HHS Public Access

Author manuscript

Int J Lab Hematol. Author manuscript; available in PMC 2024 June 01.

Published in final edited form as:

Int J Lab Hematol. 2023 June ; 45(Suppl 2): 44–49. doi:10.1111/ijlh.14098.

Full spectrum flow cytometry in the clinical laboratory

Jonathan R. Brestoff[1,2]

[1]Division of Laboratory and Genomic Medicine, Department of Pathology & Immunology, Washington University School of Medicine, Saint Louis, MO, 63110, USA

Abstract

Contemporary full spectrum or “spectral” flow cytometry is a recently developed technology that allows for high-dimensional flow cytometric analyses of cells and particles in suspension. This single-cell technology has gained popularity in research settings because it can conservatively detect 35 or more antigens simultaneously in a single-tube assay format. Recently, spectral flow cytometry has obtained regulatory approval for use as an *in vitro* diagnostic tool in China and Europe, enabling use of this technology in some clinical flow cytometry laboratories. The purpose of this review is to describe the basic principles of conventional and spectral flow cytometry, contrasting these two technologies. To illustrate the analytic power of spectral flow cytometry, we provide an example of spectral flow cytometry data analyses and the use of a machine learning algorithm to harvest the vast amount of information contained within large spectral flow cytometry datasets. Finally, we discuss the advantages of spectral flow cytometry adoption in clinical laboratories and preliminary studies comparing the performance of this technology relative to conventional flow cytometers that are currently used in clinical laboratory environments.

Introduction

Flow cytometry is powerful technology used to analyze and measure the physical, chemical, and/or gene expression characteristics of cells or particles.[1] This technique allows clinical laboratories and researchers to measure numerous properties of individual cells in a rapid, multiplexed, and quantitative manner. Given the high flexibility of flow cytometry, this technology has gained widespread adoption in numerous scientific fields, including immunology, cell biology, cancer research, developmental biology, and many other fields. In the clinical laboratory setting, flow cytometry is most commonly used to support diagnosis of primary immunodeficiencies, secondary immunodeficiencies, and hematologic malignancies; to monitor responses to therapy; and to detect disease

[2] Correspondence: **Jonathan R. Brestoff, MD, PhD, MPH,** Assistant Professor of Pathology and Immunology, Division of Laboratory and Genomic Medicine, Department of Pathology and Immunology, Washington University School of Medicine, 660 S. Euclid Ave., West Bldg #3710, Campus Box 8118, Saint Louis, MO, 63110, USA, Phone: (314) 273-1280, brestoff@wustl.edu, Twitter: @BrestoffLab.

data availability statement (if applicable): Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ethics approval statement (if applicable): not applicable

patient consent statement (if applicable): not applicable

permission to reproduce material from other sources (if applicable): not applicable

clinical trial registration (if applicable): not applicable

progression or recurrence.[2] This technology is also used to support cellular therapies, including enumeration of stem cells for storage and deployment in the setting of stem cell transplants and manufacturing engineered cells such as chimeric antigen receptor (CAR) T cells.[3,4]

Although conventional flow cytometers have been used in clinical laboratories for decades, another exciting technology called full-spectrum flow cytometry (referred to herein as *spectral flow cytometry*) has gained significant traction and popularity in research settings in the last few years and is just beginning to be used in clinical laboratories in some countries (Europe: CE-IVD NL-CA-002–2020-53432 and China: Su Xie Zhu Zhun 20212220585). The primary advantage of spectral flow cytometry is that it allows one to easily measure over 35 antigens per cell in a single-tube assay format.[2,5,6] This educational review first describes basic principles of conventional and spectral flow cytometry and contrasts these two technologies. An example of spectral flow cytometry data analyses is described, highlighting how machine learning algorithms can be used to harvest the vast amount of information contained in large spectral flow cytometry-based assays. The advantages and disadvantages of adopting spectral flow cytometry in clinical practice are discussed, and finally, the current state of clinical deployment and validation of spectral flow cytometry is reviewed.

Basic Principles of Conventional and Spectral Flow Cytometry

The most basic principle of all flow cytometry technologies is to pass a single-file stream of cells or particles in suspension through a laser beam in order to measure each cell's or particle's physical (i.e. optical) and chemical (e.g. antigen expression) features. As the cells pass through the laser light, they absorb, diffract (i.e., scatter), and emit light that is subsequently captured by an array of detectors. The amount of light that each cell scatters in the forward direction is a function of its size and refractive index. In addition, each cell has organelles, granules, and other features that diffract the light to the side. This so-called side scatter (SCC) is an indicator of the cell's overall complexity. Since some cell types have unique sizes and complexities, it is possible to identify some cell types based purely on their FSC and SSC properties, as is done in complete blood counts to identify white blood cells (WBCs), red blood cells (RBCs), and platelets (PLT) and to differentiate lymphocytes, monocytes, neutrophils, eosinophils, basophils, and some other cell types. However, there are numerous lymphocyte subsets – e.g., $CD4^+$ T cells, $CD8^+$ T cells, γ T cells, B cells, and natural killer (NK) cells – that have very similar FSC and SSC properties, making it impossible to identify them reliably without additional information about which protein antigens they express.

The primary value of flow cytometry is that it allows one to measure the expression of cell type-specific proteins to identify and quantify the abundance or activation states of specific immune cell subsets. This is accomplished by attaching a variety of distinct fluorophores to monoclonal antibodies that recognize cell type-specific protein antigens. Laser light excites these fluorophores, which then emit light at different wavelengths for subsequent detection. For example, CD4-PE and CD8-PE/Cy5 are both excited by the yellow-green laser (561 nm) but have emission spectra that peak at 576 nm and 665 nm, respectively, that allows

for detecting these two fluorophores at the same time to measure the amount of CD4 and CD8 on each cell. However, there are two problems that emerge. First, the emission spectra of these and other fluorophores can overlap considerably, such that some emitted light from one fluorophore spills over into detectors assigned to other fluorophores. Second, fluorophores can often be excited more than one laser (e.g., PE and PE/Cy5 are also excited by the 488nm blue laser). These issues create a problem called “spectral overlap.”

The fundamental difference between a conventional and spectral flow cytometer is how spectral overlap is handled. In a conventional flow cytometer, each laser has a set of light filters situated in front of photon detectors (typically photomultiplier tubes). The filters allow only certain wavelengths of emitted light to reach the detectors, and there is only one detector associated with each filter. For example, in line with the 561nm laser is one filter-detector stack for measuring PE and a different filter-detector stack for measuring PE/Cy5. This configuration accomplishes two important goals. First, it isolates “on-target” light emitted from each fluorophore. Second, it greatly reduces the ability of the detectors to see “off-target” light emitted from other fluorophores excited by the same laser or other lasers, minimizing the amount of spectral overlap. By using single-color controls for each fluorophore, the computer running the flow cytometer can calculate the degree of spectral overlap for all fluorophores involved in a panel, generating what is called a “compensation matrix.” Applying the compensation matrix allows the raw data to be adjusted such that the intended true signal from each fluorophore is reported correctly.

In contrast, in spectral flow cytometers, essentially all of the emitted light from each fluorophore is captured by a series of many detectors, for each laser. Therefore, each fluorophore is technically measured using several dozens of detectors simultaneously. This allows the spectral flow cytometer to establish a “spectral fingerprint” for each fluorophore’s emitted light. Single-color reference controls allow the computer to know the spectral fingerprint for each fluorophore and to perform spectral unmixing, an approach that is analogous to compensation on a conventional flow cytometer. The advantage of spectral flow cytometry is that fluorophores with up to 98% similarity (e.g. FITC and BB515) can technically be distinguished from each other and used in the same tube (Cytek Spectral Viewer). As a result, spectral flow cytometry can detect many more fluorophores simultaneously than conventional flow cytometry, allowing for greater flexibility in assay design.

As one example, the far-red fluorophores APC and Alexa Fluor® 647 are both primarily excited by the red laser and have emission peaks at 660nm and 665nm, respectively. On a conventional flow cytometer, these fluorophores are detected using the same laser-filter-detector stack and therefore cannot be used together in the same panel or tube. However, a spectral flow cytometer uses the many small differences in emission spectra between the two fluorophores, including minor emission spectra after excitation with other lasers (e.g., violet and yellow-green), to easily distinguish APC and AF647 and enable co-utilization in the same panel. Extension of this logic to many other fluorophore possibilities means 5-laser spectral flow cytometers have the theoretical capability of detecting well over 60 fluorophores at the same time. In practice, the number of fluorophores that one can use is more limited than this due to the availability of reagents, especially the number

of commercially available fluorophore-antibody conjugates. Conservatively, a carefully constructed spectral flow cytometry panel could include over 35 distinct markers in a single-tube assay on a 5-laser spectral flow cytometer.

Spectral flow cytometry allows for high-dimensional data analyses

Our research laboratory is equipped with a 4-laser spectral flow cytometer (Cytek Aurora 4L 16V-14B-10YG-8R) with violet, blue, yellow-green, and red lasers and 48 detectors. Although Cytek is a leading spectral flow cytometry manufacturer offering Northern Lights (3 laser) and Aurora (customizable with 3–5 lasers) analyzers and the new spectral cell sorter (Aurora CS), other companies also offer spectral flow cytometers including Sony (ID7000), Becton Dickinson (BD; Symphony A5 SE), and ThermoFisher (BigFoot Cell Sorter).

One of our lab's primary interests is to study a process called intercellular mitochondria transfer, and we use spectral flow cytometry as one tool to study this process. Specifically, we recently generated adipocyte-specific mitochondria reporter mice called MitoFat mice, which express the fluorescent reporter protein mtDendra2 only in mature adipocytes.[7] The mtDendra2 protein is attached to the mitochondria targeting sequence of cytochomre c complex VIII, targeting it to the inner mitochondrial membrane.[8] In our previous studies using a conventional flow cytometer (along with state-of-the-art imaging technologies), we were able to demonstrate that adipocytes frequently transfer their mitochondria to macrophages in healthy white adipose tissue.[7]

However, we wanted to know whether adipocytes could transfer mitochondria to other cell types *in vivo*, a task that was well-suited for spectral flow cytometry. We devised a 31-color spectral flow cytometry panel that included 1 viability dye, 1 fluorescent reporter (mtDendra2), and 29 antibody-fluorophore conjugates.[9] Using bioinformatic analyses of a single-tube assay, we were able to easily identify 21 distinct cell types, including 2 monocyte populations, 3 macrophage populations, dendritic cells, 2 B cell populations, neutrophils, eosinophils, CD8 T cells, 2 CD4 T cell populations, 2 Group 2 innate lymphoid cell (ILC2) populations, and 5 non-immune cell types. This panel allowed us to monitor the degree of mitochondria transfer from adipocytes to each of these 21 cell types and assess cell viability.[9]

We also sought to identify to 5 non-immune cell populations. To accomplish this, we used a batch-correction method originally built for analyzing single-cell RNA sequencing data[10] but adapted it for spectral flow cytometry data.[9] Briefly, we built a 2nd panel with approximately 60% overlap compared to the 1st panel, with the remaining 40% of panel space allocated to various new markers of non-immune cells and 2 known receptors that facilitate mitochondria capture. The overlapping antibodies were used to map the 2nd panel to the 1st to confidently identify the 5 original non-immune cell populations. We then used the new markers on the 2nd panel to identify the 5 non-immune cell types as 2 fibroblast lineage cell types (adipocyte progenitor cells and fibroinflammatory progenitors) and 3 heterogeneous populations of endothelial cells. This bioinformatic approach can be used to connect multiple large panels and allow for a deep phenotyping of the immune system in

patients. The code for this approach is published and freely available[9], and an alternative approach has also been developed by another group.[11]

While this example was done in mouse specimens, it illustrates the power of spectral flow cytometry and the ability to perform more complex analyses than conventional gating. This opens the possibility of using machine learning algorithms to support cell identification and potentially disease diagnosis using spectral flow cytometry. This topic is discussed in more detail below.

Advantages of spectral flow cytometry

As users of both spectral and conventional flow cytometers, we feel there are few if any significant limitations to spectral flow cytometers compared to their conventional counterparts, but there are many advantages. The example above illustrates some of the advantages of spectral flow cytometry. First, spectral flow cytometry can detect and distinguish between many more fluorophores than conventional flow cytometry, allowing for more complex and informative multicolor analyses. In the example provided, one very large panel could identify nearly every major immune cell population in a single-tube assay format. Second, it is possible to stitch together multiple panels to generate even deeper phenotypic datasets. Practically, this means it may be possible for a clinical flow cytometry laboratory to have a core panel with add-on tubes that allow for more flexible and customized depth of diagnostic analyses. Third, bioinformatic analyses may allow us to examine how various immune cell populations change as an interacting network or system, rather than examining individual cell types one at a time. This systems biology approach could potentially reveal hidden patterns or features of immune system dysregulation that could be linked to immunodeficiencies, cancer, treatment responses, disease progression, or relapse.

There are several other advantages of spectral flow cytometry that have not yet been discussed. One advantage is their increased sensitivity to detect weak signals. This benefit is conferred mainly by the use of avalanche photodiode (APD) detectors, which are more sensitive than the photomultiplier tubes (PMTs) that are used in most currently available conventional flow cytometers. This is considered a weak advantage because APDs can be installed in conventional flow cytometers, as is the case with Beckman Coulter's CytoFLEX instruments that are already available in research settings. Our prediction is that APDs will become increasingly commonplace in new flow cytometers over time and will eventually predominate in the research and clinical flow cytometer markets.

Another advantage of spectral flow cytometry is that it can account for a cell's autofluorescence characteristics and eliminate this as a variable. Autofluorescence is a common problem[12] that can make it difficult to draw gates correctly and is particularly problematic with some myeloid and plasma cell populations.[13–15] Therefore, spectral flow cytometry has the potential to increase the accuracy of gating, especially when interrogating leukemic specimens and plasma cells.[15] As there are limited use cases for spectral flow cytometry in clinical settings, it is not yet clear whether these theoretical advantages described above translate into improved diagnostic performance in clinical settings. Additional studies are needed to assess these possibilities.

From an operational perspective, the process of setting up spectral flow cytometers and performing spectral unmixing is quite easy. In my personal experience, it is easier and faster to set up a 30-color experiment on a Cytek Aurora than it is to set up a 10-color experiment on a BD Fortessa, at least in a research setting. I anticipate that the ease of instrument set-up of spectral flow cytometers will be a welcome and appreciated feature once integrated into current clinical laboratory operations and standard operating procedures, but clinical laboratories will need to budget time and resources to accomplish this.

Overall, spectral flow cytometry provides a more flexible and powerful tool for the analysis of complex cell populations, particularly when simultaneous analysis of multiple cellular features is desired. Its ability to distinguish and analyze a greater number of fluorescent dyes and to reduce compensation requirements can increase accuracy, sensitivity and reliability of multicolor flow cytometry analyses.

Clinical applications of spectral flow cytometry

While spectral flow cytometry has been in use in research settings for several years, it is just beginning to emerge in clinical markets. Spectral flow cytometers from Cytek Biosciences are currently in clinical use in China (Su Xie Zhu Zhun 20212220585), and Cytek recently received CE-IVD designation for use of their spectral flow cytometers in Europe (CE-IVD NL-CA-002–2020-53432) for using the Cytek cFluor 6-color TBNK-SL assay (CE-IVD NL-CA-002–2022-71769). To the best of our knowledge, there are no other spectral flow cytometers currently available in clinical markets. Currently, there is no Federal Drug Administration (FDA) *in vitro* diagnostic (IVD) designation for any spectral flow cytometer in the United States.

Given the recent deployment of spectral flow cytometry in clinical settings, there are limited use cases to draw upon. A particularly useful study reported a 27-color single-tube assay using a 3-laser Cytek Aurora for detection of measurable residual disease in patients diagnosed with acute myeloid leukemia.[16] The lower limit of detection for identifying abnormal myeloblasts was 0.0013% in limiting dilution studies, indicating excellent sensitivity of spectral flow cytometers for detecting rare cell types. Importantly, this study also compared the 27-color panel against an 8-color multi-tube assay run a BD Canto II and found the two assays to be concordant in terms of cell type identification. Overall, this important study provides proof-of-concept that high-dimensional single-tube flow cytometry assays can be achieved in a clinical setting using spectral flow cytometers.

There have also been some non-peer-reviewed studies that provide additional useful preliminary data. First, an abstract published as part of the 2020 American Society for Hematology conference reported that a 24-color single-tube assay with CD45, CD34, CD117, HLA-DR, CD33, CD13, CD371, CD15, CD64, CD11c, CD14, CD36, CD11b, CD4, CD19, CD7, CD2, CD56, CD96, CD123, CD38, CD200, CD72, and CD9 run on a 3-laser Cytek spectral flow cytometer with analysis in Kaluza and FlowJo.[17] The authors found that this assay and the same markers run on conventional 3- or 8-color BD FACSCanto flow cytometers were highly correlated. The t-SNE plug-in within FlowJo software was used and successfully identified abnormal cell types, suggesting that machine

learning algorithms may have diagnostic utility for high-dimensional flow cytometry datasets that may obviate the need for standard gating strategies.

Later, in a non-peer-reviewed poster presented at the International Clinical Cytometry Society (ICCS) 2021 meeting, a 23-color single-tube assay on the Cytek Northern Lights instrument was compared against a 4-tube assay with the same markers run on a BD FACSCanto II conventional flow cytometer in China (communicated with permission).[18] The two assays provided comparable results with bone marrow aspirates from n=12 multiple myeloma (MM) and n=9 non-MM patients. Among the MM patients, there was very high concordance with quantification of plasma cell abundance expressed as a percentage of total nucleated cells.[18]

Another poster was presented at ICCS 2022 in which the Cytek cFluor 6-color TBNK-SL assay performed on a Northern Lights spectral flow cytometer was compared to the BD Multitest 6-color TBNK Assay under the Lyse no-wash protocol performed on the BD FACSCanto II cytometer from n=725 patient specimens from 3 sites (locations were not disclosed; communicated with permission).[19] For CD4 cells, when the agreement cutoff was set at 350 cells/μL, there was 98.6% positive agreement, 99.3% negative agreement, and 99.2% overall agreement. There was less than 1% bias for the Northern Lights machine vs the FACSCanto II, with Bland-Altman plots showing symmetry between the two systems and an expected number of specimens outside +/− 1.96 standard deviations from the mean for relative and absolute counts of CD3+, CD3+ CD4+, CD3+CD8+, CD3-CD(16+56)+, and CD3-CD19+ cells. Deming regression analyses of revealed excellent correlations with R^2 values ~99% for all of these parameters.[19]

In addition to these studies, there are many examples in the research literature where spectral flow cytometry assays have been used successfully to immunophenotype human blood, bone marrow, or lymph nodes. While there are far too many to list, we refer the reader to several examples of non-clinical assays with human specimens.[20–26]

The above method-comparison studies provide strong preliminary evidence that spectral flow cytometers are likely functionally equivalent to conventional flow cytometers but are superior in the sense that they can perform high-dimensional assays in a single-tube format that otherwise requires 4 separate tubes on current 10-to-12-color conventional flow cytometers. It is critical that comprehensive validation studies be published in reputable peer-reviewed journals with comparisons against regulatory agency-approved predicate conventional flow cytometers. Once approved and in active clinical use, it will become possible to develop high-dimensional lab-developed tests (LDTs) and assay kits for broader deployment.

Conclusions

Spectral flow cytometry is an exciting technology that allows for high-dimensional analyses of cells with dozens of fluorophores in single-tube assays. Spectral flow cytometry has gained substantial traction in research settings and is now available to clinical laboratories in China and Europe. There is a need for additional head-to-head method comparison studies

to ensure that spectral flow cytometers perform as well as or better than conventional flow cytometry platforms in various clinical assays. These studies will increase the confidence of the clinical flow cytometry community that adopting spectral flow cytometry technology provides similar-to-superior results for currently available tests. Spectral flow cytometry has the potential to transform our capabilities in the clinical flow cytometry laboratory by opening the potential to develop and deploy ultra-high-dimensional assays of specimens from patients. This may unlock previously unrecognized opportunities and diagnostic approaches in clinical cytometry.

ACKNOWLEDGEMENTS

JRB is supported by the NIH Common Fund via the Director's Early Independence Award (DP5 OD028125) and the Burroughs Wellcome Fund Career Award for Medical Scientists (CAMS 1019648). JRB would like to thank Mark Herberger (Cytek Biosciences) for helpful discussions and Drs. Jennifer Liu (Cytek Biosciences) and Li Xiong (Wuhan Kindstar Medical Laboratory Co., Ltd.) for permission to describe results previously presented in their respective International Clinical Cytometry Society posters. JRB receives compensation from LUCA Science Inc. as a member of its Scientific Advisory Board and royalties from Springer Nature Group. He has pending patent applications related to mitochondria transfer and serum free light-chain immunoassays.

5. LIST OF REFERENCES

1. Picot J, Guerin CL, Le Van Kim C, Boulanger CM. Flow cytometry: retrospective, fundamentals and recent instrumentation. Cytotechnology. Mar 2012;64(2):109–30. doi:10.1007/s10616-011-9415-0
2. Brestoff JR, Frater JL. Contemporary Challenges in Clinical Flow Cytometry: Small Samples, Big Data, Little Time. J Appl Lab Med. Jun 30 2022;7(4):931–944. doi:10.1093/jalm/jfab176 [PubMed: 35061902]
3. Campbell JDM, Fraser AR. Flow cytometric assays for identity, safety and potency of cellular therapies. Cytometry B Clin Cytom. Sep 2018;94(5):569–579. doi:10.1002/cyto.b.21735 [PubMed: 30240161]
4. Gajkowska A, Oldak T, Jastrzewska M, et al. Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells in leukapheresis product and bone marrow for clinical transplantation: a comparison of three methods. Folia Histochem Cytobiol. 2006;44(1):53–60. [PubMed: 16584093]
5. Nolan JP. The evolution of spectral flow cytometry. Cytometry A. Oct 2022;101(10):812–817. doi:10.1002/cyto.a.24566
6. Nolan JP, Condello D. Spectral flow cytometry. Curr Protoc Cytom. Jan 2013;Chapter 1:1 27 1–1 27 13. doi:10.1002/0471142956.cy0127s63
7. Brestoff JR, Wilen CB, Moley JR, et al. Intercellular Mitochondria Transfer to Macrophages Regulates White Adipose Tissue Homeostasis and Is Impaired in Obesity. Cell Metab. Feb 2 2021;33(2):270–282 e8. doi:10.1016/j.cmet.2020.11.008 [PubMed: 33278339]
8. Pham AH, McCaffery JM, Chan DC. Mouse lines with photo-activatable mitochondria to study mitochondrial dynamics. Genesis. Nov 2012;50(11):833–43. doi:10.1002/dvg.22050
9. Borcherding N, Jia W, Giwa R, et al. Dietary lipids inhibit mitochondria transfer to macrophages to divert adipocyte-derived mitochondria into the blood. Cell Metab. Oct 4 2022;34(10):1499–1513 e8. doi:10.1016/j.cmet.2022.08.010
10. Haghverdi L, Lun ATL, Morgan MD, Marioni JC. Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors. Nat Biotechnol. Jun 2018;36(5):421–427. doi:10.1038/nbt.4091
11. Ogishi M, Yang R, Gruber C, et al. Multibatch Cytometry Data Integration for Optimal Immunophenotyping. The Journal of Immunology. 2021;206(1):206–213. doi:10.4049/jimmunol.2000854 [PubMed: 33229441]
12. Hulspas R, O'Gorman MR, Wood BL, Gratama JW, Sutherland DR. Considerations for the control of background fluorescence in clinical flow cytometry. Cytometry B Clin Cytom. Nov 2009;76(6):355–64. doi:10.1002/cyto.b.20485 [PubMed: 19575390]

13. Gupta M, Jafari K, Rajab A, et al. Radar plots facilitate differential diagnosis of acute promyelocytic leukemia and NPM1+ acute myeloid leukemia by flow cytometry. Cytometry B Clin Cytom. Jul 2021;100(4):409–420. doi:10.1002/cyto.b.21979 [PubMed: 33301193]

14. Duffield AS, Aoki J, Levis M, et al. Clinical and pathologic features of secondary acute promyelocytic leukemia. Am J Clin Pathol. Mar 2012;137(3):395–402. doi:10.1309/AJCPE0MV0YTWLUUE [PubMed: 22338051]

15. Hayden PJ, Connell NMO, Brien DAO, Rourke PO, Lawlor E, Browne PV. The value of autofluorescence as a diagnostic feature of acute promyelocytic leukemia. Haematologica. 01/01 2006;91(3):417–418. doi:10.3324/%x [PubMed: 16531269]

16. Soh KT, Conway A, Liu X, Wallace PK. Development of a 27-color panel for the detection of measurable residual disease in patients diagnosed with acute myeloid leukemia. Cytometry A. Nov 2022;101(11):970–983. doi:10.1002/cyto.a.24667 [PubMed: 35716345]

17. Chen M, Wang H, Fu M, et al. One Tube 24 Color Full Spectral Flow Cytometry and Multi-Dimensional Software to Study the Maturation Pattern and Antigen Expression of the Myeloid. Blood. 2020;136(Supplement 1):13–14. doi:10.1182/blood-2020-140600

18. Xiong L, Du W, Ma Y-K, et al. ICCS 2021: High-parameter and Effective Multiple Myeloma Diagnostic Panel on the Cytek NL-CLC Improves Sample Efficiency. Poster. International Clinical Cytometry Society (ICCS) Conference (Baltimore, MD). 2021;Poster. Communicated with written permission.

19. Liu J, Li D, Guo Y, et al. ICCS 2022: A Multi-Site Clinical Evaluation of Cytek® cFluor® 6-Color TBNK-SL Assay on Cytek® Northern LightsTM (NL) CLC Cytometers. International Clinical Cytometry Society (ICCS) Conference (Montreal, Quebec). 2022;Poster. Communicated with written permission.

20. Kjerulff B, Petersen MS, Rodrigues CM, et al. HTLV infected individuals have increased B-cell activation and proinflammatory regulatory T-cells. Immunobiology. 2020/03/01/ 2020;225(2):151878. doi:10.1016/j.imbio.2019.11.013

21. Kreutmair S, Unger S, Nunez NG, et al. Distinct immunological signatures discriminate severe COVID-19 from non-SARS-CoV-2-driven critical pneumonia. Immunity. Feb 8 2022;55(2):366–375. doi:10.1016/j.immuni.2022.01.015 [PubMed: 35139354]

22. Diebold M, Galli E, Kopf A, et al. High-dimensional immune profiling identifies a biomarker to monitor dimethyl fumarate response in multiple sclerosis. Proc Natl Acad Sci U S A. Aug 2 2022;119(31):e2205042119. doi:10.1073/pnas.2205042119

23. Odak I, Sikora R, Riemann L, et al. Spectral flow cytometry cluster analysis of therapeutic donor lymphocyte infusions identifies T cell subsets associated with outcome in patients with AML relapse. Front Immunol. 2022;13:999163. doi:10.3389/fimmu.2022.999163

24. Heeb LEM, Boyman O. Comprehensive analysis of human IL-4 receptor subunits shows compartmentalization in steady state and dupilumab treatment. Allergy. n/a(n/a)doi:10.1111/all.15576

25. Barber EL, Chen S, Pineda MJ, et al. Clinical and Biological Activity of Chemoimmunotherapy in Advanced Endometrial Adenocarcinoma: A Phase II Trial of the Big Ten Cancer Research Consortium. Cancer Research Communications. 2022;2(10):1293–1303. doi:10.1158/2767-9764.Crc-22-0147 [PubMed: 36388466]

26. Steuten J, Bos AV, Kuijper LH, et al. Distinct dynamics of antigen-specific induction and differentiation of different CD11c(+) Tbet(+) B cell subsets. J Allergy Clin Immunol. Feb 27 2023;doi:10.1016/j.jaci.2023.02.020