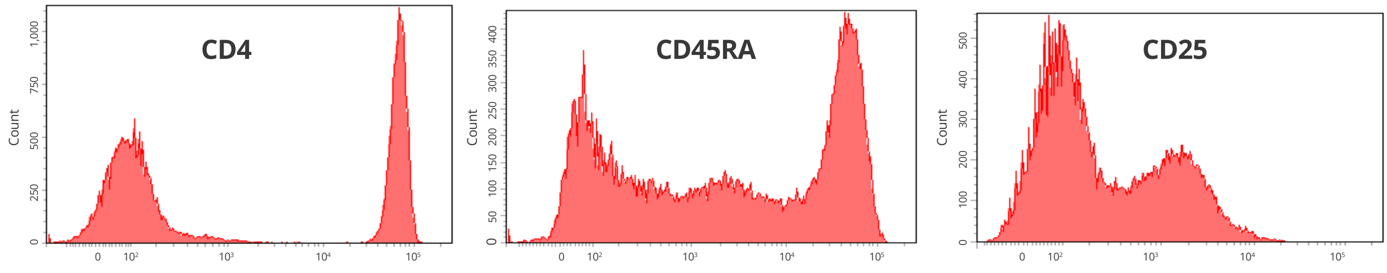
Figure 13: Schematic representation of immune cell subsets and their defining markers, highlighting the hierarchical relationships and differentiation pathways between major lymphocyte populations.



Once the target antigens and co-expression patterns have been identified, they can be divided into three antigen tiers based on expression profiles and functional roles. This tiered classification helps to prioritize marker placement in the panel:

- **Primary antigens:** These markers are well-characterized and can be classified as positive or negative. They usually indicate broad cell subsets or lineages such as T cells, B cells and NK cells. Examples include CD3, CD4, and CD19, which are characterized by bimodal expression patterns that make them ideal for gating and identifying major populations. The histogram for CD4 illustrates this bimodal expression pattern (Figure 14, left).
- **Secondary antigens:** These markers are also well-characterized; however, they are expressed at higher densities and over a continuum. They are commonly used to identify functional subsets or activation states. Examples include CD27, CD28, CD45RA, and CD45RO, characterized by expression patterns that span gradients rather than separate positive or negative populations. The histogram for CD45RA shows this continuum expression pattern (Figure 14, middle).
- **Tertiary antigens:** These indicators are expressed at low levels, can vary with activation and may have less defined expression patterns. They are often critical to separating the population of interest and typically assigned bright fluorochromes with low resolution impact. Examples include CD25, STAT5, and FoxP3, which are characterized by lower expression levels and more varied patterns. The histogram for CD25 highlights this variability (Figure 14, right).

Figure 14: Antigen tiers and expression patterns for panel design. Markers are grouped into primary, secondary, and tertiary tiers based on expression levels and functional roles. The histograms are examples of CD4 (primary, bimodal expression), CD45RA (secondary, continuum expression), and CD25 (tertiary, low and variable expression).

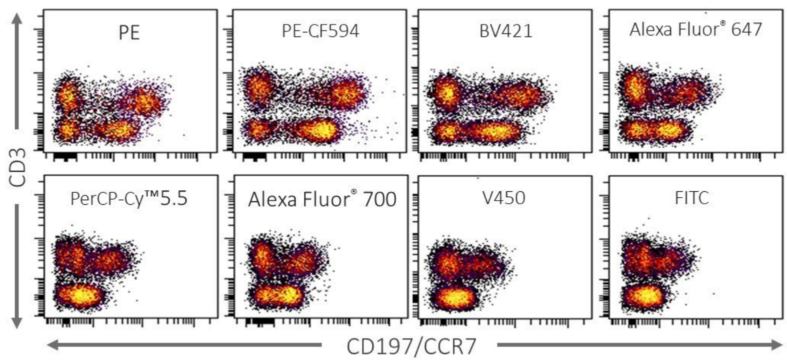


Know the properties of fluorochromes

The selection of appropriate fluorochromes is an important aspect of panel design as it directly impacts the quality of flow cytometric data. Identifying the fluorochromes available for examining the target population, as well as reviewing their characteristics—such as excitation and emission profile, brightness, and detection compatibility with the chosen instruments—is a key phase in the process. Bright fluorochromes, for example, are particularly useful for detecting antigens expressed at low levels, where signal intensity must outperform background autofluorescence or non-specific signal for proper identification. To ensure robust resolution, both the biological properties of the target antigens and the optical properties of the fluorochromes being used must be carefully considered. For example, using bright fluorochromes for low density antigens can increase separation. Resolution, which refers to the ability to discriminate between negative, dim, medium, and bright populations, can be influenced by how clearly the positive population separates from the negative population, which can mask medium or dimpositive signals.

In addition to antigen levels, the brightness of the fluorochrome used can further impact resolution. Figure 15 depicts these principles by comparing the signal resolution of different fluorochromes used to evaluate the expression of CD3 and CD197/CCR7. In this example, the CD197/CCR7 is stained with multiple fluorochromes in order of decreasing brightness. Brighter fluorochromes, such as PE, produce stronger signals that aid in the differentiation of negative, dim and bright populations, making them appropriate for detecting antigens expressed at low levels. Fluorochromes with lower brightness, such as FITC, make it difficult to identify subtle differences in antigen expression.

Figure 15: Comparison of fluorochromes (PE, PE-CF594, BV421, Alexa Fluor® 647, PerCP-Cy™5.5, Alexa Fluor® 700, V450, FITC) used to measure CD3 and CD197/CCR7 expression. The scatter plots highlight the differences in fluorochrome brightness and their impact on data resolution.



- Proper fluorochrome choices reveal more about biology
- Bright fluorochromes are important when looking at lowly expressed antigens

The brightness of commonly available fluorochromes is well known and Figure 16 is a tool that provides suggestions on how to pair these fluorochromes with antigens of varying levels of expression. Beyond fluorochrome brightness and antigen expression levels, another key factor in fluorochrome selection is spectral similarity, which can lead to fluorescence spillover in conventional flow cytometry and spectral overlap in spectral flow cytometry, ultimately affecting data quality.

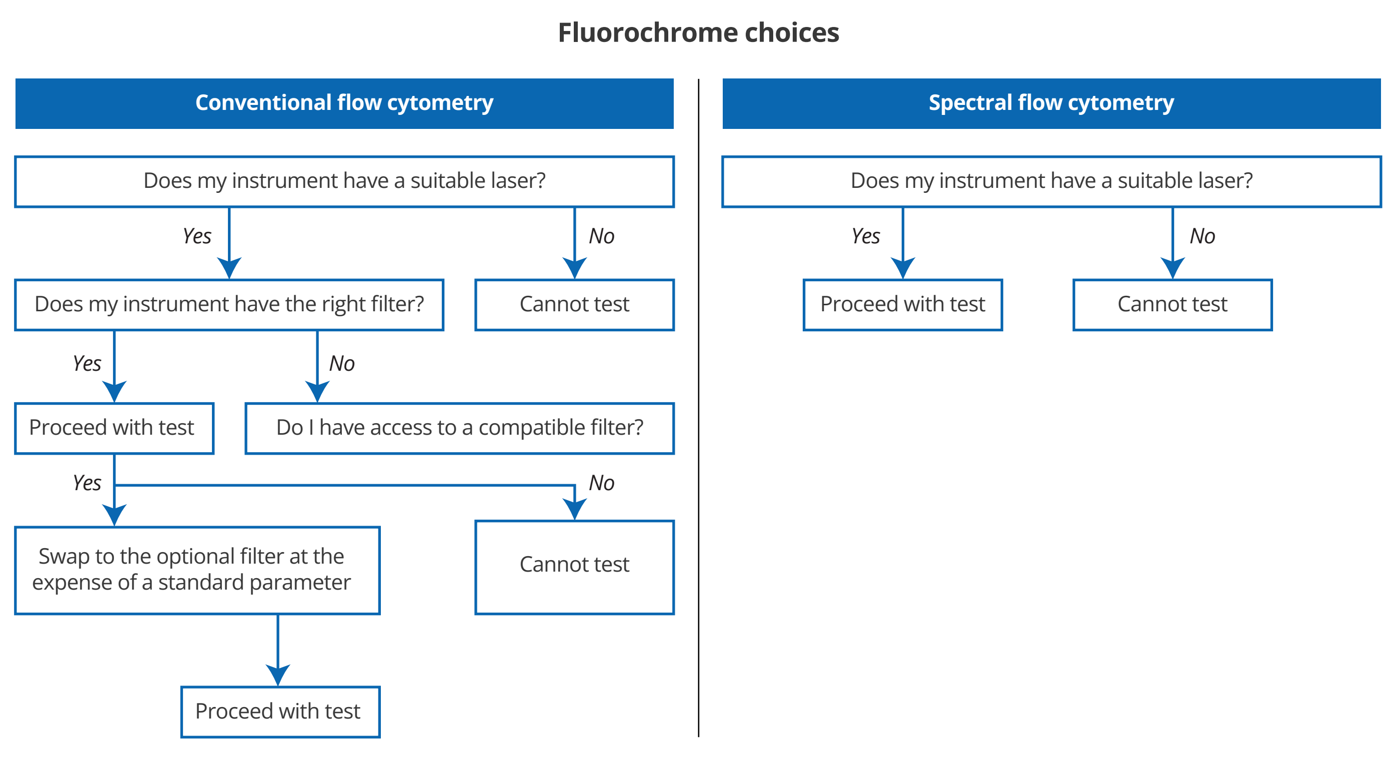
Figure 16: Antigen density and corresponding fluorochromes. Recommended fluorochromes are grouped by brightness (e.g., Very Bright 4/4, Bright 3/4, Moderate 2/4, and Dim 1/4) and matched with antigen density (low, medium, and high).

	Low	Medium		High
	Fluorochrome			
Laser	Very Bright 4/4	Bright 3/4	Moderate 2/4	Dim 1/4
Ultraviolet (355 nm)		BD Horizon™ BUV563 BD Horizon™ BUV615 BD Horizon™ BUV661 BD Horizon™ BUV737	BD Horizon™ BUV395 BD Horizon™ BUV496	BD Horizon™ BUV805
Violet (405 nm)	BD Horizon™ BV421 BD Horizon™ BV650 BD Horizon™ BV711	BD Horizon™ BV480 BD Horizon™ BV605 BD Horizon™ BV786	BD Horizon™ BV510 BD Horizon™ BV750	BD Horizon™ V450 BD Horizon™ V500
Blue (488 nm)	BD Horizon™ BB515 BD Horizon™ BB700 BD Horizon™ RB613 BD Horizon™ PE-CF594 PE-Cy5 BD Horizon™ RB705 BD Horizon™ RB744 BD Horizon™ RB780	PE PE-Cy7	FITC Alexa Fluor™ 488 BD Horizon™ RB545 PerCP-Cy5.5	PerCP
Yellow/green (561 nm)	PE BD Horizon™ PE-CF594 PE-Cy5 PE-Cy7 BD Horizon™ RY586 BD Horizon™ RY703 BD Horizon™ RY775	BD Horizon™ RY610		
Red (640 nm)		APC Alexa Fluor™ 647 BD Horizon™ APC-R700 BD Horizon™ R718		Alexa Fluor™ 700 APC-H7 APC-Cy7

Know the instrument capabilities

Understanding instrument configuration is crucial when designing experimental panels. The first step is to determine whether the instrument employs conventional or spectral flow cytometry, as each requires distinct approaches for fluorochrome selection and optimization. In conventional flow cytometry, fluorochrome compatibility relies on the availability of lasers, filters, and detector configurations (Figure 17). This setup may require filter adjustments when optimizing panels. In contrast, spectral flow cytometry provides flexibility in panel design by detecting emissions across the entire spectrum, eliminating the need to match fluorochromes to individual detectors and therefore requiring only laser compatibility to proceed with testing (Figure 17). This approach also enables better separation of autofluorescence from true signal, improving data accuracy in complex samples.

Figure 17: Instrument configuration for fluorochrome selection. Conventional flow cytometry relies on matching lasers and filters for fluorochrome detection, potentially requiring adjustments for compatibility. Spectral flow cytometry streamlines this process, relying solely on laser compatibility to support flexible panel designs.



Design the panel and select appropriate controls

After identifying the markers, reviewing the available fluorochromes, and understanding the instrument configuration, the next step in panel design involves strategically pairing fluorochromes with antigens based on their expression levels to optimize signal resolution and minimize spectral overlap. Bright fluorochromes should be assigned to antigens expressed at low levels, whereas dim fluorochromes should be paired with antigens expressed at high levels. Moreover, careful attention must be given to avoid using highly similar fluorochromes for markers that are coexpressed on the same cells, as this can result in spread of signal and loss of resolution (Figure 8).

Ensuring accurate controls

Once the panel is completed, appropriate single-color controls are required to establish the compensation and unmixing matrices for conventional and spectral flow cytometry, respectively. These controls provide the necessary reference spectral emission profiles needed for accurate compensation and spectral unmixing. Because spectral flow cytometry analyzes the entire spectral signature of each fluorochrome during unmixing, it is particularly important to use single-stain controls that precisely match the spectrum of the fluorochrome applied to the samples. One advantage of spectral flow cytometry is the ability to perform autofluorescence extraction by assigning it to a control for removal, thereby improving resolution in samples with high levels of background auto-fluorescence.

There are several sample-type options for single-color controls including cells, antibody capture beads, and synthetic cell mimics. Each of these options has advantages and disadvantages that need to be considered when selecting the best fit for the experimental set-up. For example, beads can serve as single color controls in situations where using cells is challenging—such as when sample availability is limited, or when a marker is low in abundance, dim or both, making it difficult to capture sufficient events to generate a reliable matrix. Overall, selecting the most appropriate control is critical for reducing variability, ensuring reliable data quality, and obtaining accurate compensation or spectral unmixing in flow cytometry experiments.

Conclusion

Overall, flow cytometry, whether conventional or spectral, is a series of transformations that convert biology into quantifiable data. It allows researchers to evaluate several parameters simultaneously, providing a comprehensive view of cellular identity, function, and interactions. However, obtaining high-quality data necessitates careful experimental design and execution, as each step—from panel design to fluorescence detection and data interpretation—influences the ultimate result.

Spectral flow cytometry extends the capabilities of conventional flow cytometry, providing additional flexibility in fluorochrome choice and enabling the generation of larger panels. The ability to unmix signals based on complete spectrum also introduces the ability to identify and extract auto-fluorescent signals. Careful panel design through thoughtful fluorochrome-antigen pairing, together with appropriate controls, is critical to maximizing biological resolution. These steps allow researchers to convert raw fluorescence data into biologically meaningful information, enabling the identification of populations of interest.

Appendix

Mathematical models

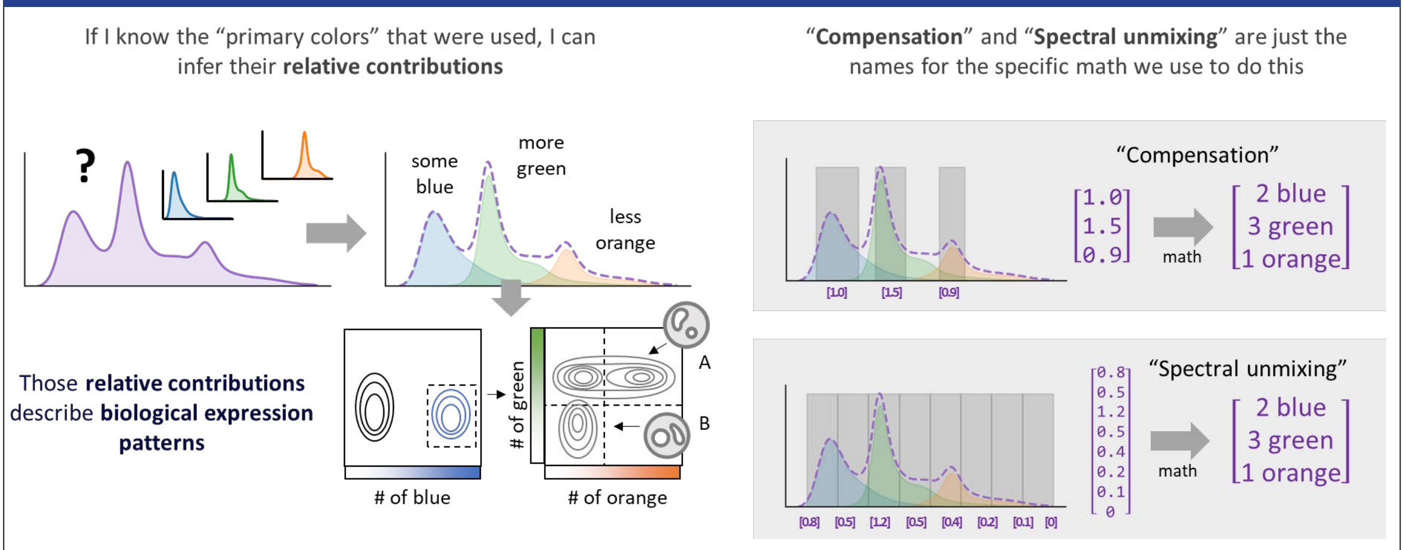
Webinar minute 27:43

Understanding how detected light signals are transformed into biologically relevant data is critical when using flow cytometry. This method entails detecting fluorescence emissions from tagged cells and processing these signals using algorithms that separate individual fluorochrome contributions. These transformations are driven by mathematical principles, with compensation and spectral unmixing serving as the primary approaches for resolving overlapping signals. By calculating how overlapping fluorescence emissions from multiple fluorochromes contribute to the measured signal, researchers can infer protein expression patterns in cells, allowing for precise phenotypic analyses.

Both compensation and spectral unmixing address the issue of spectral overlap, but their implementation and scope vary significantly. Compensation is employed in conventional flow cytometry to account for fluorescence spillover, which happens when one fluorochrome's emission can be detected in neighboring channels. As shown in the top-right portion of Figure 1A, compensation mathematically corrects for this by removing overlapping signal, ensuring that each detector appropriately displays the assigned fluorochrome. This is accomplished using spillover matrices based on single-color controls.

On the other hand, spectral unmixing, a feature unique to spectral flow cytometry, resolves overlap by evaluating each fluorochrome's entire emission profile. As shown in the bottom-right portion of Figure 1A, instead of assigning specific wavelengths to detectors, spectral flow cytometry collects the full spectral signature across multiple detectors. Advanced algorithms then deconvolute these overlapping signals by comparing them to reference spectra, extracting individual fluorochromes from the total signal. This approach enables spectral flow cytometry to handle higher-dimensional data, resulting in greater panel flexibility and more robust resolution of complex fluorescence patterns.

Figure 1A: Mathematical transformation of fluorescence signals in flow cytometry. The left panel shows how relative fluorochrome contributions are inferred from overlapping spectral signals. The right panels compare conventional compensation (top), and spectral unmixing (bottom).

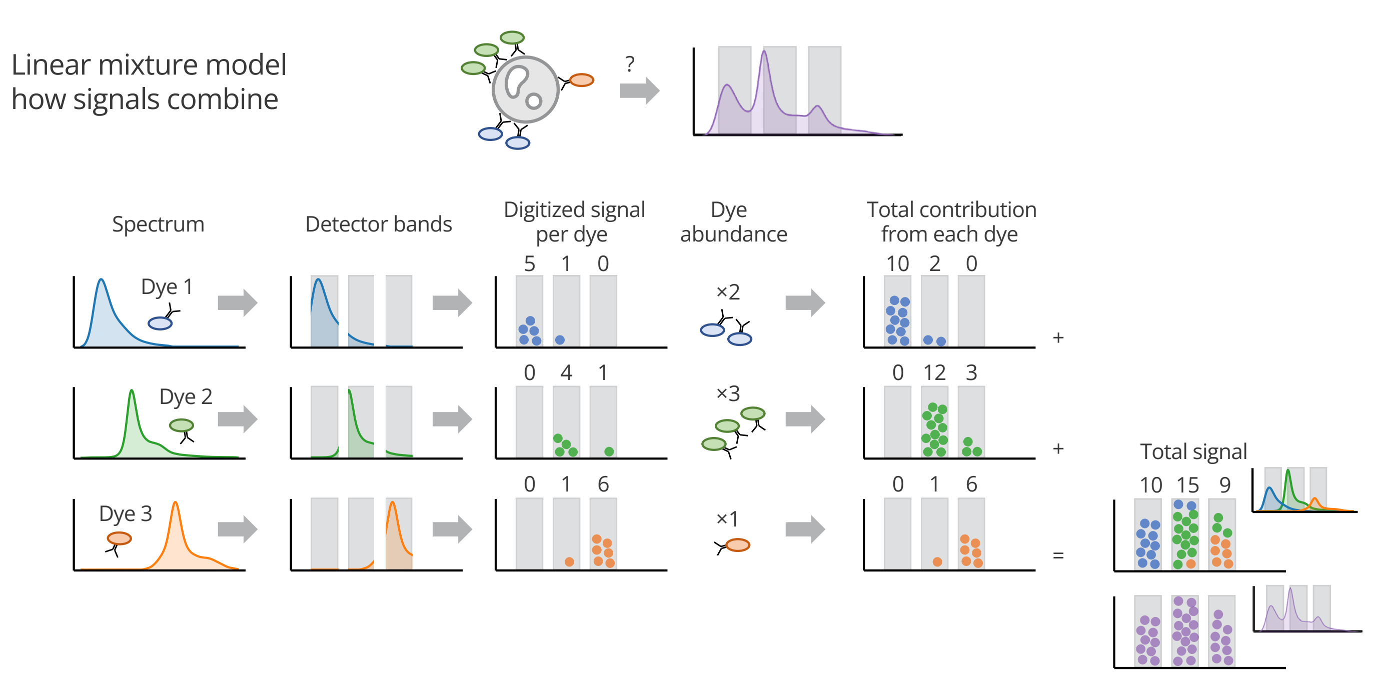


Linear mixture model

Webinar minute 27:43

The linear mixture model is a mathematical method for interpreting fluorescence data detected by flow cytometers. It describes how signals from several fluorochromes overlap and mix when detected across multiple detectors. This method is essential for resolving complex spectral data and accurately assigning signals to their corresponding fluorochromes. Figure 2A depicts this transformation process step by step, demonstrating how signals are digitized and mixed. Beginning with Dye 1 (blue), the spectrum is divided into separate detector bands that measure different parts of the emitted light. These bands are converted into discrete units (shown as blue dots in the figure), with each detector recording a specific signal strength (5, 1, and 0). Because there are two molecules of Dye 1 on the cell, the signal is multiplied by two, resulting in 10, 2, and 0 as final contributions per detector. The same procedure applies to Dye 2 (green) and Dye 3 (orange). Their individual spectra are digitized, multiplied by the number of dye molecules, and added together to calculate the overall signal measured in each band. The resulting signals (10, 15, and 9) represent the total fluorescence detected by the cytometer resulting from the three dyes (purple). However, as shown in the figure, the cytometer only detects the total signal, not the specific contributions of each dye. To resolve these contributions, unmixing methods must be used to mathematically separate overlapping spectra into their original components.

Figure 2A: Linear mixture model illustrating how signals combine. Fluorescent dyes emit spectra (left) detected in multiple bands. Each dye's signal is digitized into discrete units (dots) based on abundance, and contributions from all dyes are summed to produce the total signal detected (right).



The notion of signal combination in spectral flow cytometry can be described more concisely using a matrix form, as shown in Figure 3A. Instead of representing digitized detector signals as vertical bars, the data can be rotated by 90° and grouped into a matrix. This method simplifies the mathematical modeling of how fluorescence signals mix. In the figure, the signal is represented as the sum of contributions from each fluorochrome. The total signal observed is calculated by multiplying the spectrum of each dye (blue, green, and orange) by its abundance (the number of molecules present) and then adding them together to obtain the total signal.

Matrix representation and spectral unmixing

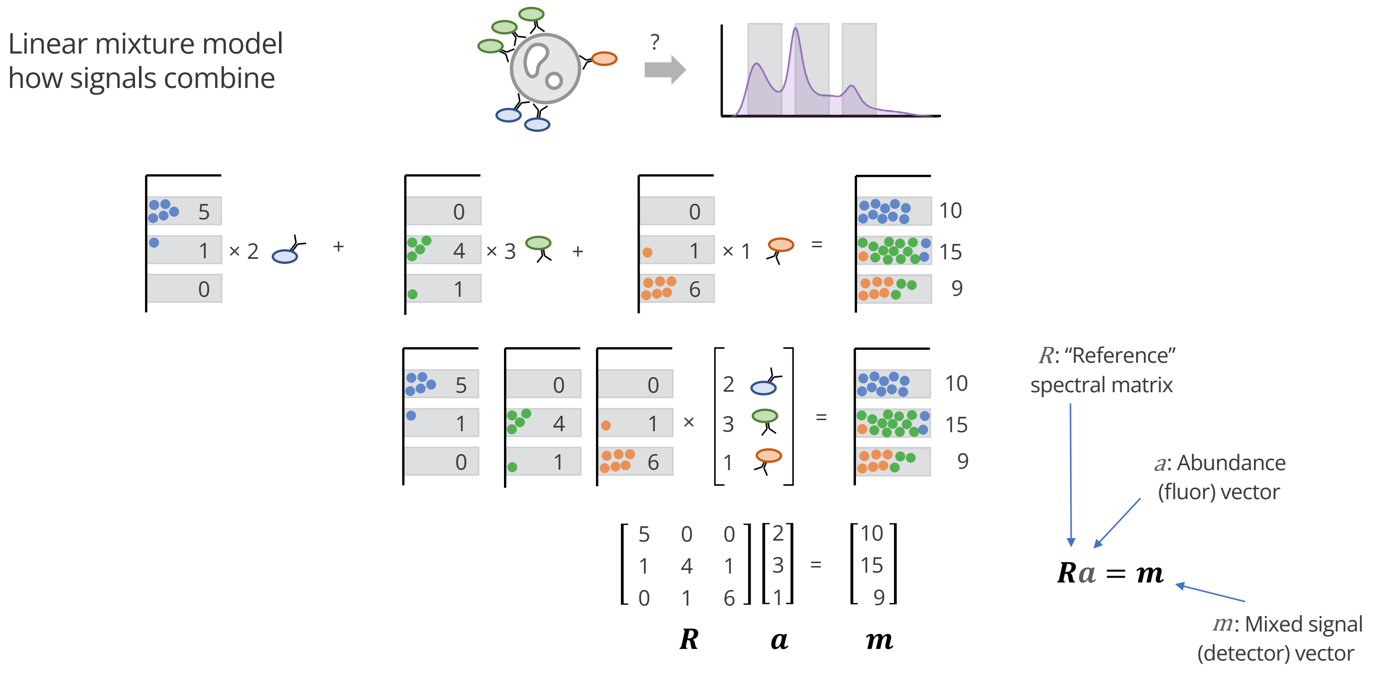
Webinar minute 30:30

Spectral flow cytometry can be mathematically defined using a linear mixture model, which reduces signal output to a compact matrix representation, as shown in Figure 3A. The figure illustrates how measured signals result from a combination of spectral signatures and fluorochrome quantities. At its core, the model is expressed as the equation:

$$\boldsymbol{R} \cdot \boldsymbol{a} = \boldsymbol{m}$$

Here $\boldsymbol{R}$ represents the "reference spectral matrix," which contains the spectral signatures of each dye across all detectors. The variable $\boldsymbol{a}$ corresponds to the "abundance vector," which quantifies the amount of each fluorochrome present in the sample, whereas $\boldsymbol{m}$ represents the "measured signal vector," which indicates the overall signal recorded across all channels. This relationship mathematically defines what is known as the "forward problem," which defines how the observed detector signals are generated depending on the dyes' spectral properties and abundances. In essence, it describes how several fluorochromes contribute to the total measured signal. In conventional flow cytometry a similar matrix, known as the "spillover matrix," is used to describe how fluorochrome signals overlap between detectors. However, spectral flow cytometry goes beyond this by accounting for the entire emission profile of each dye, allowing for greater resolution and more complex analyses.

Figure 3A: Linear mixture model and matrix representation of signal generation in spectral flow cytometry. Signals from multiple fluorochromes (blue, green, and orange dyes) combine to produce the total measured signal detected across different channels. The process is represented mathematically as $R \times a = m$, where R is the reference spectral matrix, a is the abundance vector, and m is the mixed signal detected by the instrument.



Solving the inverse problem

Webinar minute 31:35

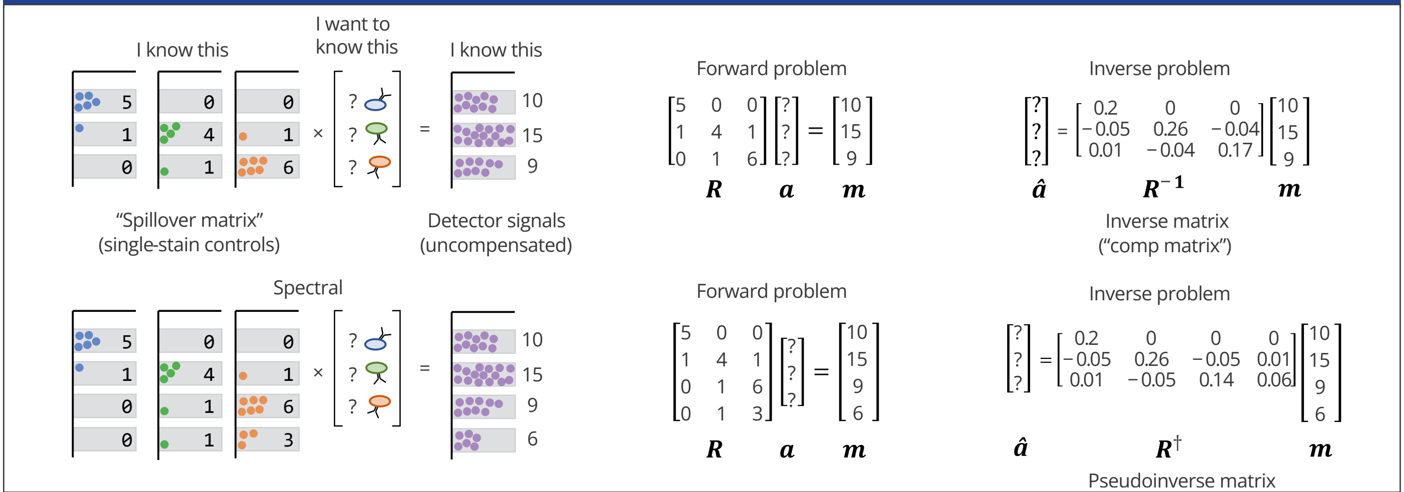
This mathematical framework lays the foundation for understanding both the process of signal production and the approach needed to analyze these signals. The forward problem describes signal generation, which involves combining known R and fluorochrome a to anticipate the m. However, in practice, the goal is often to reverse the process and calculate the abundance of each fluorochrome based on the detected signals. The process, also known as the "inverse problem," entails unmixing the overlapping signals detected by the cytometer.

As illustrated in Figure 4A, to solve the inverse problem, the process begins by constructing R, using single-color controls. Samples stained with a single fluorochrome are used to determine their unique contribution to each detector. Once this spectral matrix is established, it can be mathematically inverted (R^{-1}) to create a compensation matrix. Multiplying this compensation matrix by the m yields an estimate of fluorochrome a. The process, also known as "spectral unmixing," resolves overlapping emission signals by decomposing them into their constituent parts. It ensures that contributions from each dye are correctly detected, even when their spectra overlap considerably. As a result, the linear mixture model functions as both a signal production predictor and a corrective tool for separating complex overlapping signals during analysis.

Conventional compensation, represented in the top panel of Figure 4A, employs a 1:1 dye-to-detector ratio referred to as a "spillover matrix," which describes how signals leak between detectors. Spectral unmixing, displayed in the bottom panel of the figure, expands on this approach by using multiple detectors to capture the entire emission profile of fluorochromes, resulting in improved resolution and flexibility. When there are more detectors than fluorochromes, such as in spectral flow cytometry, the R may not be directly invertible. In such cases, a pseudoinverse matrix ($R^{\dagger}$) created via singular value decomposition (SVD), can offer a reliable solution. This enables the decomposition of complex, overlapping signals, even when traditional matrix inversion (R^{-1}) is mathematically unfeasible.

Overall, while both methods use linear mixture models, the primary distinction is in the matrix structure; while conventional compensation uses a simpler 1:1 dye-to-detector ratio, spectral unmixing uses a larger detector-to-dye ratio to resolve complex spectral overlaps.

Figure 4A: Matrix representation of spectral unmixing and compensation. Conventional compensation (top) uses a spillover matrix (R) derived from single-color controls to describe signal generation ($R \cdot a = m$). The inverse of R (R^{-1}) is then applied to resolve fluorochrome abundances (a) from the measured signals (m). Spectral unmixing (bottom) uses a more detailed spectral matrix (R) to capture full emission profiles. When the matrix is not directly invertible, a pseudoinverse ($R^{\dagger}$) provides a robust solution for resolving overlapping signals.



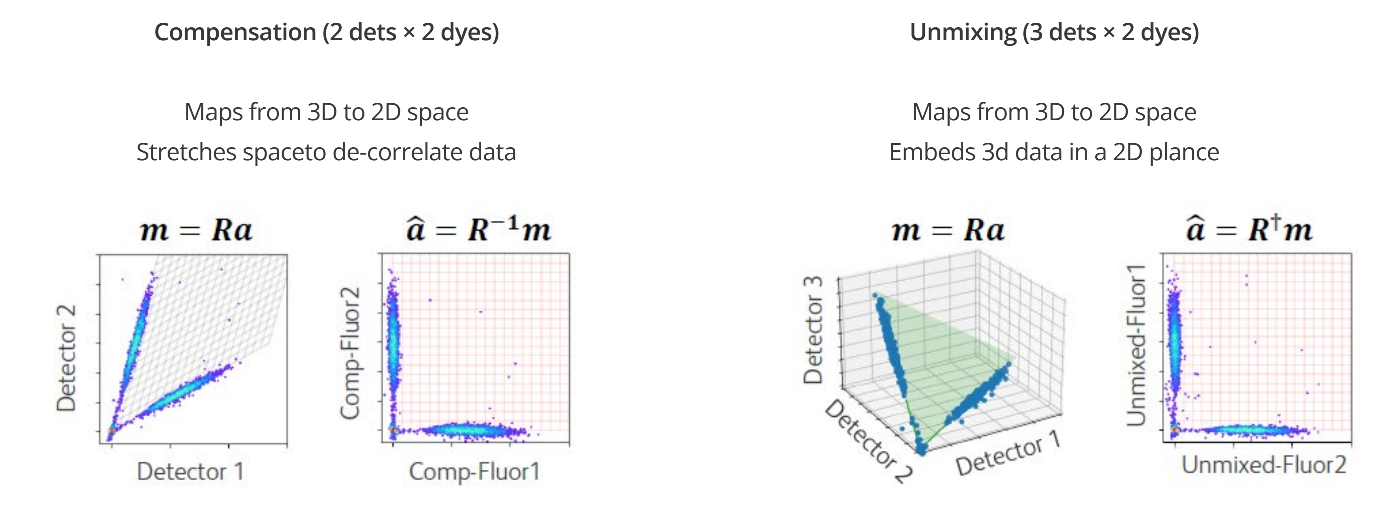
Geometric mapping in signal processing

Webinar minute 33:35

Another approach to understand compensation is through a geometric mapping process between distinct data spaces. Figure 5A (left) illustrates a two-detector, two-dye system, with signal spillover causing associated signals to appear as skewed populations in the uncompensated plot. The compensation procedure expands the two-dimensional detector space into an orthogonal space, where dye signals are de-correlated and represented as separate populations. This technique efficiently eliminates spillover artifacts by splitting signals geometrically to reflect actual dye quantities. This transformation gives the dot plot results that are commonly seen in flow cytometry analysis.

Figure 5A (right) illustrates how spectral unmixing extends this concept to higher-dimensional spaces. Unlike compensation, which maps equal-dimensional spaces, spectral unmixing maps from a higher-dimensional detector space (where the number of dimensions matches the number of detectors) to a lower-dimensional fluorochrome space (where dimensions correspond to the number of fluorochromes). Adding a third detector to collect additional spectral data broadens the data set into a higher-dimensional space. Nonetheless, the data is still displayed on a two-dimensional plane. Spectral unmixing then mathematically embeds this higher-dimensional dataset, in a lower-dimensional space that efficiently resolves spectrum overlaps, in a process similar to compensation but designed for more complex spectral data.

Figure 5A: Comparison of compensation and spectral unmixing. Compensation (2 detectors, 2 dyes) transforms detector space into an orthogonal plane to de-correlate signals (left). Spectral unmixing (3 detectors, 2 dyes) projects higher-dimensional data into a lower-dimensional space using a pseudoinverse matrix ($R^{\dagger}$) to resolve spectral overlaps (right).



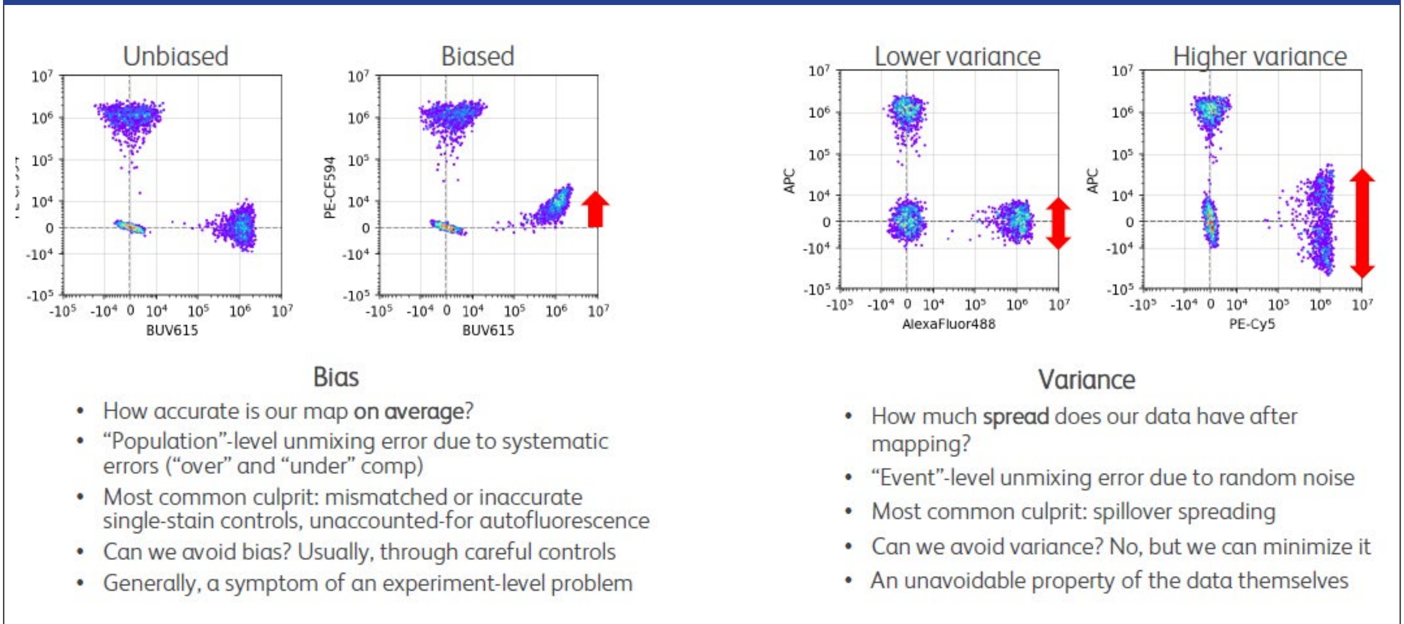
Assessing data accuracy (bias) and precision (variance)

Webinar minute 40:15

The framework of linear mapping in the mathematical model provides an effective approach for evaluating how the mapping process affects the quality of unmixed data. As spectral unmixing relies on a mathematical model to interpret data, its performance may be quantitatively assessed using terms like "bias" and "variance" to define the model's accuracy and consistency. As represented in Figure 6A (left), "bias" reflects the accuracy of the mapping process. Systematic errors, such as under- or overcompensation, cause data distortions at the population level. These errors are frequently caused by discrepancies between the expected and actual spectra of dyes, which may result from poor-quality single-stain controls or unaccounted autofluorescence. Avoiding bias requires meticulous preparation of controls, accurate acquisition during instrument setup, and careful evaluation of single-stain data.

On the other hand, as it can be observed in Figure 6A (right), "variance" reflects the precision of the mapping process. Rather than focusing on average accuracy, variance evaluates the spread of individual events relative to their predicted values. This spread is caused by random noise inherent in measurements, which is sometimes amplified when using dyes with overlapping spectra, resulting in spillover spreading. Variance cannot be completely avoided, but it can be minimized through optimized panel design, precise instrument calibration, and robust algorithm development.

Figure 6A: Assessing data accuracy (bias) and precision (variance). Bias reflects systematic population-level errors caused by spectral mismatches or poor single-stain controls, leading to under- or overcompensation (left). Variance reflects random event-level errors due to measurement noise, leading to data spread, especially with dyes having similar spectra (right).



BD FACSDiscover™ A8 Cell Analyzer with BD CellView™ Image Technology and BD SpectralFX™ Technology

Coming soon

Technologies

BD CellView™ Image Technology

This technology implements orthogonal frequency domain multiplexing to image cells with the electronic and optical components used in flow cytometers. This unique technology makes it possible to produce images without a camera, enabling real time imaging for analysis.

BD SpectralFX™ Technology

This technology combines full spectrum optics, next-gen QC, and system-aware spectral unmixing that manages spread by adapting to instrument performance and sample conditions in real-time.

Pre-launch product information

Technical specifications

Optics

Excitation optics

Lasers

Excitation optics

349 nm – nominal 30 mW; output power: 27mw

405 nm – nominal 50 mW; output power: 45mw

488 nm – nominal 100 mW; output power: 95mw

561 nm – nominal 50 mW; output power: 45mw

637 nm – nominal 100 mW; output power: 90mw

Note: 488nm laser is optically divided to support signal detection with BD CellView™ Image Technology

Optical platform

Fixed optical assembly with the capacity to be configured with up to five spatially separated laser beams and six beam spots. Laser delays are automatically adjusted during instrument QC.

Flow cell

The quartz cuvette flow cell is coupled to the fluorescence objective lens by a refractive index-matching gel for optimal light collection.

Beam geometry

Flat top laser beam profile

Emission optics

Optical coupling

Emitted light from the gel-coupled cuvette is delivered by fiber optics to the detector arrays. The optical pathways use signal reflection to maximize signal detection.

Scatter detectors

Blue laser: Forward scatter (FSC) / Side scatter (SSC) / Axial light loss (ALL)

Violet laser: ALL, SSC

Fluorescence detectors for spectral flow cytometry

Spectral arrays – 78 APD detectors paired with algorithmically optimized filter bandwidths covering full spectrum:

UV 349nm laser – 22 UV detectors, covering 365nm – 860nm

Violet 405nm laser – 20 Violet detectors, covering 410nm – 860nm

Blue 488nm laser – 16 Blue detectors, covering 495nm – 860nm

Y/G 561nm laser – 12 Yellow-green detectors, covering 570nm – 860nm

Red 637nm laser – 8 Red detectors, covering 645nm – 860nm

Imaging optics

Image-enabled detectors

Blue laser scatter detectors

Forward scatter (FSC) / Side scatter (SSC) / Axial light loss (ALL)

Fluorescence detectors for imaging

FL1: LP505: 534/46

FL2: LP570: 600/60

FL3: LP675: 788/225

Imaging features

Center of mass X, Center of mass Y, Correlation, Delta center of mass, Diffusivity, Eccentricity, Max intensity, Moment (long), Moment (short), Radial moment, Size, Total intensity

Fluidics

Flow cell

Quartz cuvette

Sample acquisition rate*

Imaging mode: 10K events/sec

High speed mode: 35K events/sec

Sample injection tube (SIT) flush

Each SIT flush, when performed, cleans the inside and outside of the sample line tubing and sends flushed fluids to waste. It is performed after removal of the manual port tube or after each sample acquisition on the loader, by default.

Customization:

Manual port: additional SIT flushes through the FACSChorus UI.

Loader: option to choose between 1–3 SIT flushes to be performed automatically

Sample input

Manual port

Sample carrier: 5.0-mL polystyrene tubes

Carryover*

<0.1%

Dead volume*

<20uL

Aerosol containment

No aerosols or hazardous material exits the system

Fluidic reservoirs

One sheath tank (10L) that contains sheath fluid (distilled water)

One waste tank (10L) that collects waste from the cytometer

Auto-loader

Sample carrier: Standard 96 well plates, deep well 96 well plates, 40-tube rack (12x75mm Falcon Tubes)

Sample agitation: Orbital mixing (400 – 1400rpm)

Sample temperature control: 4°C, 20°C, 37°C, room temperature

Flow sensor

In sample line path; detects sample flow rate up to 120 uL/min.

Flow rate

Imaging mode

Low sample flow rate: 12 +/- 4 uL/min

High sample flow rate: 30 +/- 5 uL/min

Sheath velocity: < 1.1 m/sec

High speed mode

Low sample flow rate: 12 +/- 4 uL/min

Medium sample flow rate: >40 and <90 uL/min

High sample flow rate: 100 +/- 10 uL/min

Sheath velocity: 4.5 – 5.5 m/sec

Common QC for imaging and high-speed modes

Automated daily single tube QC with BD FACSDiscover™ Setup Beads

Biweekly image calibration QC with BD CellView™ Calibration Beads

Installation requirements

Dimensions (W x D x H)

Cell analyzer: 91.4 x 62.7 x 53.6 cm (36 x 24.7 x 21.1 in)

Electronics box: 50.8 x 55.9 x 48.3 cm (20 x 22 x 19 in.)

Weight

Cell analyzer: 81.65 kg (180 lb)

Electronics box: 45.35 kg (100 lb)

Power

Total power: 1000 W

VAC-Hz: 100 – 240 VAC (50/60 Hz)

Circuit breaker: 10 A

Operating temperature range

Between 17.5°C (63.5°F) and 27.5°C (81.5°F) +/-2.5°C variation in the same day

Operating humidity

40–60% relative humidity (noncondensing)

Audible noise

<65dB

System/Software/Support

Operating system

Microsoft® Windows® 10 IoT Enterprise LTSC (Long-term Servicing Channel) Version 21H2

Monitor

32-in with 3840 x 2160 resolution (4K UHD)

Memory

32 GB RAM

Storage

OS Drive: 500 GB NVMe SSD

2nd Drive: 4.0 TB NVMe SSD

Software

BD FACSChorus™ software

Software guides researchers throughout the entire cell analysis process.

Exported file types

FCS 3.2; CSV, CVW

Offline data analysis

Supported by FlowJo™ Software with the CellView Lens plugin, which enables offline analysis of image and flow parameters.

*Based on characterization testing data, pending formal verification testing.

BD flow cytometers are Class 1 Laser Products.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Pair the BD FACSymphony™ AS SE Cell Analyzer with the BD FACSymphony™ S6 SE Cell Sorter

Now you can easily transfer your experiment from analyzer to sorter. The matched configuration between the BD FACSymphony™ AS SE and the BD FACSymphony™ S6 SE allows you to transfer panels between instruments without having to worry about differences between lasers or detectors. With both compensation and spectral workflows you can run all of your standard high parameter conventional panels and design high parameter spectral panels. And you can do both with the industry standard BD FACSDiva™ Software workflow.

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