Transformation in Cell Analysis: From Conventional to Spectral Flow Cytometry

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ABSTRACT

Flow cytometry has been essential in cell biology, allowing real-time analysis of individual cells, however, limitations in spectral resolution and the ability to measure multiple parameters simultaneously restricted its application, spectral flow cytometry (SCF) solved these limitations by allowing complete capture of emission spectra, which increased the accuracy of the analysis and allowed the evaluation of up to 40 parameters in one cell. This has driven advances in immunology, oncology and autoimmune diseases, but despite its advantages, the high initial investment and complexity in data management remain barriers to its adoption. It is proposed that the combination with emerging technologies such as nanotechnology and artificial intelligence could revolutionize personalized medicine, improving the speed and accuracy of diagnoses.

Keywords: Spectral flow cytometry, Multiparametric analysis, Precision diagnostics, Minimal residual disease, High-dimensional bioinformatics

INTRODUCTION

Since its conception in the 1960s, flow cytometry has been a revolutionary tool in cellular and molecular biology, enabling multiparametric analysis of individual cells in real time (1), this breakthrough transformed our understanding of complex biological systems and cemented itself as a mainstay in fields such as immunology, oncology, clinical diagnosis and microbiology, among others (2). Over the years, this technology has evolved significantly, not only in terms of accuracy and analytical resolution, but also in its ability to characterize cells and particles from a morphological, phenotypic, functional, and molecular perspective with an unprecedented level of detail (3,4).

The initial development of conventional flow cytometry (FC) was based on relatively simple optical principles: the excitation of fluorochromes by lasers and the detection of fluorescence through optical filters and photodetectors (3), thanks to this approach, heterogeneous cell populations can be characterized based on their size, granularity and expression of surface and intracellular markers (5), However, with the increasing complexity of biological studies and the need for higher analytical resolution, several technical limitations emerged, including spectral overlap between fluorochromes, cellular autofluorescence, and restriction in the number of measurable parameters represented critical challenges that limited the potential of this technology.

In response to these limitations, spectral flow cytometry (SFC) emerged in the last decade as an innovation that marked a turning point in the field (5–7); Unlike the conventional approach, this technique allows the capture of

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the entire emission spectrum of each fluorochrome instead of being restricted to specific channels (3), this is possible thanks to the use of multi-channel detectors and advanced spectral demixing algorithms, which not only minimizes the need for signal compensation, but also allows a significantly larger number of parameters to be analyzed in a single sample (6,8,9).

This evolution has had a significant impact on various scientific and clinical applications. SCF has made it possible to identify previously unexplored cell subpopulations, which has facilitated advances in immunotherapy, diagnosis of hematological diseases and functional characterization of immune cells in autoimmune and chronic pathologies (10–14). In addition, it has allowed the study of tumour microenvironments to be deepened, contributing to the development of personalised therapies based on a more detailed cell characterization (10,15).

However, despite its many advantages, the implementation of SCF is not without its challenges. Factors such as the high cost of equipment and reagents (16), the need for specialized training for their correct handling (17,18) and the complexity of data analysis (19) have slowed down their widespread adoption. In addition, the lack of standardization in protocols and methodologies for their clinical application continues to be a relevant obstacle (16).

Historical context and current relevance of flow cytometry

Flow cytometry has been one of the most revolutionary tools in biomedicine since its conception in the twentieth century. Its origin dates back to 1934, when Moldavan proposed the idea of counting cells using optical flow sensing (20). However, the true materialization of this technology would not come until the 1960s, when Wolfgang Göhde developed the first fluorescent flow cytometer at the University of Münster, Germany (21). That rudimentary instrument, known as ICP 11, was the starting point for what we know today as modern cytometry.

The decisive breakthrough came in the 1970s with the advent of the FACS (Fluorescence-Activated Cell Sorter), developed by Leonard Herzenberg (22). This device not only made it possible to detect cells, but also to separate them according to their characteristics, which exponentially expanded the applications of cytometry. The emergence of monoclonal antibodies around the same time created a key synergy that boosted their use in immunology, oncology, and clinical diagnostics. Today, flow cytometry has reached levels of sophistication that initially seemed unthinkable. Modern cytometers can simultaneously analyze up to 50 parameters per cell and process tens of thousands of events per second with exceptional accuracy (23).

The growth of the flow cytometry market reflects its growing relevance. It is estimated to reach $8.61 billion by 2032, with an annual growth rate of 8.3%. This boom is due to the increase in applications in clinical diagnostics, the development of targeted therapies and the need for high-performance analysis platforms in pharmaceutical research (24–26).

Despite its undeniable advantages, conventional flow cytometry faces certain limitations that have driven the search for technological innovations, one of the main barriers being the spectral limitation imposed by its design. Traditional cytometers use fixed optical filters and photomultipliers (PMTs) to detect fluorescence in specific bands, which causes spectral overlap between fluorochromes with nearby emissions (5), this forces the application of mathematical compensation algorithms, which can introduce errors and affect the quantification of cell populations, especially in panels with more than 10 markers (27,28).

Another difficulty lies in the detection of rare cells, such as circulating tumor cells or those involved in minimal residual disease in leukemias (29), conventional CF can be limited by background noise (30) and interference between fluorochromes (31), which compromises its diagnostic sensitivity and clinical utility in decision-making (32).

In addition, conventional cytometry demands a high level of technical expertise, designing multicolored panels and optimizing the configuration of a cytometer requires specialized knowledge and considerable time (27), despite standardization efforts, such as those promoted by Euroflow (33–35) and the Human ImmunoPhenotyping Consortium (36), variability between laboratories remains a challenge (37).

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Faced with these limitations, CSF has emerged as an innovative solution that redefines the approach to data acquisition and analysis, unlike conventional cytometry, which relies on discrete filters, spectral cytometry captures the full emission spectrum of each fluorochrome, allowing for more accurate signal discrimination and greater ability to analyze multiple parameters without the constraints imposed by spectral overlap (Figure 1). This evolution marks a turning point in the field, with promising applications that are transforming biomedical research and clinical diagnostics.

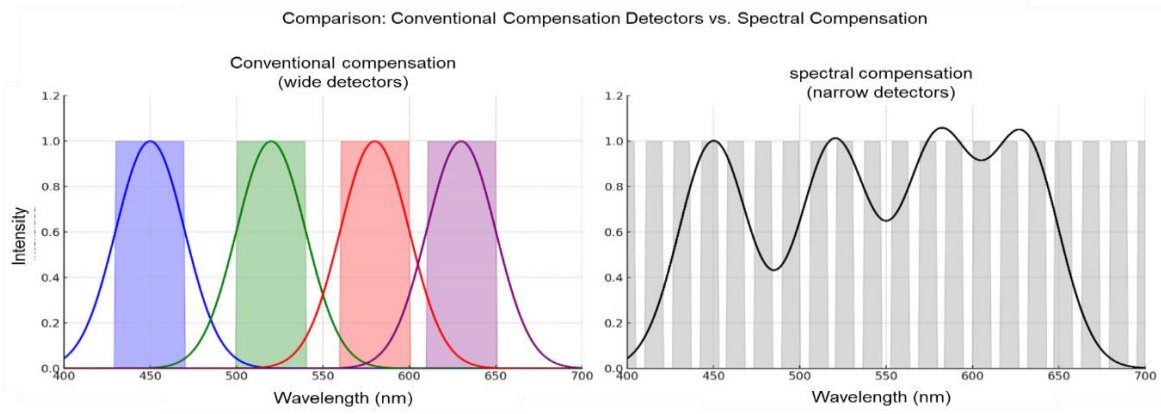

Figure 1 Comparison between conventional compensation and spectral deconvolution detectors. On the left is conventional compensation, where the detectors have a wide range of wavelengths, causing the signals to overlap. On the right, spectral deconvolution uses narrow detectors that allow emission spectra to be captured more accurately, reducing overlap and improving the resolution of the analysi

Technical Fundamentals: Comparison between Conventional and Spectral Cytometry

Detection mechanisms: Filters vs. full spectrum

Conventional CF and SCF differ fundamentally in their approach to capturing and analyzing fluorescent signals, conventional systems use fixed optical filters and dichroic mirrors to direct specific wavelengths to individual detectors, usually photomultipliers (PMTs), although this design has been widely used, it has limitations due to the spectral overlap between fluorochromes (spillover), which makes it necessary to apply compensation algorithms that can introduce variability in the results (8,23).

In contrast, SCF uses full-scanning spectroscopy technology, capturing all of the fluorescent signal emitted by the particles (usually in the range of 400 to >800 nm) and scattering it using diffraction grating or optical prisms. This approach allows the full emission spectrum of each fluorochrome to be recorded, generating a unique spectral signature for each marker, even in the presence of highly overlapping signals (38). The spectral information is processed by advanced spectral unmixing algorithms, which mathematically decompose the overlapping signals using previously established reference spectra (39). This strategy not only maximizes the resolution of complex cell populations, but also minimizes the need for traditional trade-offs, improving the accuracy and reproducibility of experiments (Figure 3) (40).

Comparative studies have shown that SCF systems have the ability to efficiently resolve combinations of up to 40 simultaneous fluorochromes, far exceeding the practical limit of approximately 18 colors in conventional systems (41,42). This significant improvement in resolution capability allows for greater accuracy and detail in analyses, which is particularly valuable in a variety of advanced applications. Highlights include:

- High-dimensional immunophenotyping: Spectral cytometry allows for a comprehensive characterization of rare immune subpopulations, providing a broader and more accurate view of the immune system. This ability is crucial for studies of autoimmune diseases, hematological cancers, and inflammatory disorders, where identifying specific cellular phenotypes can directly influence diagnosis and clinical prognosis (43).
- Multiplexed functional studies: The ability to simultaneously analyze intracellular cytokines, transcription factors, and surface markers provides an integrated perspective on cellular processes. This

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functional multiplexing capability is critical to understanding the dynamics of immune responses and pathological mechanisms in conditions such as chronic infections, immunotherapy, and degenerative diseases (39,44).

- Translational research: Spectral cytometry has revolutionized the analysis of volume-limited samples, such as cerebrospinal fluid or sparse tissue biopsies, allowing multiparametric evaluations with minimal cell counts. This has facilitated advances in clinical research and has promoted the personalization of treatments in oncological, neurological and autoimmune pathologies (45)

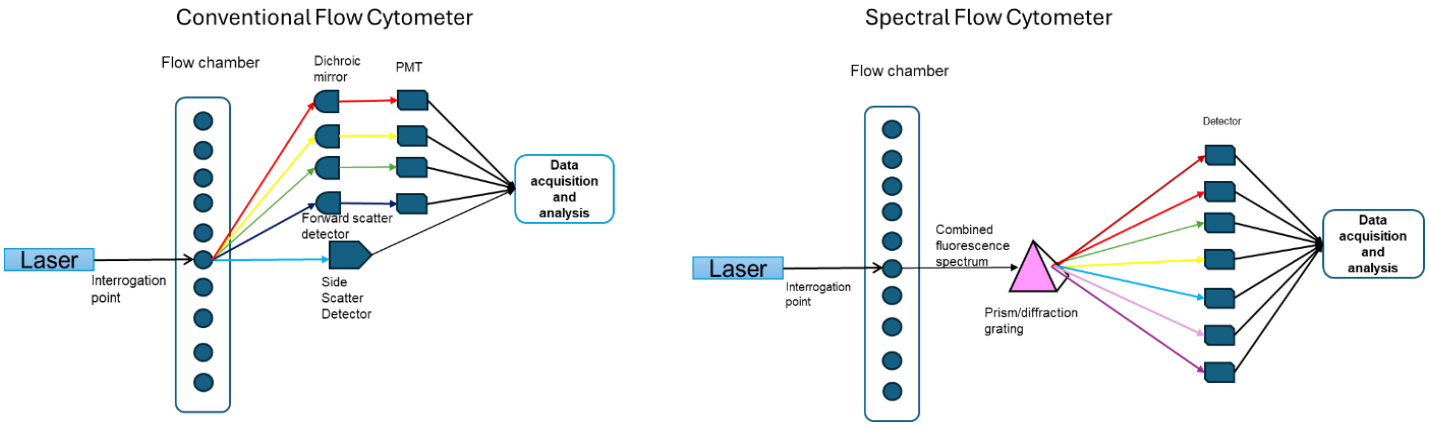

Figure 2 Operation of a conventional and spectral flow cytometer. In conventional cytometry, lasers excite particles and emitted light is filtered by dichroic mirrors and detected by PMTs. Spectral cytometry disperses combined fluorescence via a prism or grating, capturing full emission spectra with multiple detectors, enabling more precise multidimensional analys

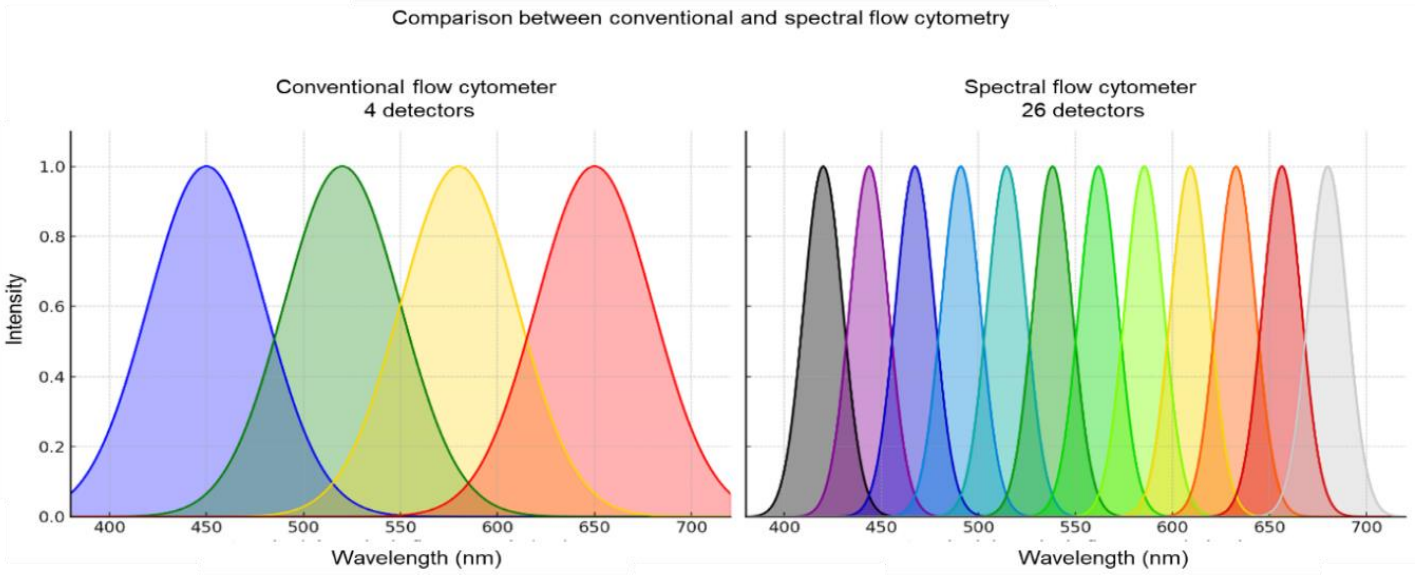

Figure 1 Comparison between polychromatic and spectral cytometry. On the left (A), polychromatic cytometry with 4 detectors is shown, where the emission spectra of different fluorochromes overlap, limiting the number of parameters analyzed. On the right (B), spectral cytometry uses 26 detectors, allowing a larger number of parameters to be analyzed at higher resolution and without overlapping signals

Optical system: Lasers and diffraction grating

While conventional cytometers employ fixed configurations of bandpass filters (typically 20-30 nm bandwidth) (46), spectral systems incorporate more sophisticated optical elements:

Excitation sources: Both systems use solid-state lasers (e.g., 405 nm, 488 nm, 640 nm), but spectral lasers typically employ higher-power lasers (50-100 mW vs. 20-50 mW in conventional ones) to improve excitation in complex panels (47).

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Collection optics: In conventional systems, dichroic mirrors divide light into discrete optical paths. In contrast, spectral systems use diffraction grates or prisms to scatter light in its full spectrum (300-800 nm), allowing for more accurate analysis (48,49).

Geometric configuration: Modern designs such as the Cytek Aurora employ single-mode fiber optics, reducing signal distortion and eliminating alignment issues present in conventional systems with multiple PMTs (41,50,51).

Detectors: PMTs vs. APDs

The evolution in detection technologies has been key to the development of spectral cytometry. The following is a comparison of the characteristics of the main detectors used:

PMTs (Conventional)	APDs (Spectral)
Spectral Range	300–800 nm (expanded)
Sensitivity (with thermal noise)	Very high (sinde molecule detection)
Linieaity Limited at high intensities	Wide dynamic range (> 5 decades)
Temporal Resolution –1 ns	–100 ps (ideal for fast flow)
Moderate	High (but require fewer units)

Figure 2 Comparison between PMTs (Conventional) and APDs (Spectral). Table summarizing the main differences between photomultipliers (PMTs) used in conventional cytometry and avalanche photodiodes (APDs) in spectral cytometry, including spectral range, sensitivity, linearity, temporal resolution, and cost. (52–54).

Avalanche photodiodes (APDs) have emerged as key detectors in SCF due to their high quantum efficiency and sensitivity in the near-infrared range. Unlike photomultiplier tubes (PMTs), which exhibit limited quantum efficiency at wavelengths above 650 nm, APDs maintain a quantum efficiency of over 80% in this range, making them particularly suitable for detecting fluorochromes such as Cy7 and APC-Cy7, whose emissions are above 700 nm (52,55). In addition to their ability to detect near-infrared emissions, APDs generate less electronic noise compared to PMTs. This feature allows the detection of cell populations with low marker expression, improving resolution and accuracy in complex sample analysis (53–55).

These advantages make APDs especially suitable for applications that require high sensitivity and detection at extended spectral ranges, such as in studies of rare cell populations or in analysis of samples with weak fluorescence.

Fluid System Evolution

The evolution of fluid systems in SCF has been instrumental in achieving previously unattainable levels of sensitivity and accuracy. Although the basic principle of hydrodynamic flow remains, innovations in microfluidics have transformed the way cells are manipulated and analyzed in these systems.

In traditional cytometers, the hydrodynamic approach is achieved by injecting the sample into a sheath fluid, which centers the cells for analysis. However, this method requires large volumes of fluid and additional pumping systems, limiting the portability and efficiency of the system (56).

Advances in microfluidics have allowed the miniaturization and improvement of these systems. For example, the use of microfluidic chips with high-precision flow controllers, such as the OB1 MK3+, allows for more stable and accurate hydrodynamic focusing, with variations of less than 0.1 µm in the position of the cells (57).

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These systems have also demonstrated greater than 99.9% cell recovery, which is crucial for limited sample analysis (58).

In addition, the integration of technologies such as acoustic focusing has further improved the accuracy in cell alignment. This method uses acoustic waves to pre-focus cells prior to their introduction into the hydrodynamic flow, improving data accuracy even at high sample input rates (59).

These fluid system improvements have expanded the applications of spectral flow cytometry. For example, in the analysis of liquid biopsies, it is now possible to detect one tumor cell among a million leukocytes, which represents unprecedented sensitivity (58). Likewise, in cellular pharmacodynamics, multiple functional parameters can be quantified simultaneously, which facilitates real-time monitoring of the efficacy of therapies (60).

The evolution of fluid systems in SCF has been key to its transformation into an essential tool in personalized medicine and advanced biomedical research.

Signal Processing: Traditional vs. Offsetting Spectral unmixing

One of the most significant differences between conventional and spectral flow cytometry lies in the processing of fluorescent signals. In conventional systems, compensation is based on experimentally constructed matrices using individual staining controls. Each combination of fluorochrome and detector requires manual adjustments, assuming a linearity that does not always fully correct for nonlinear spectral overlap. This methodology limits the number of parameters that can be analyzed simultaneously, usually up to 15-18, before cumulative errors affect data quality (Figure 5) (61–63).

In contrast, SCF employs predefined spectral libraries for each fluorochrome and spectral unmixing algorithms that automatically separate spectral contributions. These algorithms allow correcting cellular autofluorescence, overlap between channels and instrumental variations, improving the accuracy and reproducibility of the experiments. In addition, they offer the possibility of retrospectively re-evaluating data without the need for repeat experiments, which represents a significant advantage in longitudinal or limited sample studies (Figure 5) (64,65).

Recent studies have demonstrated quantifiable advantages of the spectral approach. For example, a 40-60% reduction in coefficients of variation (CVs) has been observed in rare populations, as well as improved recovery of weak signals in the presence of bright fluorochromes. Likewise, the ability to reuse panels with different combinations of fluorochromes without the need to reconstruct compensation matrices has been a significant improvement in experimental flexibility (66–68).

These advances reflect how flow cytometry has evolved since its first implementations, enabling unprecedented explorations of cellular heterogeneity in diverse health and disease contexts.

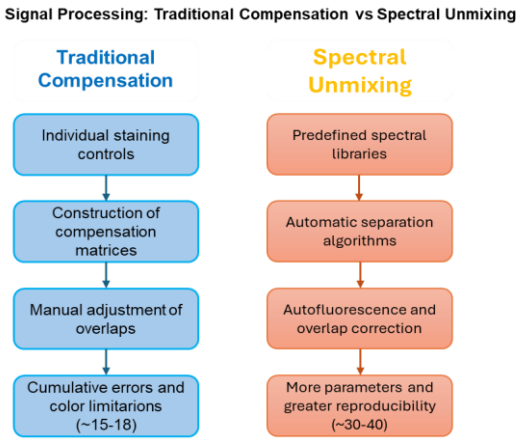

Figure 3 Signal Processing: Traditional Compensation vs. Spectral Unmixing. Comparison diagram between traditional and spectral unmixing compensation methods. On the left, traditional processing involves individual

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staining controls, manual construction of compensation matrices, and manual adjustments of overlaps, which limits the number of colors. On the right, spectral unmixing uses predefined spectral libraries, automatic separation algorithms, and correction for autofluorescence and overlapping, allowing more parameters to be analyzed with greater reproducibility.

Advantages of spectral cytometry

SCF has emerged as a disruptive technology in the last decade, redefining the boundaries of multidimensional cellular analysis; unlike conventional systems, which operate under optical and computational paradigms established half a century ago, SCF represents a radical reconceptualization of how we capture, process, and interpret biological signals (69,70).

Spectral resolution

The ability of SCF to digitally correct cellular autofluorescence represents a significant advance in the analysis of complex biological samples, this process involves the mathematical characterization of the spectral signatures of intrinsic cellular components, such as flavoproteins and lipofuscin, allowing the selective subtraction of these background signals. As a result, the signal-to-noise ratio is markedly improved, facilitating the accurate detection of rare cell populations or those with low marker expression (71).

In particular, lipofuscin, known as the "age pigment", is an endogenous fluorophore that accumulates in cells over time and fluoresces in the yellow-orange range (72), its presence can interfere with the detection of specific signals in conventional CF, however, SCF allows autofluorescence to be treated as an independent signal, similar to a "virtual fluorochrome", and extract it from the set of fluorescent signals. This capability improves the accuracy of spectral unmixing and fluorochrome resolution in samples with high autofluorescence (73).

In addition, it has been shown that autofluorescence can interfere with conventional CF, as its signal results in a background that can hinder the detection and specific analysis of exogenous marker emissions (74). SCF allows for separate measurement and analysis of autofluorescence, thereby improving the resolution of weak signals and the precise definition of cell phenotypes (71,75) .

These capabilities position spectral flow cytometry as an advanced tool to unravel the biochemical complexity of cell samples, overcoming the limitations imposed by traditional techniques and opening up new possibilities in biomedical research.

Reduction of technical errors

Spectral resolution is one of the fundamental pillars that distinguish spectral flow cytometry from conventional methodologies. While traditional cytometers employ fixed optical filters, spectral systems use diffraction gratings and array detectors to record the full emission spectrum of each particle. This capability allows the application of spectral unmixing algorithms, based on non-negative least squares (NNLS), which mathematically decompose the overlapping signals using reference spectral libraries, facilitating the accurate identification of fluorochromes with highly overlapping emissions (69,70).

An example of this capability is seen in the distinction between PE-Cy7 and APC-Cy7, two tandem fluorochromes commonly used in immunophenotyping that exhibit maximum emissions separated by only 5 nm. Spectral systems have been shown to achieve efficient separation of these signals, which is challenging in conventional configurations due to the high degree of spectral overlap. This fine resolution has allowed the design of experimental panels that take advantage of structurally related, but functionally distinct fluorochromes, optimizing the use of spectral space without compromising accuracy (76).

In addition, spectral technology introduces a unique capability: digital correction of cellular autofluorescence. By mathematically characterizing the spectral signature of intrinsic cellular components, such as flavoproteins or lipofuscin, algorithms can selectively subtract these background signals. This process significantly improves the signal-to-noise ratio in complex samples, such as dissociated tumor tissue or primary stem cells, allowing for more accurate detection of rare or low-marker expression cell populations (77,78).

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Advanced applications of spectral cytometry

Spectral cytometry has revolutionized the analysis of samples with low cellular content, such as cerebrospinal fluid (CSF), thanks to its high sensitivity and multiplexing capacity. Below are innovative applications supported by recent studies:

Detection of neoplastic infiltration in CSF

Spectral flow cytometry has emerged as a critical tool in the detection of tumor cells in the cerebrospinal fluid (CSF), especially in the context of acute leukemias and lymphomas, thanks to its ability to identify neoplastic infiltrations with a sensitivity far superior to conventional cytology, it has transformed the diagnostic and prognostic approach to central nervous system (CNS) disease in cancer patients (79).

A retrospective study of relevance, which analyzed the CSF of 165 children with B-lineage acute lymphoblastic leukemia (ALL), compared the sensitivity of multiparametric flow cytometry (MFC) versus conventional cytology. The results showed that CFM detected neoplastic infiltration in a significantly higher percentage of cases compared to cytology, thus highlighting its diagnostic superiority in the context of CNS involvement in pediatric patients (80,81).

The usefulness of spectral flow cytometry has also been validated in adult populations. In a study that evaluated adult patients with ALL treated under the hyperCVAD regimen, it was observed that, of the 92 patients analyzed, 18 (20%) were positive for CSF infiltration by CFM at the time of diagnosis, while only six of these cases were detected by conventional cytology (82).. Importantly, CFM positivity was associated with a higher incidence of CNS relapse, suggesting that this technique is not only more sensitive, but also possesses superior predictive value compared to traditional cytology (83).

In the case of primary CNS lymphomas, the combination of conventional cytology with flow cytometry has been shown to substantially increase CSF disease detection rates, a retrospective study in 35 cases of CNS lymphoproliferative diseases showed that the detection of infiltration increased by 50% when immunophenotyping by flow cytometry was used in conjunction with cytopathology, compared to cytopathology alone (83) .

Together, these findings support the implementation of spectral flow cytometry as a tool of choice for the diagnosis and follow-up of hematological malignancies affecting theCNS, the improved sensitivity and specificity in the detection of tumor cells in CSF not only improve early diagnosis but also allow for better risk stratification and the design of more adaptive therapeutic strategies for patients.

Diagnosis of autoimmune diseases

Spectral flow cytometry is an essential tool for the detailed characterization of immunological subpopulations in various autoimmune diseases. Its ability to analyze multiple parameters simultaneously allows for a deeper understanding of immune states in different pathologies.

One study implemented a 37-parameter panel using spectral flow cytometry to analyze synovial fluid mononuclear cells in patients with juvenile idiopathic arthritis (JIA). The results revealed elevated expression of activation markers such as CD69 in B cells, monocytes, NK cells, dendritic cells, and CD4+ and CD8+ T cell subpopulations, compared to healthy controls. These findings suggest an activated immune status characteristic of JIA patients, providing critical functional information for diagnosis and dynamic monitoring of the disease (84).

In the therapeutic field, spectral cytometry has been instrumental in evaluating the efficacy of advanced immunomodulatory treatments. One study investigated the potential of multiparametric spectral flow cytometry

to identify patients with autoimmune diseases and its usefulness as an evaluation tool for in vitro drug detection approaches. Combinations of 22-color immunophenotyping panels were designed to profile cell subset distribution and cell activation. In vitro testing of epigenetic immunomodulatory drugs revealed an altered activation state following treatment, supporting the use of spectral flow cytometry as a high-throughput drug screening tool (85).

More recently, spectral cytometry has facilitated deep immunophenotyping studies in large cohorts of autoimmune and autoinflammatory diseases. In an analysis that included 443 patients with 15 different diseases, cytometry panels were used to characterize more than 600 cell populations. This high-resolution approach allowed the identification of disease segregation based on the expression profile of markers such as LAG3 and ICOS in regulatory T cells, highlighting the ability of spectral cytometry to define specific immunological signatures that could be used as diagnostic or prognostic biomarkers in different autoimmune pathologies (86).

These clinical applications of spectral flow cytometry highlight its potential to improve the diagnosis, monitoring, and treatment of autoimmune diseases through detailed and accurate immunological characterization.

Minimal Residual Disease (EMR) Monitoring

Spectral flow cytometry has established its position as an essential tool for the detection of minimal residual disease (MRD) in various pediatric hematological malignancies and solid tumors. Unlike conventional cytometry, its ability to analyze multiple parameters simultaneously and with high sensitivity allows the identification of residual tumor cells that would otherwise go undetected in traditional evaluations. This technical advantage has significantly transformed risk stratification and therapeutic adjustment, particularly in pediatric oncology.

In the setting of pediatric acute lymphoblastic leukemia (ALL), a recent study implemented a 23-color panel in a single tube, achieving sensitivity and specificity superior to those of conventional 8-color multicolor systems. This approach allowed the detection of residual leukemia cells with a sensitivity of up to 0.01%, equivalent to identifying one tumor cell among 10,000 normal, directly impacting clinical decision-making earlier and more accurately (87).

In acute myeloid leukemia (AML), spectral cytometry has driven the evolution of panels that include up to 24 parameters, integrating relevant biomarkers such as CD96 and CD200. The incorporation of these new markers has improved not only the ability to identify residual leukemic cells, but also the understanding of changes in the tumour immune microenvironment, with important prognostic implications and in the selection of targeted therapy strategies (88–90).

In the field of lymphomas, especially those with bone marrow involvement, spectral cytometry has demonstrated fundamental value for the precise characterization of minority cell subpopulations. Panels have been developed that make it possible to distinguish functional subtypes of regulatory T cells through the simultaneous analysis of more than 30 parameters, providing critical information for a more accurate assessment of RMS and its correlation with relapse risk (91).

In pediatric solid tumors, such as neuroblastoma, spectral cytometry has also shown relevant applications. Using combinations of markers such as GD2 and CD56, it has been possible to identify residual tumor cells in bone marrow with a sensitivity comparable to advanced molecular techniques such as quantitative PCR. This ability is crucial to more accurately assess therapeutic response and anticipate relapses, in a clinical context where early detection of residual disease can radically modify treatment strategies (92–94).

Together, these advances consolidate spectral flow cytometry as a robust, high-resolution platform for monitoring RMS in a broad spectrum of oncological pathologies. Its ability to simultaneously assess multiple cellular parameters not only improves diagnostic sensitivity but also drives the transition to a more dynamic and adaptive personalized medicine model, where clinical decisions can be adjusted in real time based on accurate and detailed biological information.

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Immunological studies in limited samples

Spectral flow cytometry has extended its impact beyond the oncology field, becoming an essential tool in the evaluation of primary immunodeficiencies, autoimmune disorders and lymphoproliferative syndromes. Its ability to perform multiparametric analyses with extremely small sample volumes has allowed to optimize immunological characterization in clinical scenarios that traditionally presented significant operational limitations.

In the study of immunodeficiencies, spectral cytometry has allowed the absolute quantification of critical lymphocyte subpopulations using minimal blood volumes, frequently less than 0.5 mL. The accurate identification of NK cells (CD56+/CD3−), memory B lymphocytes (CD27+) and differentiated subtypes of regulatory or effector T cells is now carried out in a more reliable and sensitive manner, which has significantly improved the early diagnosis of immunological alterations in pediatric and young adult patients (95–98).

In the functional field, spectral cytometry has opened up the possibility of simultaneously measuring, in individual cells, both the intracellular production of cytokines and the expression of activation markers. Thus, parameters such as IFN-γ and IL-6 secretion, along with CD25 and HLA-DR expression, can be evaluated in an integrated manner, allowing a more complete view of immune activation and dysfunction in autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis (95,99–101).

In the diagnosis of lymphoproliferative syndromes, spectral cytometry has also demonstrated key advantages, particularly in the evaluation of biological fluids such as CSF or pleural fluids. By identifying clonal restriction of κ/λ light chains in B populations, it has been possible to differentiate between reactive inflammatory processes and early malignant expansions, increasing diagnostic accuracy in highly complex clinical scenarios (102).

Thus, spectral flow cytometry is emerging not only as a high-resolution technique for the monitoring of malignant diseases, but also as a versatile platform for functional and phenotypic characterization in the diagnosis of immunological dysfunctions, consolidating itself as an indispensable tool in precision medicine.

Studies in infectious diseases

The COVID-19 pandemic represented a catalyst for the widespread application of spectral flow cytometry in the study of immune responses to emerging pathogens. The need to simultaneously analyze multiple immunological parameters in large cohorts and efficiently consolidated this technology as an essential tool in infectious disease immunology.

A comparative study between patients with severe COVID-19 and those with pneumonia not related to SARS-CoV-2 employed spectral flow cytometry to delineate specific immune profiles. This analysis revealed that although both groups showed signs of emergency myelopoiesis and adaptive immune paralysis, pathological immunosignatures suggestive of T-cell depletion were unique to COVID-19. In addition, it was identified that the frequency of circulating NKT cells could serve as a predictive biomarker of clinical outcome in patients with severe COVID-19 (103).

Beyond COVID-19, the applications of spectral flow cytometry in infectious diseases are expanding rapidly. In tuberculosis, it has been shown to be useful in detecting IFN-γ-producing Tγδ cells directly in sputum samples, facilitating the monitoring of local immune responses at the site of lung infection. In the context of HIV, the technology enables the precise quantification of viral reservoirs in CD4+ memory T cell subpopulations, a critical advance for the evaluation of functional cure strategies. In malaria, it has been used to identify erythrocytes infected by Plasmodium spp. by detecting oxidative stress markers in combination with cell surface profiles, opening new avenues for research in immunopathology and therapeutic monitoring (104,105).

Thus, spectral flow cytometry has not only demonstrated its value in the analysis of complex immune responses to acute viral infections, but is also emerging as an indispensable tool in translational research of chronic and emerging infectious diseases. Its ability to comprehensively capture the functional and phenotypic heterogeneity of the immune system promises to transform the way these pathologies are studied, monitored, and eventually controlled at the population and individual level.

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Applications in veterinary medicine

The expansion of spectral flow cytometry into veterinary medicine represents a significant advance in the application of high-resolution technologies in non-human species. Through the adaptation of specific multiparametric protocols and panels, it has been possible to overcome the historical limitations of immunophenotyping in animals, opening new opportunities both in comparative medicine and in biodiversity conservation.

In veterinary oncology, spectral cytometry has allowed the most accurate diagnosis of lymphomas in canines through the use of multi-marker panels, including specific combinations such as CD45, CD34 and CD79a, which allow discriminating between myeloid and lymphoid lineages with greater sensitivity than traditional methods. Similarly, in domestic cats, the immunophenotypic characterization of mast cells has been optimized by simultaneously identifying CD117 and c-KIT expression, providing critical information for the diagnosis and prognosis of these tumors (106–109).

In the field of infectious diseases, spectral cytometry has proven to be a valuable tool in monitoring the immune response in felines infected with feline immunodeficiency virus (FIV), allowing the functional evaluation of T-cell subpopulations with minimal sample volumes. Likewise, in horses, it has been used to improve the diagnosis of equine infectious anemia by characterizing phenotypic alterations in erythrocytes, increasing the sensitivity of traditional laboratory tests (110–113).

In wildlife conservation programs, spectral technology has opened up new possibilities for the non-invasive study of the immune status of endangered species. An emblematic case is the analysis of immune stress in polar bears, where immune dysfunction has been correlated with exposure to environmental pollutants and the impact of climate change in the Arctic (114). Likewise, in threatened amphibian populations, spectral cytometry has allowed the early detection of subclinical infections by fungal pathogens, contributing to the design of intervention and biodiversity management strategies (115).

In addition, flow cytometry has been used to assess immune function in marine mammals exposed to environmental pollutants. For example, studies have employed this technique to analyze the phagocytic activity of monocytes and granulocytes in species such as bottlenose dolphins, beluga whales, Patagonian sea lions, walruses, and harbor seals (116).

These developments consolidate spectral flow cytometry not only as a tool of clinical excellence in human medicine, but also as an instrument of increasing relevance in modern veterinary medicine and in the global conservation of species.

Challenges and emerging solutions in spectral cytometry

Spectral cytometry, despite its revolutionary advantages, faces technical, economic, and ethical challenges that require multidisciplinary attention.

Complexity in the analysis of high-dimensional data

The analysis of data generated by spectral flow cytometry represents one of the greatest contemporary challenges in applied bioinformatics. A single experiment involving 40 parameters measured in approximately one million cells can generate up to 4.8 GB of raw data, a volume equivalent to the simultaneous processing of more than 10,000 high-resolution microscopy images. This magnitude of information poses critical challenges both in terms of storage and analytical interpretation (117–119).

Traditionally, classical dimensionality reduction algorithms such as t-distributed Stochastic Neighbor Embedding (t-SNE) or Principal Component Analysis (PCA) have been used for the visualization and clustering of flow cytometry data (120,121). However, recent studies have highlighted the limitations of these methods in high-dimensional scenarios. For example, t-SNE may lose rare cell subpopulations and does not adequately preserve the overall structure of the data (122–124).

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Faced with these challenges, quantum computing has begun to offer innovative solutions. The QFlow algorithm uses the ability of qubits to process high-dimensional arrays more efficiently than traditional systems (125,126). In practical applications, QFlow has demonstrated the ability to optimize a large number of wavefunction parameters using modest quantum resources, suggesting significant potential for spectral flow cytometry data analysis (127,128) .

In parallel, platforms such as Spectre have emerged as integrative tools in spectral cytometry bioinformatics. Spectre combines automated systems that integrate dimensionality reduction algorithms such as UMAP, clustering methods such as PhenoGraph, and unsupervised learning techniques, achieving high accuracy in the identification of rare immune populations, even in datasets with more than 30 simultaneous parameters (129).

The adoption of FAIR (Findable, Accessible, Interoperable, Reusable) principles in the handling of spectral flow cytometry data has been instrumental in improving the quality, interoperability, and reuse of the data generated in these experiments.

A prominent initiative in this area is the MIFlowCyt (Minimum Information about a Flow Cytometry Experiment) standard, developed by the International Society for the Advancement of Cytometry (ISAC). This standard sets out the minimum requirements for reporting flow cytometry experiments, including details about the samples, reagents, instrument configuration, and data analysis methods. The implementation of MIFlowCyt facilitates the interpretation, comparison, and reuse of flow cytometry data, aligning with FAIR principles (130,131).

In addition, the FlowRepository platform provides a public repository for sharing annotated flow cytometry datasets according to MIFlowCyt standards. This tool allows researchers to access, review, download, deposit, annotate, share, and analyze flow cytometry datasets, promoting transparency and reuse of data in the scientific community (132).

Integrating FAIR principles and standards such as MIFlowCyt into spectral flow cytometry data management not only improves data quality and reproducibility but also facilitates collaboration and advancement in biomedical and clinical research. These innovations not only overcome the technical hurdles associated with high-dimensional analysis but are also accelerating the transition from spectral flow cytometry to fully reproducible, scalable, and discovery-oriented data science for new cell biologies.

Although spectral cytometry offers significant technical advantages, the transition from conventional workflows is not without practical difficulties, especially in laboratories with limited experience in multicolor cytometry. Common problems include a steep learning curve for panel design, errors in spectral deconvolution due to poor fluorochrome selection, and difficulties in quality control due to a lack of standardization in the handling of autofluorescence or internal controls. These technical barriers are not always mitigated by the infrastructure or training available in laboratories with limited resources, which can compromise the reproducibility and reliability of results obtained with SFC.

Technical Requirements and Start-Up Costs: Barriers to Democratization

Despite technological advances and obvious clinical advantages, the widespread adoption of spectral flow cytometry faces substantial barriers, especially related to its infrastructure and associated costs. A basic system, such as the Cytek Aurora, requires initial investments ranging from US$350,000 to US$500,000, in addition to annual maintenance costs of more than US$70,000, a figure that represents a challenge for most laboratories in low- and middle-income countries, where the average annual budget for equipment procurement is usually less than US$100,000 (133–136).

Faced with this limitation, solutions have emerged based on shared access models and the development of technologies that seek to democratize access to high-resolution spectral cytometry. A notable example is the expansion of Core Facility platforms, such as EuroFlow Nexus, which offers remote access to spectral cytometers for affordable rates of approximately $50 per hour. This model has made it possible to process more than 1,200 samples annually through a network of 32 countries, optimizing the use of resources and promoting technological equity at the international level (137).

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An emerging strategy to make spectral flow cytometry more accessible is the use of modular devices that allow conventional cytometers to be upgraded. These modular converters incorporate advanced technologies such as diffraction gratings and low-cost CMOS avalanche detectors, transforming traditional equipment into platforms capable of performing high-resolution spectral analysis. There are technologies that seek to reduce economic barriers in the adoption of spectral cytometry. For example, Beckman Coulter's CytoFLEX Mosaic Spectral Detection Module, which connects to existing cytometers such as the CytoFLEX LX or S, enables spectral analysis of up to 88 parameters, significantly improving detection capability without the need to purchase an entirely new system (138,139). This modular solution makes spectral cytometry more accessible, especially for laboratories with limited budgets.

These initiatives demonstrate that, although upfront infrastructure costs represent a challenge, solutions based on technological innovation, global collaboration, and virtual training can effectively democratize access to spectral cytometry, expanding its impact on biomedical research and precision clinical medicine

Future Directions: Beyond Spectral Cytometry

CFS has revolutionized multidimensional cell analysis, enabling the accurate characterization of individual cells by simultaneously detecting multiple parameters. This technology has set a new standard in biomedical research and personalized medicine. However, its evolution continues by integrating disruptive advances from disciplines such as nanotechnology, quantum computing, and advanced artificial intelligence, promising to radically transform both research and clinical practice in the next decade.

One of the main vectors of innovation in flow cytometry is the miniaturization of detection systems using nanotechnology. The development of smartphone-based wearable cytometric biosensors has proven to be suitable for point-of-care (POC) applications, such as on-site disease diagnosis and personal health monitoring. These compact devices incorporate microfluidic technology and smartphone cameras to perform multi-color cellular analysis with high accuracy, making it easier to detect diseases in resource-limited settings (140).

On the other hand, quantum computing is emerging as a powerful tool to improve spectral analysis in flow cytometry. The ability of quantum processors to handle large volumes of data and perform complex calculations in short times allows us to model interactions between fluorochromes and cellular autofluorescence with a precision unattainable by classical algorithms. This integration promises to accelerate the spectral demixing and cellular clustering of millions of events, opening up new possibilities for large-scale systemic biology and immunological epidemiology studies (141).

In addition, artificial intelligence (AI) is playing a crucial role in the evolution of flow cytometry, enabling faster and more accurate analyses of complex data. Machine learning models, such as deep neural networks, are applied directly to flow cytometry data to predict clinical or biological outcomes without the need for cell type identification steps. This ability to apply models directly to raw data makes it easier to streamline workflows and improve diagnostic accuracy in clinical settings (142).

The combination of CFS and mass cytometry (CyTOF) is marking a new era in single-cell immunological analysis, enabling a more detailed and accurate characterization of cell populations. Both technologies offer complementary advantages: while SFC allows the simultaneous evaluation of multiple parameters using fluorochrome-conjugated antibodies, CyTOF uses metal isotopes to avoid spectral overlap, making it possible to measure more than 40 parameters in a single cell (143–145).

Recently, it has been shown that the integration of these two platforms can improve resolution and reproducibility in immune cell analysis. In a comparative study, a high correlation was observed between the results obtained with CFS and CyTOF in the identification and characterization of innate myeloid cells in blood, with a Pearson correlation coefficient of 0.99. However, differences were also identified in intra-measurement variability and in the cell recovery rate, with SFC being more efficient in these aspects (146).

The growing demand for fast and accurate diagnostics in remote environments is driving the development of miniaturized spectral cytometry systems, capable of being integrated into portable devices the size of a mobile

phone. These advances open a new frontier for real-time personalized medicine, especially in global health and high-precision surgery contexts.

An example of this evolution is the CytoTrack-Mini 2.0, which weighs just 800 grams and is capable of analyzing up to 15 parameters simultaneously using only 5 microliters of blood in less than 8 minutes (147,148). This level of wearable performance would have been unthinkable just a few years ago and reflects the maturation of multiple disruptive technologies.

CONCLUSION

In spectral flow cytometry, various components and technological advances have allowed a better understanding of cellular heterogeneity and its role in complex pathologies such as cancer. Through improved resolution and the ability to analyze multiple parameters simultaneously, spectral cytometry has provided powerful tools for detailed analysis of cells in the tumor microenvironment, immune system, and other biological conditions. However, this technology also faces significant challenges, such as high equipment costs and the need for specialized training for proper handling. Despite these barriers, its integration with emerging technologies such as nanotechnology and artificial intelligence promises to optimize data analysis and democratize access to this powerful tool. In this article, we highlight the importance of spectral cytometry in personalized medicine and early diagnosis, suggesting that its potential in biomedical research is vast and that its future adoption could significantly change the way we treat and diagnose various diseases, including oncology.

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REFERENCES

1. C. AR. Molecular biology and flow cytometry as new diagnostic tools. Oncology [Internet]. 2006 Dec 1 [cited 2025 Mar 31]; 16(3–4). Available from: https://doaj.org/article/c4e8b1cdc1d1490188daff34aed6a99c
2. Gajendra S, Sachdev R, Dorwal P, Goel S, Jha B, Sahni T. Mixed-phenotypic acute leukemia: Cytochemically myeloid and phenotypically early T-cell precursor acute lymphoblastic leukemia. Blood Res. 2014; 49(3):196–8.
3. McKinnon KM. Flow Cytometry: An Overview. Curr Protoc Immunol [Internet]. 2018 Jan 1 [cited 2025 Mar 31]; 120(1):5.1.1-5.1.11. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cpim.40
4. Cereceda K, Jorquera R, Villarroel-Espíndola F. Advances in mass cytometry and applicability in digital pathology for clinical-translational studies in oncology. Advances in Laboratory Medicine [Internet]. 2021 Mar 1 [cited 2025 Mar 31]; 3(1):17. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10197305/
5. Siddiqui S, Livák F. Principles of Advanced Flow Cytometry: A Practical Guide. Methods Mol Biol [Internet]. 2023 [cited 2025 Mar 31];2580:89. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10802916/
6. Spies NC, Rangel A, English P, Morrison M, O'Fallon B, Ng DP. Machine Learning Methods in Clinical Flow Cytometry. Cancers 2025, Vol 17, Page 483 [Internet]. 2025 Feb 1 [cited 2025 Mar 31]; 17(3):483. Available from: https://www.mdpi.com/2072-6694/17/3/483/htm
7. Jaimes MC, Leipold M, Kraker G, Amir E ad, Maecker H, Lannigan J. Full spectrum flow cytometry and mass cytometry: A 32-marker panel comparison. Cytometry A [Internet]. 2022 Nov 1 [cited 2025 Mar 31]; 101(11):942–59. Available from:https://pubmed.ncbi.nlm.nih.gov/35593221/
8. Robinson JP, Ostafe R, Iyengar SN, Rajwa B, Fischer R. Flow Cytometry: The Next Revolution. Cells [Internet]. 2023 Jul 1 [cited 2025 Mar 31]; 12(14):1875. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10378642/

9. Telford WG. Lasers in flow cytometry. Methods Cell Biol [Internet]. 2011 [cited 2025 Mar 31]; 102:373–409. Available from: https://pubmed.ncbi.nlm.nih.gov/21704847/
10. Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. Nat Rev Cancer [Internet]. 2021 Jun 1 [cited 2025 Mar 31]; 21(6):345–59. Available from: https://pubmed.ncbi.nlm.nih.gov/33837297/
11. Cheng Y, Newell EW. Deep Profiling Human T Cell Heterogeneity by Mass Cytometry. Adv Immunol [Internet]. 2016 [cited 2025 Mar 31]; 131:101–34. Available from: https://pubmed.ncbi.nlm.nih.gov/27235682/
12. Lucas F, Hergott CB. Advances in Acute Myeloid Leukemia Classification, Prognostication and Monitoring by Flow Cytometry. Clin Lab Med [Internet]. 2023 Sep 1 [cited 2025 Mar 31]; 43(3):377–98. Available from: https://pubmed.ncbi.nlm.nih.gov/37481318/
13. Terranova-Barberio M, Pawlowska N, Dhawan M, Moasser M, Chien AJ, Melisko ME, et al. Exhausted T cell signature predicts immunotherapy response in ER-positive breast cancer. Nat Commun [Internet]. 2020 Dec 1 [cited 2025 Mar 31]; 11(1):3584. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7367885/
14. Inamo J, Keegan J, Griffith A, Ghosh T, Horisberger A, Howard K, et al. Deep immunophenotyping reveals circulating activated lymphocytes in individuals at risk for rheumatoid arthritis. J Clin Invest [Internet]. 2025 Mar 17 [cited 2025 Mar 31]; 135(6):e185217. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11910230/
15. Bendall SC, Nolan GP. From single cells to deep phenotypes in cancer. Nat Biotechnol [Internet]. 2012 Jul 10 [cited 2025 Mar 31]; 30(7):639–47. Available from: https://europepmc.org/article/med/22781693
16. Brestoff JR, Jonathan Brestoff CR. Full spectrum flow cytometry in the clinical laboratory. Int J Lab Hematol [Internet]. 2023 Jun 1 [cited 2025 Mar 31]; 45(S2):44–9. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/ijlh.14098
17. Spasic M, Ogayo ER, Parsons AM, Mittendorf EA, van Galen P, McAllister SS. Spectral Flow Cytometry Methods and Check for updates Pipelines for Comprehensive Immunoprofiling of Human Peripheral Blood and Bone Marrow. Cancer Research Communications [Internet]. 2024 Mar 1 [cited 2025 Mar 31]; 4(3):895–910. Available from: /cancerrescommun/article/4/3/895/741931/Spectral-Flow-Cytometry-Methods-and-Pipelines-for
18. Vorobjev IA, Kussanova A, Barteneva NS. Development of Spectral Imaging Cytometry. Methods in Molecular Biology [Internet]. 2023 [cited 2025 Mar 31]; 2635:3–22. Available from: https://link.springer.com/protocol/10.1007/978-1-0716-3020-4_1
19. Vardaman D, Ali MA, Hasanul M, Siam B, Bolding C, Tidwell H, et al. Development of a Spectral Flow Cytometry Analysis Pipeline for High-dimensional Immune Cell Characterization. The Journal of Immunology [Internet]. 2024 Dec 1 [cited 2025 Mar 31]; 213(11):1713–24. Available from: https://dx.doi.org/10.4049/jimmunol.2400370
20. Moldavan A. PHOTO-ELECTRIC TECHNIQUE FOR THE COUNTING OF MICROSCOPICAL CELLS. Science [Internet]. 1934 [cited 2025 Mar 31]; 80(2069):188–9. Available from: https://pubmed.ncbi.nlm.nih.gov/17817054/
21. 50 years LASERS: in vitro diagnostics, clinical applications and perspectives - PubMed [Internet]. [cited 2025 Mar 31]. Available from: https://pubmed.ncbi.nlm.nih.gov/21500720/
22. Bonner WA, Hulett HR, Sweet RG, Herzenberg LA. Fluorescence activated cell sorting. Rev Sci Instrum [Internet]. 1972 [cited 2025 Mar 31]; 43(3):404–9. Available from: https://pubmed.ncbi.nlm.nih.gov/5013444/
23. Drescher H, Weiskirchen S, Weiskirchen R. Flow Cytometry: A Blessing and a Curse. Biomedicines [Internet]. 2021 Nov 1 [cited 2025 Mar 31]; 9(11):1613. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8615642/
24. Flow Cytometry, Analysis, and Forecast Market Size 2022-2032. [Internet]. [cited 2025 Mar 31]. Available from: https://www.sphericalinsights.com/es/reports/flow-cytometry-market?utm_source=chatgpt.com
25. Analytical Flow Cytometry Market Analysis (2032) [Internet]. [cited 2025 Mar 31]. Available from: https://www.wiseguyreports.com/es/reports/analytical-flow-cytometry-market?utm_source=chatgpt.com

26. Flow Cytometry Market Size & Share | Industry Report, 2030 [Internet]. [cited 2025 Mar 31]. Available from: https://www.grandviewresearch.com/industry-analysis/flow-cytometry-market
27. Edwards JM, Andrews MC, Burridge H, Smith R, Owens C, Edinger M, et al. Design, optimisation and standardisation of a high-dimensional spectral flow cytometry workflow assessing T-cell immunophenotype in patients with melanoma. Clin Transl Immunology [Internet]. 2023 [cited 2025 Mar 31]; 12(9):e1466. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10484688/
28. Roederer M. Compensation in flow cytometry. Curr Protoc Cytom [Internet]. 2002 [cited 2025 Mar 31]; Chapter 1. Available from: https://pubmed.ncbi.nlm.nih.gov/18770762/
29. Rastogi P, Sachdeva MUS. Flow Cytometric Minimal Residual Disease Analysis in Acute Leukemia: Current Status. Indian Journal of Hematology & Blood Transfusion [Internet]. 2019 Jan 1 [cited 2025 Mar 31]; 36(1):3. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7042470/
30. Sabines-Chesterking J, Burenkov IA, Polyakov S V. Quantum measurement enables single biomarker sensitivity in flow cytometry. Scientific Reports 2024 14:1 [Internet]. 2024 Feb 16 [cited 2025 Mar 31]; 14(1):1–8. Available from: https://www.nature.com/articles/s41598-023-49145-7
31. Theunissen P, Consortium on behalf of the E, Mejstrikova E, Consortium on behalf of the E, Sedek L, Consortium on behalf of the E, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood [Internet]. 2017 Jan 19 [cited 2025 Mar 31]; 129(3):347–57. Available from: https://dx.doi.org/10.1182/blood-2016-07-726307
32. F. McGowan P, D. Hyter S, Cui W, Plummer RM, Godwin AK, Zhang D. Comparison of flow cytometry and next-generation sequencing in minimal residual disease monitoring of acute myeloid leukemia: One institute's practical clinical experience. Int J Lab Hematol [Internet]. 2022 Feb 1 [cited 2025 Mar 31]; 44(1):118–26. Available from: https://pubmed.ncbi.nlm.nih.gov/34585519/
33. Kalina T, Flores-Montero J, Lecrevisse Q, Pedreira CE, van der Velden VHJ, Novakova M, et al. Quality assessment program for EuroFlow protocols: summary results of four-year (2010-2013) quality assurance rounds. Cytometry A [Internet]. 2015 Feb 1 [cited 2025 Mar 31]; 87(2):145–56. Available from: https://pubmed.ncbi.nlm.nih.gov/25345353/
34. Van Dongen JJM, Orfao A. EuroFlow: Resetting leukemia and lymphoma immunophenotyping. Basis for companion diagnostics and personalized medicine. Leukemia 2012 26:9 [Internet]. 2012 Sep 5 [cited 2025 Mar 31]; 26(9):1899–907. Available from: https://www.nature.com/articles/leu2012121
35. Kalina T, Flores-Montero J, Van der Velden VHJ, Martin-Ayuso M, Böttcher S, Ritgen M, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia [Internet]. 2012 Sep [cited 2025 Mar 31]; 26(9):1986–2010. Available from: https://pubmed.ncbi.nlm.nih.gov/22948490/
36. Finak G, Langweiler M, Jaimes M, Malek M, Taghiyar J, Korin Y, et al. Standardizing Flow Cytometry Immunophenotyping Analysis from the Human ImmunoPhenotyping Consortium. Scientific Reports 2016 6:1 [Internet]. 2016 Feb 10 [cited 2025 Mar 31]; 6(1):1–11. Available from: https://www.nature.com/articles/srep20686
37. Kalina T, Flores-Montero J, Lecrevisse Q, Pedreira CE, van der Velden VHJ, Novakova M, et al. Quality assessment program for EuroFlow protocols: summary results of four-year (2010-2013) quality assurance rounds. Cytometry A [Internet]. 2015 Feb 1 [cited 2025 Mar 31]; 87(2):145–56. Available from: https://pubmed.ncbi.nlm.nih.gov/25345353/
38. Bene L, Damjanovich L. The other side of the coin: time-domain fluorescence lifetime in flow. Cytometry A [Internet]. 2015 Feb 1 [cited 2025 Apr 20]; 87(2):101–3. Available from: https://pubmed.ncbi.nlm.nih.gov/25515417/
39. Weber LM, Robinson MD. Comparison of clustering methods for high-dimensional single-cell flow and mass cytometry data. Cytometry Part A [Internet]. 2016 Dec 1 [cited 2025 Apr 20]; 89(12):1084–96. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.23030
40. Bengtsson E, Danielsen H, Treanor D, Gurcan MN, MacAulay C, Molnár B. Computer-aided diagnostics in digital pathology. Cytometry A [Internet]. 2017 Jun 1 [cited 2025 Apr 20]; 91(6):551–4. Available from: https://pubmed.ncbi.nlm.nih.gov/28640523/
41. Nolan JP, Condello D. Spectral flow cytometry. Curr Protoc Cytom [Internet]. 2013 [cited 2025 Mar 31]; Chapter 1(SUPPL.63). Available from: https://pubmed.ncbi.nlm.nih.gov/23292705/
42. Vardaman D, Ali MA, Siam MHB, Bolding C, Tidwell H, Stephens HR, et al. Development of a Spectral Flow Cytometry Analysis Pipeline for High-dimensional Immune Cell Characterization. J

Immunol [Internet]. 2024 Dec 1 [cited 2025 Mar 31]; 213(11):1713–24. Available from: https://pubmed.ncbi.nlm.nih.gov/39451039/

43. Ferrer-Font L, Small SJ, Lewer B, Pilkington KR, Johnston LK, Park LM, et al. Panel Optimization for High-Dimensional Immunophenotyping Assays Using Full-Spectrum Flow Cytometry. Curr Protoc [Internet]. 2021 Sep 1 [cited 2025 Apr 20]; 1(9):e222. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cpz1.222
44. Proto JD, Doran AC, Gusarova G, Yurdagul A, Sozen E, Subramanian M, et al. Regulatory T Cells Promote Macrophage Efferocytosis during Inflammation Resolution. Immunity [Internet]. 2018 Oct 16 [cited 2025 Apr 20]; 49(4):666-677.e6. Available from: https://pubmed.ncbi.nlm.nih.gov/30291029/
45. Spasic M, Ogayo ER, Parsons AM, Mittendorf EA, van Galen P, McAllister SS. Spectral Flow Cytometry Methods and Pipelines for Comprehensive Immunoprofiling of Human Peripheral Blood and Bone Marrow. Cancer Research Communications [Internet]. 2024 Mar 1 [cited 2025 Apr 20]; 4(3):895. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10962315/
46. Ferrer-Font L, Pellefigues C, Mayer JU, Small SJ, Jaimes MC, Price KM. Panel Design and Optimization for High-Dimensional Immunophenotyping Assays Using Spectral Flow Cytometry. Curr Protoc Cytom. 2020 Mar 1; 92(1).
47. Telford WG. Laser Sources for Traditional and Spectral Flow Cytometry. Methods Mol Biol [Internet]. 2024 [cited 2025 Mar 31]; 2779:33–68. Available from: https://pubmed.ncbi.nlm.nih.gov/38526781/
48. Kwok SJJ, Forward S, Fahlberg MD, Assita ER, Cosgriff S, Lee SH, et al. High-dimensional multi-pass flow cytometry via spectrally encoded cellular barcoding. Nature Biomedical Engineering 2023 8:3 [Internet]. 2023 Nov 30 [cited 2025 Mar 31]; 8(3):310–24. Available from: https://www.nature.com/articles/s41551-023-01144-9
49. Kare AJ, Nichols L, Zermeno R, Raie MN, Tumbale SK, Ferrara KW. OMIP-095: 40-Color spectral flow cytometry delineates all major leukocyte populations in murine lymphoid tissues. Cytometry A [Internet]. 2023 Nov 1 [cited 2025 Mar 31]; 103(11):839–50. Available from: https://pubmed.ncbi.nlm.nih.gov/37768325/
50. Nolan JP. The evolution of spectral flow cytometry. Cytometry Part A [Internet]. 2022 Oct 1 [cited 2025 Mar 31]; 101(10):812–7. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24566
51. Cytek ® Aurora User's Guide. 2021;
52. Lawrence WG, Varadi G, Entine G, Podniesinski E, Wallace PK. Enhanced red and near infrared detection in flow cytometry using avalanche photodiodes. Cytometry Part A [Internet]. 2008 Aug 1 [cited 2025 Apr 20]; 73A(8):767–76. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.20595
53. Stewart CC, Woodring ML, Podniesinski E, Gray B. Flow cytometer in the infrared: Inexpensive modifications to a commercial instrument. Cytometry Part A. 2005 Oct; 67(2):104–11.
54. Lawrence WG, Varadi G, Entine G, Podniesinski E, Wallace PK. A comparison of avalanche photodiode and photomultiplier tube detectors for flow cytometry. Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues VI. 2008 Feb 7; 6859:68590M.
55. Deiters K, Flügel T, Renker D, Wirtz HP, Kirn T, Denes P. Comparison of photomultiplier and avalanche photodiode readout of PbWO4. Nucl Instrum Methods Phys Res A. 1997 Mar 1; 387(1–2):211–3.
56. Adan A, Alizada G, Kiraz Y, Baran Y, Nalbant A. Flow cytometry: basic principles and applications. Crit Rev Biotechnol. 2017 Feb 17; 37(2):163–76.
57. Shrirao AB, Fritz Z, Novik EM, Yarmush GM, Schloss RS, Zahn JD, et al. Microfluidic flow cytometry: The role of microfabrication methodologies, performance and functional specification. Technology (Singap World Sci) [Internet]. 2018 Mar [cited 2025 Apr 20]; 6(1):1. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5907470/
58. Irish JM, Doxie DB. High-dimensional single-cell cancer biology. Curr Top Microbiol Immunol. 2014;377.
59. Turaç G, Hindley CJ, Thomas R, Davis JA, Deleidi M, Gasser T, et al. Combined Flow Cytometric Analysis of Surface and Intracellular Antigens Reveals Surface Molecule Markers of Human Neuropoiesis. PLoS One. 2013 Jun 24; 8(6).

60. Henel G, Schmitz JL. Basic theory and clinical applications of flow cytometry. Lab Med. 2007 Jul; 38(7):428–36.
61. Novo D. A comparison of spectral unmixing to conventional compensation for the calculation of fluorochrome abundances from flow cytometric data. Cytometry Part A [Internet]. 2022 Nov 1 [cited 2025 Apr 20]; 101(11):885–91. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24669
62. Bhowmick D, Lowe SK, Ratliff ML. Side-by-Side Comparison of Compensation Beads Used in Polychromatic Flow Cytometry. Immunohorizons [Internet]. 2023 Dec 1 [cited 2025 Apr 20]; 7(12):819–33. Available from: https://dx.doi.org/10.4049/immunohorizons.2300066
63. Vardaman D, Ali MA, Hasanul M, Siam B, Bolding C, Tidwell H, et al. Development of a Spectral Flow Cytometry Analysis Pipeline for High-dimensional Immune Cell Characterization. The Journal of Immunology [Internet]. 2024 Dec 1 [cited 2025 Apr 20]; 213(11):1713–24. Available from: https://dx.doi.org/10.4049/jimmunol.2400370
64. Vorobjev IA, Kussanova A, Barteneva NS. Development of Spectral Imaging Cytometry. Methods in Molecular Biology [Internet]. 2023 [cited 2025 Apr 20]; 2635:3–22. Available from: https://link.springer.com/protocol/10.1007/978-1-0716-3020-4_1
65. Nolan JP, Condello D. The evolution of spectral flow cytometry. Cytometry Part A [Internet]. 2022 Oct 1 [cited 2025 Apr 20]; 101(10):812–7. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24566
66. Robinson JP. Flow Cytometry: Past and Future. Biotechniques [Internet]. 2022 Apr 1 [cited 2025 Apr 20]; 72(4):159–69. Available from: https://www.tandfonline.com/doi/abs/10.2144/btn-2022-0005
67. Paul Robinson J, Rajwa B. Spectral flow cytometry: Fundamentals and future impact. Methods Cell Biol. 2024 Jan 1; 186:311–32.
68. Nolan JP, Condello D. Spectral Flow Cytometry. Current protocols in cytometry / editorial board, J Paul Robinson, managing editor . [et al] [Internet]. 2013 [cited 2025 Apr 20]; CHAPTER(SUPPL.63):Unit1.27. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3556726/
69. den Braanker H, Bongenaar M, Lubberts E. How to Prepare Spectral Flow Cytometry Datasets for High Dimensional Data Analysis: A Practical Workflow. Front Immunol [Internet]. 2021 Nov 19 [cited 2025 Apr 20];12:768113. Available from: https://github.com/HdBraanker/Spectral_
70. Kröger C, Müller S, Leidner J, Kröber T, Warnat-Herresthal S, Spintge JB, et al. Unveiling the power of high-dimensional cytometry data with cyCONDOR. Nature Communications 2024 15:1 [Internet]. 2024 Dec 19 [cited 2025 Apr 20]; 15(1):1–16. Available from: https://www.nature.com/articles/s41467-024-55179-w
71. Croce AC, Bottiroli G. Autofluorescence Spectroscopy and Imaging: A Tool for Biomedical Research and Diagnosis. Eur J Histochem [Internet]. 2014 Dec 12 [cited 2025 Apr 20]; 58(4):2461. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4289852/
72. Semenov AN, Yakimov BP, Rubekina AA, Gorin DA, Drachev VP, Zarubin MP, et al. The Oxidation-Induced Autofluorescence Hypothesis: Red Edge Excitation and Implications for Metabolic Imaging. Molecules [Internet]. 2020 Apr 1 [cited 2025 Apr 20]; 25(8):1863. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7221974/
73. Bertolo A, Baur M, Guerrero J, Pötzel T, Stoyanov J. Autofluorescence is a Reliable in vitro Marker of Cellular Senescence in Human Mesenchymal Stromal Cells. Scientific Reports 2019 9:1 [Internet]. 2019 Feb 14 [cited 2025 Apr 20]; 9(1):1–15. Available from: https://www.nature.com/articles/s41598-019-38546-2
74. Spasic M, Ogayo ER, Parsons AM, Mittendorf EA, van Galen P, McAllister SS. Spectral Flow Cytometry Methods and Check for updates Pipelines for Comprehensive Immunoprofiling of Human Peripheral Blood and Bone Marrow. Cancer Research Communications [Internet]. 2024 Mar 1 [cited 2025 Apr 20]; 4(3):895–910. Available from: /cancerrescommun/article/4/3/895/741931/Spectral-Flow-Cytometry-Methods-and-Pipelines-for
75. Schmutz S, Valente M, Cumano A, Novault S. Analysis of Cell Suspensions Isolated from Solid Tissues by Spectral Flow Cytometry. J Vis Exp [Internet]. 2017 May 5 [cited 2025 Apr 20]; 2017(123):55578. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5607894/

76. Seong Y, Nguyen DX, Wu Y, Thakur A, Harding F, Nguyen TA. Novel PE and APC tandems: Additional near-infrared fluorochromes for use in spectral flow cytometry. Cytometry [Internet]. 2022 Oct 1 [cited 2025 Apr 20]; 101(10):835. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9790705/
77. Longhini ALF, Fernández-Maestre I, Kennedy MC, Wereski MG, Mowla S, Xiao W, et al. Development of a customizable mouse backbone spectral flow cytometry panel to delineate immune cell populations in normal and tumor tissues. Front Immunol. 2024 Mar 27;15:1374943.
78. Iyengar SN, Patsekin V, Rajwa B, Bae E, Dowden B, Ragheb K, et al. Measuring autofluorescence spectral signatures for detecting antibiotic-resistant bacteria using Thermofisher's Bigfoot spectral flow cytometer. 2024 May 15 [cited 2025 Apr 20]; Available from: http://biorxiv.org/lookup/doi/10.1101/2024.05.13.593944
79. Tang X, Mai H, Wang L, Chen S, Chen F, Li T, et al. Diagnostic significance of cerebrospinal fluid flow cytometry in Chinese children with B lineage acute lymphoblastic leukemia. BMC Pediatr [Internet]. 2024 Dec 1 [cited 2025 Apr 20]; 24(1):1–8. Available from: https://bmcpediatr.biomedcentral.com/articles/10.1186/s12887-024-04684-4
80. Zhao H, Ma M, Zhang L, Zheng G, Lv H, Liu J, et al. Diagnosis of central nervous system lymphoma via cerebrospinal fluid cytology: a case report. BMC Neurol [Internet]. 2019 May 7 [cited 2025 Apr 20]; 19(1):90. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6505083/
81. Tu S, Zhang K, Liu C, Wang N, Chu J, Yang L, et al. Cerebrospinal Fluid Flow Cytometry in Pediatric Acute Lymphoblastic Leukemia: A Multicenter Retrospective Study in China. Cancer Med [Internet]. 2024 Dec 1 [cited 2025 Apr 20]; 13(23):E70452. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cam4.70452
82. Garcia KLA, Cherian S, Stevenson PA, Martino CH, Shustov AR, Becker PS, et al. Cerebrospinal fluid flow cytometry and risk of central nervous system relapse after hyperCVAD in adults with acute lymphoblastic leukemia. Cancer [Internet]. 2022 Apr 1 [cited 2025 Apr 20]; 128(7):1411–7. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cncr.34073
83. Malikova H, Burghardtova M, Koubska E, Mandys V, Kozak T, Weichet J. Secondary central nervous system lymphoma: Spectrum of morphological mri appearances. Neuropsychiatr Dis Treat. 2018 Mar 12; 14:733–40.
84. Szaflarska A, Lenart M, Rutkowska-Zapała M, Siedlar M. Clinical and experimental treatment of primary humoral immunodeficiencies. Clin Exp Immunol [Internet]. 2024 Apr 23 [cited 2025 Apr 21]; 216(2):120–31. Available from: https://dx.doi.org/10.1093/cei/uxae008
85. Attrill MH, Shinko D, Alexiou V, Kartawinata M, study C, Al-Abadi E, et al. The immune landscape of the inflamed joint defined by spectral flow cytometry. Clin Exp Immunol [Internet]. 2024 Nov 12 [cited 2025 Apr 21]; 218(3):221–41. Available from: https://dx.doi.org/10.1093/cei/uxae071
86. Tchitchek N, Binvignat M, Roux A, Pitoiset F, Dubois J, Marguerit G, et al. Deep immunophenotyping reveals that autoimmune and autoinflammatory disorders are spread along two immunological axes, capturing disease, inflammation, levels and types. Ann Rheum Dis [Internet]. 2024 May 1 [cited 2025 Apr 21]; 83(5):638–50. Available from: https://ard.bmj.com/content/83/5/638
87. Wu K, Wang Z, Xie Y, Wang X, Hu W, Cai J, et al. One Tube 23 Color Full Spectral Flow Cytometry Panel in Detecting Minimal Residual Disease in Pediatric B-Cell Acute Lymphoblastic Leukemia. Blood [Internet]. 2023 Nov 2 [cited 2025 Apr 21]; 142(Supplement 1):1607–1607. Available from: https://dx.doi.org/10.1182/blood-2023-181565
88. Fokken H, Waclawski J, Kattre N, Kloos A, Müller S, Ettinger M, et al. A 19-color single-tube full spectrum flow cytometry assay for the detection of measurable residual disease in acute myeloid leukemia. Cytometry Part A [Internet]. 2024 Mar 1 [cited 2025 Apr 21]; 105(3):181–95. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24811
89. Coustan-Smith E, Song G, Shurtleff S, Yeoh AEJ, Chng WJ, Chen SP, et al. Universal monitoring of minimal residual disease in acute myeloid leukemia. JCI Insight [Internet]. 2018 May 3 [cited 2025 Apr 21]; 3(9):98561–98561. Available from: https://europepmc.org/articles/PMC6012500
90. Chen M, Fu M, Gong M, Gao Y, Wang A, Zhao W, et al. Twenty-four-color full spectrum flow cytometry panel for minimal residual disease detection in acute myeloid leukemia. Open Medicine (Poland). 2023 Jan 1; 18(1).

91. Zhang S, Wang X, Yang Z, Ding M, Zhang M, Young KH, et al. Minimal residual disease detection in lymphoma: methods, procedures and clinical significance. Front Immunol [Internet]. 2024 [cited 2025 Apr 21];15:1430070. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11345172/
92. Furlanetto G, Spagnol F, Alegretti AP, Farias MG, Soares VJ, Daudt LE, et al. Flow cytometry as a diagnostic tool in neuroblastoma. J Immunol Methods. 2021 Nov 1;498.
93. Chen C, Hu C, He B, Bai Y, He F, Li S, et al. Functionalized GD2 Electrochemical Immunosensor to Diagnose Minimum Residual Disease of Bone Marrow in Neuroblastoma Effectively. Biosensors 2023, Vol 13, Page 920 [Internet]. 2023 Oct 10 [cited 2025 Apr 21]; 13(10):920. Available from: https://www.mdpi.com/2079-6374/13/10/920/htm
94. Theodorakos I, Paterakis G, Papadakis V, Vicha A, Topakas G, Jencova P, et al. Interference of bone marrow CD56+ mesenchymal stromal cells in minimal residual disease investigation of neuroblastoma and other CD45−/CD56+ pediatric malignancies using flow cytometry. Pediatr Blood Cancer. 2019 Aug 1; 66(8).
95. Kwon WK, Choi SI, Kim HJ, Huh HJ, Kang JM, Kim YJ, et al. Flow Cytometry for the Diagnosis of Primary Immunodeficiency Diseases: A Single Center Experience. Allergy Asthma Immunol Res [Internet]. 2020 Mar 1 [cited 2025 Apr 21]; 12(2):292–305. Available from: https://doi.org/10.4168/aair.2020.12.2.292
96. Vardaman D, Ali MA, Hasanul M, Siam B, Bolding C, Tidwell H, et al. Development of a Spectral Flow Cytometry Analysis Pipeline for High-dimensional Immune Cell Characterization. The Journal of Immunology [Internet]. 2024 Dec 1 [cited 2025 Apr 21]; 213(11):1713–24. Available from: https://dx.doi.org/10.4049/jimmunol.2400370
97. Lamb ER, Glomski IJ, Harper TA, Solga MD, Criss AK. High-dimensional spectral flow cytometry of activation and phagocytosis by peripheral human polymorphonuclear leukocytes. bioRxiv [Internet]. 2024 Dec 5 [cited 2025 Apr 21];2024.12.01.626241. Available from: https://www.biorxiv.org/content/10.1101/2024.12.01.626241v1
98. Tahiat A, Belbouab R, Yagoubi A, Hakem S, Fernini F, Keddari M, et al. Flow cytometry-based diagnostic approach for inborn errors of immunity: experience from Algeria. Front Immunol [Internet]. 2024 Jul 12 [cited 2025 Apr 21];15:1402038. Available from: www.esid.org
99. Attrill MH, Shinko D, Alexiou V, Kartawinata M, study C, Al-Abadi E, et al. The immune landscape of the inflamed joint defined by spectral flow cytometry. Clin Exp Immunol [Internet]. 2024 Nov 12 [cited 2025 Apr 21]; 218(3):221–41. Available from: https://dx.doi.org/10.1093/cei/uxae071
100. Delmonte OM, Fleisher TA. Flow cytometry: Surface markers and beyond. Journal of Allergy and Clinical Immunology [Internet]. 2019 Feb 1 [cited 2025 Apr 21]; 143(2):528–37. Available from: https://www.jacionline.org/action/showFullText?pii=S0091674918311990
101. Poloni C, Schonhofer C, Ivison S, Levings MK, Steiner TS, Cook L. T-cell activation–induced marker assays in health and disease. Immunol Cell Biol [Internet]. 2023 Jul 1 [cited 2025 Apr 21]; 101(6):491–503. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/imcb.12636
102. De Graaf MT, De Jongste AHC, Kraan J, Boonstra JG, Smitt PAES, Gratama JW. Flow cytometric characterization of cerebrospinal fluid cells. Cytometry B Clin Cytom [Internet]. 2011 Sep 1 [cited 2025 Apr 21]; 80B(5):271–81. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.b.20603
103. Kreutmair S, Unger S, Núñez NG, Ingelfinger F, Alberti C, De Feo D, et al. Distinct immunological signatures discriminate severe COVID-19 from non-SARS-CoV-2-driven critical pneumonia. Immunity [Internet]. 2021 Jul 13 [cited 2025 Apr 21]; 54(7):1578. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8106882/
104. Fu Y, Tilley L, Kenny S, Klonis N. Dual labeling with a far red probe permits analysis of growth and oxidative stress in P. falciparum-infected erythrocytes. Cytometry A [Internet]. 2010 Mar [cited 2025 Apr 21]; 77(3):253–63. Available from: https://pubmed.ncbi.nlm.nih.gov/20091670/
105. Butzloff S, Groves MR, Wrenger C, Müller IB. Cytometric quantification of singlet oxygen in the human malaria parasite Plasmodium falciparum. Cytometry Part A. 2012 Aug; 81 A(8):698–703.
106. Deravi N, Berke O, Woods JP, Bienzle D. Specific immunotypes of canine T cell lymphoma are associated with different outcomes. Vet Immunol Immunopathol [Internet]. 2017 Sep 1 [cited 2025 Apr 21]; 191:5–13. Available from: https://pubmed.ncbi.nlm.nih.gov/28895866/

107. Stokol T, Nickerson GA, Shuman M, Belcher N. Dogs with Acute Myeloid Leukemia Have Clonal Rearrangements in T and B Cell Receptors. Front Vet Sci [Internet]. 2017 May 31 [cited 2025 Apr 21]; 4(MAY). Available from: https://pubmed.ncbi.nlm.nih.gov/28620611/
108. Avery PR, Burton J, Bromberek JL, Seelig DM, Elmslie R, Correa S, et al. Flow cytometric characterization and clinical outcome of CD4+ T-cell lymphoma in dogs: 67 cases. J Vet Intern Med [Internet]. 2014 [cited 2025 Apr 21]; 28(2):538–46. Available from: https://pubmed.ncbi.nlm.nih.gov/24495161/
109. Meichner K, Stokol T, Tarigo J, Avery A, Burkhard MJ, Comazzi S, et al. Multicenter flow cytometry proficiency testing of canine blood and lymph node samples. Vet Clin Pathol [Internet]. 2020 Jun 1 [cited 2025 Apr 21]; 49(2):249–57. Available from: https://pubmed.ncbi.nlm.nih.gov/32246538/
110. Wang XF, Wang YH, Bai B, Zhang M, Chen J, Zhang X, et al. Truncation of the Cytoplasmic Tail of Equine Infectious Anemia Virus Increases Virion Production by Improving Env Cleavage and Plasma Membrane Localization. J Virol [Internet]. 2021 Nov 9 [cited 2025 Apr 21]; 95(23):E01087-21. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8577380/
111. Davis EG, Wilkerson MJ, Rush BR. Flow Cytometry: Clinical Applications in Equine Medicine. J Vet Intern Med [Internet]. 2002 Jul 1 [cited 2025 Apr 21]; 16(4):404–10. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1939-1676.2002.tb01257.x
112. Zwicklbauer K, von la Roche D, Krentz D, Kolberg L, Alberer M, Zablotski Y, et al. Adapting the SMART tube technology for flow cytometry in feline full blood samples. Front Vet Sci. 2024 Jun 26;11:1377414.
113. Hoffmann-Fezer G, Mortelbauer W, Hartmann K, Mysliwietz J, Thefeld S, Beer B, et al. Comparison of T-cell subpopulations in cats naturally infected with feline leukaemia virus or feline immunodeficiency virus. Res Vet Sci [Internet]. 1996 [cited 2025 Apr 21]; 61(3):222–6. Available from: https://pubmed.ncbi.nlm.nih.gov/8938851/
114. Bourque J, Desforges JP, Levin M, Atwood TC, Sonne C, Dietz R, et al. Climate-associated drivers of plasma cytokines and contaminant concentrations in Beaufort Sea polar bears (Ursus maritimus). Sci Total Environ [Internet]. 2020 Nov 25 [cited 2025 Apr 21];745. Available from: https://pubmed.ncbi.nlm.nih.gov/32738684/
115. Hauser KA, Garvey CN, Crow RS, Hossainey MRH, Howard DT, Ranganathan N, et al. Amphibian mast cells serve as barriers to chytrid fungus infections. Elife. 2023 Jul 31;12.
116. Panza G, Frontalini F, Ciacci C, Protano G, Montanari M, Lopez D, et al. Environmental Diagnosis through a Flow Cytometric Approach. Int J Mol Sci [Internet]. 2024 Oct 1 [cited 2025 Apr 21]; 25(20):11069. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11507161/
117. Park LM, Lannigan J, Jaimes MC. OMIP-069: Forty-Color Full Spectrum Flow Cytometry Panel for Deep Immunophenotyping of Major Cell Subsets in Human Peripheral Blood. Cytometry Part A [Internet]. 2020 Oct 1 [cited 2025 Apr 22]; 97(10):1044–51. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24213
118. Weber LM, Robinson MD. Comparison of clustering methods for high-dimensional single-cell flow and mass cytometry data. Cytometry Part A [Internet]. 2016 Dec 1 [cited 2025 Apr 22]; 89(12):1084–96. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.23030
119. den Braanker H, Bongenaar M, Lubberts E. How to Prepare Spectral Flow Cytometry Datasets for High Dimensional Data Analysis: A Practical Workflow. Front Immunol [Internet]. 2021 Nov 19 [cited 2025 Apr 22];12:768113. Available from: https://github.com/HdBraanker/Spectral_
120. Belkina AC, Ciccolella CO, Anno R, Halpert R, Spidlen J, Snyder-Cappione JE. Automated optimized parameters for T-distributed stochastic neighbor embedding improve visualization and analysis of large datasets. Nature Communications 2019 10:1 [Internet]. 2019 Nov 28 [cited 2025 Apr 21]; 10(1):1–12. Available from: https://www.nature.com/articles/s41467-019-13055-y
121. Huang H, Wang Y, Rudin C, Browne EP. Towards a comprehensive evaluation of dimension reduction methods for transcriptomic data visualization. Communications Biology 2022 5:1 [Internet]. 2022 Jul 19 [cited 2025 Apr 21]; 5(1):1–11. Available from: https://www.nature.com/articles/s42003-022-03628-x
122. Lin L, Frelinger J, Jiang W, Finak G, Seshadri C, Bart PA, et al. Identification and visualization of multidimensional antigen-specific T-cell populations in polychromatic cytometry data. Cytometry

Part A [Internet]. 2015 Jul 1 [cited 2025 Apr 21]; 87(7):675–82. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.22623
123. Van Unen V, Höllt T, Pezzotti N, Li N, Reinders MJT, Eisemann E, et al. Visual analysis of mass cytometry data by hierarchical stochastic neighbour embedding reveals rare cell types. Nature Communications 2017 8:1 [Internet]. 2017 Nov 23 [cited 2025 Apr 21]; 8(1):1–10. Available from: https://www.nature.com/articles/s41467-017-01689-9
124. Mazza EMC, Brummelman J, Alvisi G, Roberto A, De Paoli F, Zanon V, et al. Background fluorescence and spreading error are major contributors of variability in high-dimensional flow cytometry data visualization by t-distributed stochastic neighboring embedding. Cytometry Part A [Internet]. 2018 Aug 1 [cited 2025 Apr 21]; 93(8):785–92. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.23566
125. Kowalski K, Bauman NP. Resource-adaptive quantum flow algorithms for quantum simulations of many-body systems: sub-flow embedding procedures. 2024 Oct 15 [cited 2025 Apr 21]; Available from: https://arxiv.org/abs/2410.11992v1
126. Kowalski K, Bauman NP. Quantum Flow Algorithms for Simulating Many-Body Systems on Quantum Computers. Phys Rev Lett [Internet]. 2023 Nov 17 [cited 2025 Apr 21]; 131(20):200601. Available from: https://journals.aps.org/prl/abstract/10.1103/PhysRevLett.131.200601
127. Chen S, Weddell J, Gupta P, Conard G, Parkin J, Imoukhuede PI. qFlow Cytometry-Based Receptoromic Screening: A High-Throughput Quantification Approach Informing Biomarker Selection and Nanosensor Development. Methods Mol Biol [Internet]. 2017 [cited 2025 Apr 21]; 1570:117–38. Available from: https://pubmed.ncbi.nlm.nih.gov/28238133/
128. Fang Y, Malik M, England SK, Imoukhuede PI. Absolute Quantification of Plasma Membrane Receptors Via Quantitative Flow Cytometry. Methods Mol Biol [Internet]. 2022 [cited 2025 Apr 21]; 2475:61–77. Available from: https://pubmed.ncbi.nlm.nih.gov/35451749/
129. Ashhurst TM, Marsh-Wakefield F, Givanna |, Putri H, Spiteri AG, Shinko D, et al. Integration, exploration, and analysis of high-dimensional single-cell cytometry data using Spectre. Cytometry Part A [Internet]. 2022 Mar 1 [cited 2025 Apr 21]; 101(3):237–53. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24350
130. Welsh JA, Van Der Pol E, Arkesteijn GJA, Bremer M, Brisson A, Coumans F, et al. MIFlowCyt-EV: a framework for standardized reporting of extracellular vesicle flow cytometry experiments. J Extracell Vesicles [Internet]. 2020 Jan 1 [cited 2025 Apr 21]; 9(1). Available from: https://www.tandfonline.com/doi/abs/10.1080/20013078.2020.1713526
131. Lee JA, Spidlen J, Boyce K, Cai J, Crosbie N, Dalphin M, et al. MIFlowCyt: the minimum information about a Flow Cytometry Experiment. Cytometry A [Internet]. 2008 Oct [cited 2025 Apr 21]; 73(10):926–30. Available from: https://pubmed.ncbi.nlm.nih.gov/18752282/
132. Spidlen J, Breuer K, Brinkman R. Preparing a Minimum Information about a Flow Cytometry Experiment (MIFlowCyt) Compliant Manuscript Using the International Society for Advancement of Cytometry (ISAC) FCS File Repository (FlowRepository.org). [cited 2025 Apr 21]; Available from: http://www.mozilla.org
133. Flow Cytometer Pricing: A Researcher's Personal Guide - Merkel Technologies Ltd [Internet]. [cited 2025 Apr 22]. Available from: https://merkel.co.il/flow-cytometer-price/
134. Belkina AC, Roe CE, Tang VA, Back JB, Bispo C, Conway A, et al. Guidelines for establishing a cytometry laboratory. Cytometry Part A [Internet]. 2024 Feb 1 [cited 2025 Apr 22]; 105(2):88–111. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24807
135. Cytek ® Aurora CS User's Guide. 2022;
136. Spectral Flow Cytometer Market Growth and Analysis 2032 [Internet]. [cited 2025 Apr 22]. Available from: https://www.wiseguyreports.com/reports/spectral-flow-cytometer-market
137. EuroFlow [Internet]. [cited 2025 Apr 22]. Available from: https://euroflow.org/
138. CytoFLEX mosaic [Internet]. [cited 2025 Apr 22]. Available from: https://www.beckman.mx/flow-cytometry/research-flow-cytometers/cytoflex-mosaic
139. Explore the CytoFLEX mosaic: Spectral cytometry redefined [Internet]. [cited 2025 Apr 22]. Available from: https://www.news-medical.net/news/20250318/Beckman-Coulter-Life-Sciences-launches-industry-first-modular-spectral-flow-cytometry-solution.aspx

140. Wang Z, Yi S, Chen A, Zhou M, Luk TS, James A, et al. Single-shot on-chip spectral sensors based on photonic crystal slabs. Nature Communications 2019 10:1 [Internet]. 2019 Mar 4 [cited 2025 Apr 22]; 10(1):1–6. Available from: https://www.nature.com/articles/s41467-019-08994-5
141. Yun HG, Cadierno A, Kim TW, Muñoz-Barrutia A, Garica-Gonzalez D, Choi S, et al. Computational Hyperspectral Microflow Cytometry. Small [Internet]. 2024 Jul 1 [cited 2025 Apr 22]; 20(30):2400019. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/smll.202400019
142. Hu Z, Bhattacharya S, Butte AJ. Application of Machine Learning for Cytometry Data. Front Immunol [Internet]. 2022 Jan 3 [cited 2025 Apr 22];12:787574. Available from: www.frontiersin.org
143. Iyer A, Hamers AAJ, Pillai AB. CyTOF® for the Masses. Front Immunol [Internet]. 2022 Apr 14 [cited 2025 Apr 22];13:815828. Available from: www.frontiersin.org
144. Arnett LP, Rana R, Chung WWY, Li X, Abtahi M, Majonis D, et al. Reagents for Mass Cytometry. Chem Rev [Internet]. 2023 Feb 8 [cited 2025 Apr 22]; 123(3):1166–205. Available from: https://pubs.acs.org/doi/abs/10.1021/acs.chemrev.2c00350
145. Tan T, Gray DHD, Teh CE. Single-Cell Profiling of the Intrinsic Apoptotic Pathway by Mass Cytometry (CyTOF). Methods in Molecular Biology [Internet]. 2022 [cited 2025 Apr 22]; 2543:83–97. Available from: https://link.springer.com/protocol/10.1007/978-1-0716-2553-8_8
146. van der Pan K, Khatri I, de Jager AL, Louis A, Kassem S, Naber BAE, et al. Performance of spectral flow cytometry and mass cytometry for the study of innate myeloid cell populations. Front Immunol. 2023 May 19;14:1191992.
147. Tárnok A. Cytometry-on-the-chip. Cytometry Part A [Internet]. 2021 Oct 1 [cited 2025 Apr 22]; 99(10):964–964. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.24502
148. Li Z, Zhang S, Wei Q. Smartphone-based flow cytometry. Smartphone Based Medical Diagnostics. 2020 Jan 1; 67–88.
149. Evaluating mHealth technology in HIV to improve Empowerment and healthcare utilisation: Research and innovation to Generate Evidence for personalised care. 2015 May 1 [cited 2025 Apr 22]; Available from: https://cordis.europa.eu/project/id/643736
150. Liu P, Mu X, Zhang XD, Ming D. The Near-Infrared-II Fluorophores and Advanced Microscopy Technologies Development and Application in Bioimaging. Bioconjug Chem [Internet]. 2020 Feb 19 [cited 2025 Apr 22]; 31(2):260–75. Available from: https://pubs.acs.org/doi/full/10.1021/acs.bioconjchem.9b00610
151. Kalogeropoulou M, Díaz-Payno PJ, Mirzaali MJ, van Osch GJVM, Fratila-Apachitei LE, Zadpoor AA. 4D printed shape-shifting biomaterials for tissue engineering and regenerative medicine applications. Biofabrication [Internet]. 2024 Apr 1 [cited 2025 Apr 22]; 16(2). Available from: https://pubmed.ncbi.nlm.nih.gov/38224616/