



# MASS CYTOMETRY PUBLICATIONS TRENDING NOW

July 2021

## ON HIGH-PARAMETER CYTOMETRY

Welcome to the July issue of Trending Now, a quarterly anthology of recent impactful publications by researchers using CyTOF technology.

This month's edition overviews how investigators have successfully incorporated panels with 30 to 50 markers or more into their studies of infectious disease and cancer and highlights the novel insights revealed.



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# WHAT'S NEW IN **MASS** CYTOMETRY

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**High-parameter analysis of single cells can achieve unprecedented levels of dimensionality.**

Biological research regularly presents a debate: Generate as much information as possible from a broad investigation at a particular cellular level or focus the approach for an in-depth look at a specific item of importance. With compromise on either path, there is not one true winner.

Mass cytometry bridges the gap between these two approaches, with the capability for single-cell resolution, simultaneous high-parameter analysis and throughput of millions of cells per sample. This winning combination empowers research from every angle. Biological complexity can be unraveled, where identification of major cell subsets, discovery of rare cell populations and examination of cell behavior are possible.

## High-dimensional cytometric analysis opens doors to answering once-formidable questions, provoking fresh perspectives and creative solutions.

Armed with a tool that frees thinking from the limitations of conventional technologies, new research into the variability and individuality of the immune system and its response to disease has successfully achieved a greater biological understanding and unprecedented insights into clinical outcome.

Multiparametric mass cytometry has sufficient sensitivity, dynamic range and resolution to independently measure a wide number of targets simultaneously, benefiting from extremely low levels of the signal crosstalk that is common in mass spectrometry and from relative uniformity of detection across all channels.

The significance of this approach lies in its flexibility for comprehensive analysis, whether it is used alone or as a contributor in complementary multi-omics studies and systems biology applications. Driven by the desire to understand not only what, but how, why and where, the blending of technologies has incited the exploration of coordinated systems in the human body, defying the limits of siloed mechanisms.

Here, we review only a sampling of recent publications that highlight the high-parameter capabilities of mass cytometry. These studies benefit from investigating multiple layers of cell behavior during infections or cancer progression, detecting surface and intracellular molecules for phenotypic and functional study and broadening research for a cohesive view.

### Cohort comparisons leveraging multiple markers

Initial studies on vaccine efficacy related to SARS-CoV-2 infection have prompted further research into differences in mild vs. severe COVID-19 cases, vaccinated vs. unvaccinated response, vaccination of previously infected individuals, and so on. New research from University of California, San Francisco, (Neidleman et al.) explored the differences between infection-naïve and COVID-19 convalescent individuals before and after vaccination.

Using high-parameter CyTOF® analysis with a 39-marker panel to phenotype SARS-CoV-2

specific T cells across multiple time points pre- and post-vaccination, the study describes T cell response to the original SARS-CoV-2 strain in addition to B.1.1.7 and B.1.351 variant strains. Data showed that vaccine-elicited T cells respond to all currently tested variants, suggest that convalescent individuals have no added benefit from a second vaccine dose and indicate that vaccinated convalescents appear to have heightened respiratory tract-homing SARS-CoV-2 specific T cells compared to infection-naïve individuals.

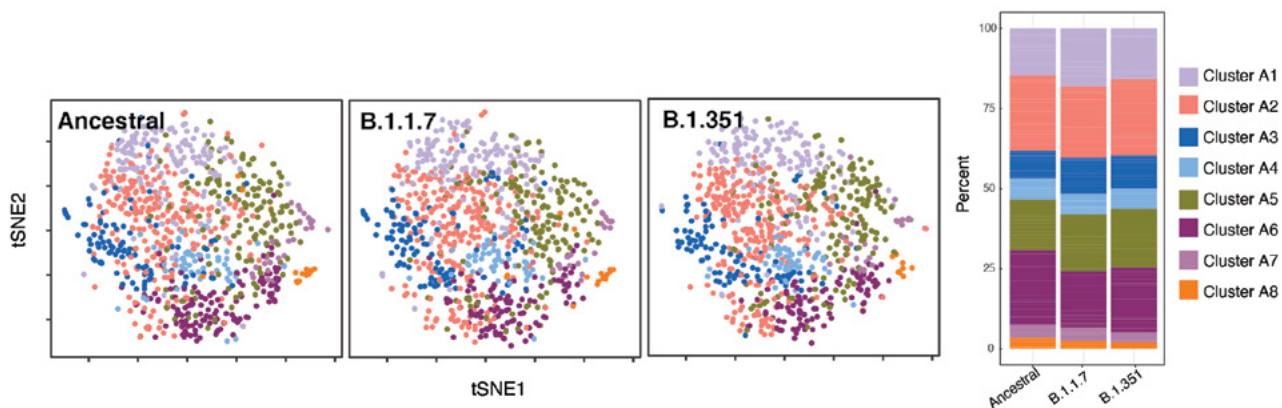


Figure 1. SARS-CoV-2 specific CD4+ T cells exhibit same phenotypes responding to ancestral, B.1.1.7 and B.1.351 spikes. (Neidleman et al.)

With little known about the homeostatic and functional properties of T cells in the context of mild vs. severe COVID-19, scientists at the University Hospital Zurich (Adamo et al.) used 40-parameter mass cytometry, flow cytometry, targeted proteomics, and functional assays in a cross-sectional analysis of the peripheral T cell compartment. The team found extensive T cell dysfunction and apoptosis and showed T cell lymphopenia and redistribution of T cell proportions, where naive T cells were reduced and activated and exhausted T cells expanded.

Given that T cells are known to be central to antiviral immunity, the inefficiency of the T cell response in severe COVID-19 supports observations that perturbed adaptive antiviral immunity and the hyperinflammation that follows could help further explain its pathogenesis.

In a separate cohort comparison of immune signatures from malaria-naïve volunteers to those with lifelong malaria exposure, a group at Leiden

University Medical Center (de Jong et al.) used controlled human infection in an effort to develop better response and treatment options for parasitemia, a typical consequence of malaria infection.

Prior studies examining immune cell profiles during infection could focus on only a limited number of cell subsets using flow cytometry. Mass cytometry enabled in-depth and broad immune profiling using a 36-marker panel, allowing the team to reveal distinct European and African immune signatures marked by enrichment of memory cells and expression of specific activation/differentiation markers on both adaptive and innate immune cells. These signatures are indicative of the variation in exposure to microorganisms and parasites in different locations.

“Mass cytometry was used to show, at an unprecedented depth, the detailed cellular immunological profiles at baseline, as well as the dynamics of immune responses to malaria parasites,” the researchers reported.

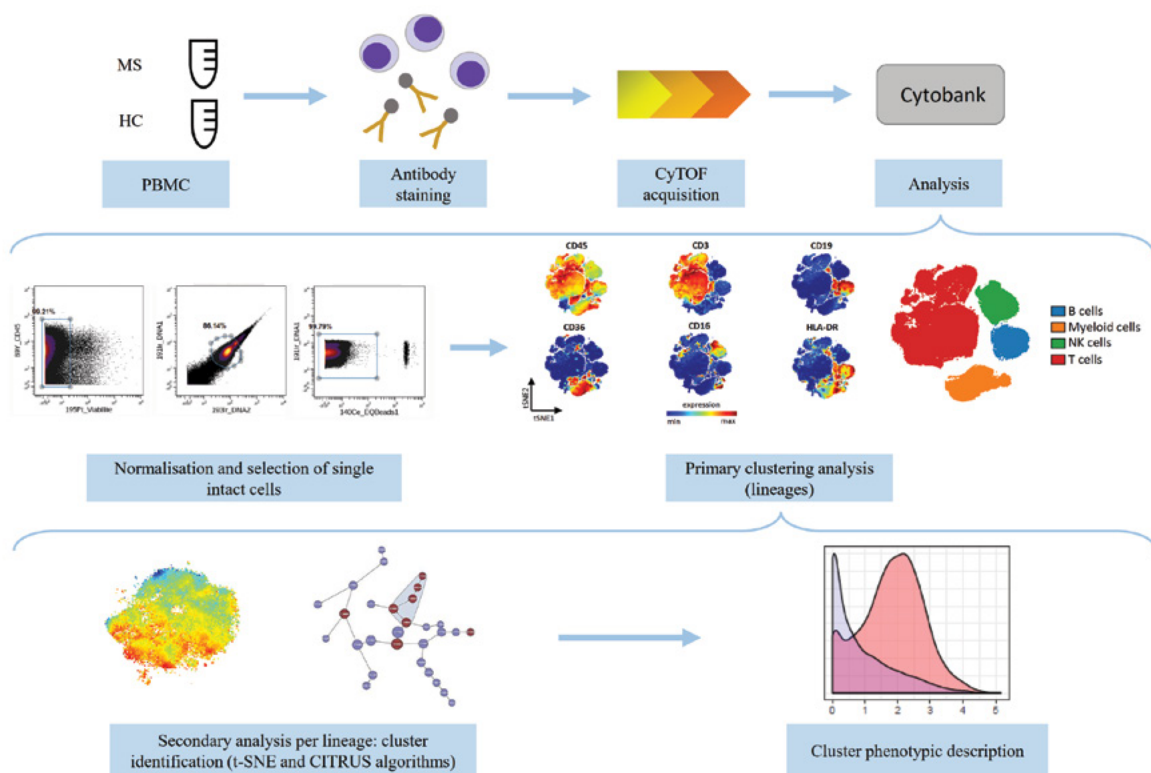


Figure 2. Experimental design (Couloume et al.)

## Efficacy of treatment to support clinical decisions

Application of mass cytometry to strategies improving precision medicine demonstrates its widespread utility in translational and clinical research. For example, a group from the Université Rennes (Couloume et al.) was able to gain a better understanding of the heterogeneity of immune cells and diversity in immune response associated with multiple sclerosis (MS). Two large CyTOF panels that included 62 markers covering myeloid and lymphoid cells were applied to the analysis of PBMC samples from early untreated MS patients.

The study describes the detection of subsets of immune cells, including T-bet<sup>+</sup> B cells, CD206<sup>+</sup> classical monocytes and pro-inflammatory NK cells, that are significantly increased in early MS patients. Cell analysis provided insight into the pathophysiology of MS and the potential for immunological biomarkers to be used in the identification of neuroinflammation and MS progression.

“CyTOF, combined with high-dimensional analysis, is a robust method to identify numerous and poorly-described cell subsets from heterogeneous populations, including in the autoimmune context,” the team reported.

Mass cytometry is ideal for studies collecting valuable samples from patients, where sample type or volume must be limited. Taking advantage of the ability to detect 40 immune cell markers, researchers at Southern Medical University in Guangzhou, China, (Liu et al.) explored the immunological profiles of kidney transplant patients before and after immunosuppressive treatment. This important study supports treatment strategies for transplant patients for more successful graft acceptance.

The technology enabled classification and comparison of the proportions of immune cells between pre-treatment and post-treatment groups. Findings revealed an abundance of activated immune cell subsets in the peripheral blood of patients post-treatment and an increase in the proportion of tolerant immune cells.

While NK cell immunotherapy holds much promise as an effective treatment for a variety of cancers, NK-resistant cancers remain a significant challenge. Scientists at MD Anderson Cancer Center (Kerbauf et al.) used mass cytometry and cytotoxicity assays to identify an enhanced response to NK therapy by adding the tetravalent bispecific antibody AFM13. Two CyTOF panels containing 36 and 37 markers targeting NK activation and signaling revealed that AFM13–NK complex cells exhibited enhanced responses to CD30<sup>+</sup> lymphomas *in vitro* and *in vivo*.

Interested in mechanisms of therapeutic resistance, Roswell Park Cancer Institute scientists (Emmons et al.) recently used mass cytometry to ascertain how neutrophils acquire a complement-dependent T cell suppressor phenotype in the tumor microenvironment. This adapted phenotype results in inhibited T cell proliferation and activation. Using ascites fluid supernatants (ASC) from patients with ovarian cancer as an accurate sampling of the TME, a 43-marker CyTOF panel helped the team determine that ASC could stimulate complement deposition and signaling in neutrophils, activating specific granule components that initiate a suppressor function in these cells.

## New systems and approaches to investigation

High-parameter analysis using mass cytometry has opened doors to asking more detailed questions and to generating a multitude of data from one experiment. The versatile capabilities of such a cytometric platform encourage a shift in perspective and prompt creative approaches.

New research from the University of Zurich (Tognetti et al.) shows how single-cell measurements of cellular signaling responses can be used to build signaling network models for cancer and immune cells that can help predict cancer cell resistance or sensitivity to drug treatments.

The comprehensive study used mass cytometry to characterize the single-cell signaling landscapes of 62 breast cancer cell lines and 5 cell lines from healthy tissue, quantifying 34 markers in each cell line upon epithelial growth factor stimulation in the presence or absence of 5 kinase inhibitors. The group obtained

data on 29 phosphorylation events and more than 80 million single cells from 4,000 conditions and generated mechanistic signaling network models to explore how cancer cells process information.

Similarly, mechanisms behind cancer metastasis can provide a basis for understanding why clinical responses can vary among metastatic patients. Won Jin Ho and colleagues at Johns Hopkins School of Medicine recently investigated the molecular and cellular landscape within tumor microenvironment (TME) metastasis of pancreatic ductal adenocarcinomas (PDAC) to the liver and lung, the most common sites of PDAC spread. A combination of mass cytometry, immunohistochemistry and RNA sequencing helped identify key regulatory pathways distinguishing liver and lung TMEs in a preclinical mouse model of metastatic PDAC.

Initially the team used a 33-marker panel, including 16 for subtyping, 11 for functional analysis, 4 for barcoding, 1 for cell identification and 1 for viability. Four unique CD45 barcodes were employed with a batching strategy for multiplexed staining and data acquisition. In order to more thoroughly characterize key immune regulatory chemokines and validate the immune profiles from their original model, they performed CyTOF again with an easily revised 48-marker panel. This is the first study to reveal tissue-specific differences in the TME, demonstrating higher levels of immune activation and infiltration in

the lung and enriched immunoregulatory networks in the liver for a greater extent of immune suppression.

Most studies investigating progression and mechanisms of COVID-19 have focused on peripheral blood analysis, with only a select few exploring effects on multiple organs. Recent work from Leiden University Medical Center (Roukens et al.) shifted investigations to nasal mucosa and the effects of COVID-19 on mucosal immunity in patients during hospitalization and later recovery.

A very different picture of the immune cell landscape was observed in the nasal mucosa compared with what is seen in blood, with no general depletion of T cells. There were, however, pro-inflammatory responses associated with viral load during acute COVID-19, with cell numbers normalizing in convalescents, aside from persisting CD127+ granulocytes and activated T cells, demonstrating both transient and long-term response in the upper airway.

Immune cells were analyzed using a 39-marker mass cytometry panel, allowing identification of 8 main lineages of nasal CD45+ immune cells subclustered into 28 populations and in-depth analysis of cell types and proportions. Overall, 875,564 nasal CD45+ immune cells were analyzed from 56 samples, consisting of 44 samples collected from 29 COVID-19 patients and 12 samples from healthy donors.

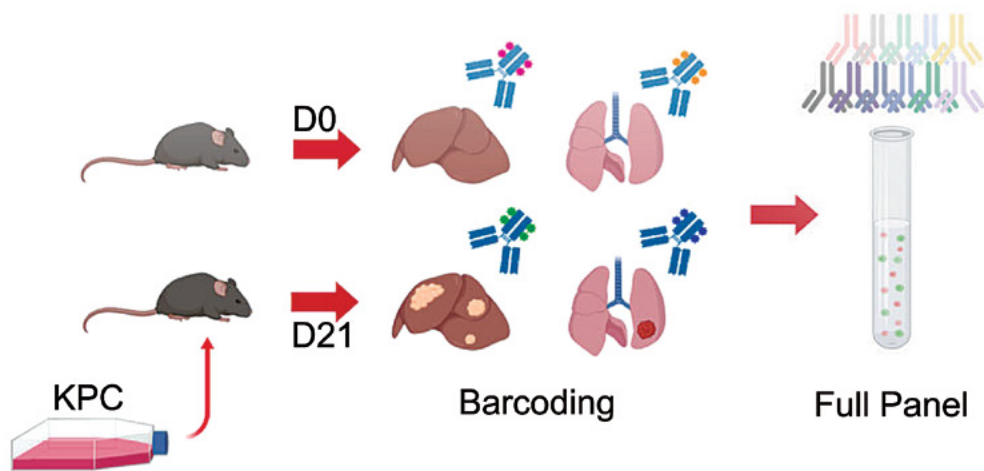


Figure 3. Global immune profiling of metastatic liver and lung TME with CyTOF (Ho et al.)

An interesting look into acute respiratory distress syndrome (ARDS), a primary complication of COVID-19, from the Centre Hospitalier Universitaire de Rennes (Roussel et al.) found differences between those cases associated with SARS-CoV-2 infection and those related to other causes. The team built 3 patient cohorts based on COVID-19-ARDS+, COVID-19+ARDS+ and COVID-19+ARDS- and characterized and compared the immune landscapes by high-dimensional mass cytometry.

Myeloid cell alterations, including an increase in a subpopulation of activated monocytes with upregulated CD169 expression, were common across all COVID-19 patients and absent from non-COVID-19 ARDS patients. The characterization of PBMC from COVID-19+ vs. COVID-19- patients using two 37- and 36-marker mass cytometry panels for myeloid and lymphoid subsets, respectively, provided

a reproducible, broad and unbiased approach that showed immune markers related to monocytes segregated the two classes of patients, with specific changes of T, plasma and NK cell subsets.

## Translating cell behavior and interactions

High-parameter immune profiling aids in the discovery of immune correlates to disease and predictive biomarkers by learning about differentially regulated proteins and how these proteins relate to clinical outcome. The large amounts of immunologic data generated by mass cytometry, even from limited sample sizes, offers a comprehensive view of biological systems, therapeutic response and signatures of health vs. disease.

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