Welcome to the inaugural issue of a quarterly anthology of recent impactful publications by researchers using CyTOF® technology to help expand our understanding of human health and disease.
As we learn more about cellular systems, once thought to be homogeneous, we gain better insights into true cellular heterogeneity in both healthy and disease states. This discovery has driven our desire to continually expand the number of cellular features we quantify with single-cell resolution. And technology is keeping up. Mass cytometry blends established flow cytometry methods with the precision of mass spectrometry. This enhanced hybrid enables in-depth multiplexed evaluation of complex cellular networks and processes, resulting in unparalleled insight into individual cells at a systems level. Here, we look at the most recent successes that are opening our eyes to new biological interactions, exposing detailed correlations and identifying new cell populations.
A helpful guide

With so much growing interest in mass cytometry, the highly regarded *Methods in Molecular Biology* series has released its newest edition, *Mass Cytometry: Methods and Protocols*. As a compilation of proven mass cytometry protocols, the book provides a guide to new and expert users alike on facility setup, panel design, reagent and sample preparation, detailed applications and data analysis.

Beginning with initial integration of mass cytometry into a lab environment and best practices for data acquisition, the chapters follow a representative workflow including both necessary and recommended steps. Numerous protocols detail proper panel design, optimized antibody tagging and staining techniques for a variety of sample types. For those interested in scaling their mass cytometry assays, experts weigh in on tips for better multiplexing with barcoding and automation to improve cell recovery from smaller sample sizes. By reviewing current protocols and introducing new methods, the book serves as a resource for the CyTOF community to successfully obtain high-quality, reproducible data, implement best practices and benefit from expert knowledge.

“These chapters capture the experience of those who pioneered the approach, as well as build on the collective knowledge that has seen it [mass cytometry] now actively embraced in the wider research community.”

— *Mass Cytometry: Methods and Protocols*
Beyond conventional methodology

Flow cytometry has been a traditional approach to immune profiling and biomarker discovery for decades. However, as experiments have evolved with the need to more thoroughly capture cellular diversity, the limitations inherent in flow cytometry have become evident. Primarily, its utility is restricted by the number of parameters that can be analyzed simultaneously. In part because of the need for complicated panel design to ensure that bright and dim fluorophores are matched with appropriate variably expressed markers, time spent performing flow cytometry assays has steadily increased as experiments have grown in size and complexity.

To address the fundamental properties of mass cytometry that are triggering a shift in experimental approach away from flow cytometry, a recent publication (Gadalla et al.) compared and validated its accuracy and reproducibility. CyTOF technology provides a platform for over 50 parameters (Simoni et al.) to be interrogated at once from a single sample, whereas in flow cytometry the same number of markers must be broken into batches and separately analyzed, requiring larger sample sizes and introducing room for technical error. In contrast to fluorophores, which are sensitive to photobleaching or degradation, the metal-tagged antibodies used in mass cytometry are very robust, providing the opportunity to store stained samples for later acquisition without notable signal loss. This last point is especially useful for clinical trials, where samples are often not collected all at once and need long-term preservation for comprehensive study.

Given that large-scale immune monitoring by mass cytometry provides many capabilities that support clinical trial success, scientists at the Icahn School of Medicine at Mount Sinai set out to create a standardized protocol for the largest validated mass cytometry dataset to date (Amir et al.). The group developed a comprehensive 350-surface marker screen that encompassed all major circulating immune cell subsets down to single-cell resolution, and included two-tiered barcoding to enable multiplexing, broad dried panels for streamlined sample processing and automated data analysis. Successful workflow standardization and automated analysis for such a large dataset offers a significant step toward CyTOF based immune monitoring for biomarker discovery and immunotherapy response in clinical trials.

CyTOF has enormous potential to discover disease-associated immunologic changes in cancer, identify functional changes to guide subsequent therapy and ultimately predict therapeutic outcomes.

—Gadalla et al.

More than 50 isotopic metal tags with discrete, non-overlapping signals are commercially available for use in mass cytometry experiments.
Accelerating research for better understanding of the immune system in human disease

Reflecting recent progress enabled by CyTOF technology, *Frontiers in Immunology* created a special research topic focusing on the use of mass cytometry to study immune-related human diseases. The nine papers currently listed under this topic include investigations of cancer, autoimmune disorders and infectious disease, among others.

In recent years, mass cytometry has evolved into a powerful platform for high-dimensional single-cell analysis and is uniquely positioned for implementation in clinical studies.  

— *Frontiers in Immunology*

Studies of multiple myeloma (Marsh-Wakefield et al.) and colon cancer (Zhang et al.) advanced our understanding of disease-associated properties of regulatory T cell populations and cancer-specific immune profiles in patient samples. A study of rheumatoid arthritis (RA), a disease where treatment fosters high interpatient variability, identified markers that may be used in the future to more precisely distinguish untreated RA patients from healthy donors (Bader et al.). Finally, a group from the French Alternative Energies and Atomic Energy Commission (CEA) combined phenotypic information from multiple CyTOF panels to characterize the immune response to chronic HIV infection, which has a complex interaction network of over 70 cell markers (Pereira et al.).

Taking on the challenge of early diagnosis of preeclampsia, one of the most severe pregnancy complications and a leading cause of maternal death worldwide, a lab at Stanford University used mass cytometry to validate current findings as well as discover novel and dynamic changes in immune profiles (Han et al.). Researchers used a high-dimensional mass cytometry immunoassay to learn about dynamic changes in over 370 immune cell features in both healthy and preeclamptic pregnancies. The study aimed to create a more relevant immune profile for the prediction and prevention of preeclampsia and discovered a set of eight cell-specific immune features that accurately identified patients before clinical diagnosis. A better understanding of immune response leads to opportunities to expand current therapeutic concepts and develop new approaches.

Han et al. *Frontiers in Immunology*. Experimental workflow using mass cytometry for deep immune profiling.
**Toward monitoring therapeutic success**

Riding the wave of accomplishments in biological research, the clinical research community is beginning to translate mass cytometry to its objectives, evidenced by over 50 clinical trials listed at [clinicaltrials.gov](http://clinicaltrials.gov) as employing the technology. Several publications validate mass cytometry as a valuable tool for immune monitoring in clinical studies, benefitting from analysis of cells in their original complexity. In one recent study investigating obstacles to the efficacy of T cell-based immunotherapy ([Hammerich et al.](#)), mass cytometry was used to test and confirm cell-specific response to enhancing checkpoint blockade therapy for indolent non-Hodgkin’s lymphomas by cross-priming T cells with an *in situ* vaccine.

T cell characterization has led to expanding options in immunotherapy, such as use of chimeric antigen receptors (CAR) in T cells. CAR T cell design and delivery is experiencing some success in hematological malignancies, but expanding the therapy to different cancer types has been difficult. Applying the improved sensitivity and systems analysis available with mass cytometry to immune monitoring helps identify new pathways like the use of anti-CD33 CAR T cells for acute myeloid leukemia that are associated with therapeutic response ([Kim et al.](#)). The resulting understanding of the tumor microenvironment and cell subset profiles further supports the success of a new therapy.

**Completing the story with data analysis**

Mass cytometry is consistently and repeatedly being proven for more applications, driving the development of new algorithms for analysis of cytometric data. A recently published beginner’s guide to data analysis ([Kimball et al.](#)) offers a summary of commonly used algorithms to support researchers exploring different analytical approaches for high-dimensional data. In addition to basic considerations for five primary algorithms (viSNE, SPADE, X-shift, PhenoGraph and Citrus), the resource lists relevant steps in implementing and interpreting CyTOF data, such as integrating at least three of these algorithms for visualization, stratification and confirmation of statistical significance.

One of the more popular tools specific to visualizing high-parameter data by dimensionality reduction uses t-distributed stochastic neighbor embedding (t-SNE). A comparison of t-SNE to conventional manual gating ([Eshghi et al.](#)) in stratifying and quantitating frequencies of diverse cell populations confirms that t-SNE is a valid and more automated method for larger and higher-dimensional datasets, where manual gating becomes impractical.
Conclusion

While we have touched on only a small selection of current publications, mass cytometry continues to expand its reach and push the boundaries of what we can learn from single cells. From working toward a deeper understanding of diverse cellular processes to applying this knowledge to disease evaluation and therapy design, mass cytometry has become an established and valued platform for high-dimensional cell analysis. We will continue to highlight successes and use of mass cytometry across the sciences in this quarterly series. We also invite you to explore the more than 800 publications and reviews in our mass cytometry bibliography and the many recordings of webinars and seminars by investigators using CyTOF technology in a wide variety of research applications.

Sources


