Main Submission

A Lens Into Mass Cytometry: A Multifaceted Technology Used to Delve Deep into Single-Cell Analysis

Matthew Boroditsky, Mobeen Mubasher, John-Paul Oliveria

Division of Respirology, Department of Medicine, McMaster University

Introduction

Modern bioanalytical chemistry has enabled us to simultaneously detect multiple cellular markers, including proteins, genes, or small molecules within or on the surface of individual cells (1). These markers can be objectively measured and used as an indicator of disease state (1). Mass cytometry, or cytometry by time of flight mass spectrometry (CyTOF), is a versatile technology used for multiplexed single-cell analysis. Compared to its predecessor, flow cytometry, CyTOF allows for simultaneous measurement of more biomarkers with greater precision. These developments allow for exciting applications within the clinical, pharmaceutical, and research fields. In this article, we describe the methods underlying CyTOF, as well as discuss its emerging applications and limitations.

How Does Mass Cytometry Work?

Before understanding how mass cytometry works, it is important to understand the fundamental concepts of its predecessor, flow cytometry. In flow cytometry, cells of interest are labeled with fluorochrome-conjugated antibodies and measured with lasers. Fluorescent labels are excited at various wavelengths, which allows for the detection and quantification of up to 20 cellular markers (2). Mass cytometry involves the labeling of cells with metal-tagged (e.g. lanthanide earth metals) antibodies for the detection and quantification of up to 50 cellular markers (2). Simultaneously exploring more parameters per cell allows for a better understanding of complex cellular systems and signalling pathways, contributing to our current understanding of immunology and stem cell

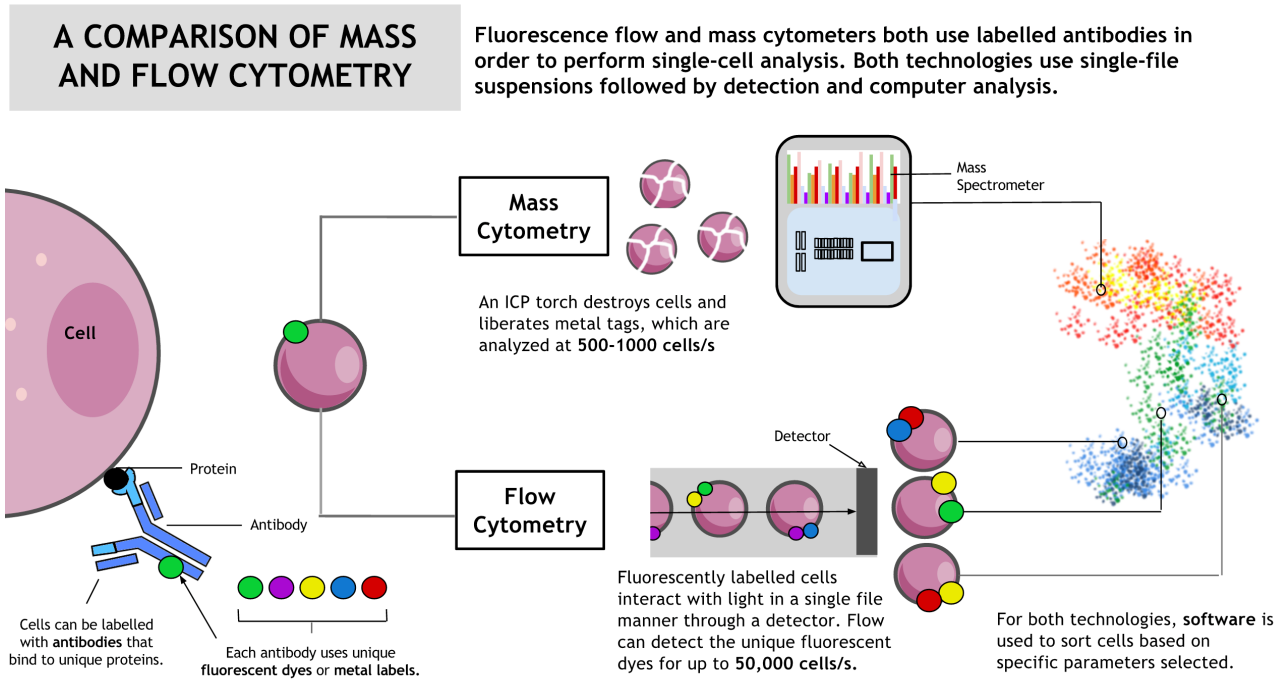

Figure 1: A comparison of mass cytometry and flow cytometry. Adapted from (2).

biology. Furthermore, CyTOF utilizes high temperature plasma to nebulize cells into a "cloud" of elemental ions, which contains the lanthanide metals that can be electronically analyzed based on mass and charge (Figure 1) (2). Quantifying cells based on mass and charge allows us to circumvent fluorochrome light detection overlap and autofluorescence, limitations of flow cytometry, thereby enabling the measurement of significantly more biomarkers for each cell (2).

Applications Of Mass Cytometry

Mass cytometry is a ground-breaking technological advancement in science, as it allows for highly detailed single-cell profiling that can be used to track progression of disease, determine specific immune signatures of patients, and assess the success of therapies (3,4). Currently, the measurement of gene expression levels and mass spectrometric analysis of human serum are used to identify biomarkers for the aid in diagnosis of a wide array of diseases. However, gene expression levels do not always correlate with protein levels in serum. Mass cytometry, a more sensitive technology, overcomes these inconsistencies by quantifying protein levels produced by single cells (5). Furthermore, mass cytometry shows promise for clinical application at the patient bedside (4). This technology allows clinicians to monitor immune cell signatures in patient blood samples in order to determine diagnostic and prognostic patterns, and to identify potential therapeutic targets (4). For example, a study by Fragiadakis *et al.* (2015) used mass cytometry to assess pre-operative immune cell distributions and intracellular signalling responses amongst a pool of patients undergoing hip arthroplasty (4). Quantitative analysis of intracellular signalling pathways that are vital to surgical recovery, such as pSTAT4, pCREB, and pNF-κB, were compared to successful post-operative immune profiles (4). Studies found that pre-operative immune states were predictive of successful surgical outcomes, suggesting that pre-operative implementation of mass cytometric analysis can be used as a screening tool to predict successful hip arthroplasty (4). However, a limitation of the study is that the patient cohort had minimal comorbidities and underwent the same surgical procedure, thereby reducing generalizability of the results to more heterogeneous patients with complex comorbidities. Lastly, although mass cytometry has tremendous potential in clinical applicability, the technology generates complex data sets, and scientists are still exploring statistical algorithms to better translate findings to clinical relevance, which is currently lacking consistency in present literature (6,7).

On a scientific scale, mass cytometry allows for cellular barcoding, which improves the efficiency and sensitivity of single-cell analysis; this allows researchers to better understand shifts and functional potential of specific cellular subsets and subphenotypes (5). Capitalizing on the utility of cellular barcoding to increase the efficiency in the acquisition of cells from multiple patients and/or cells from different compartments (e.g. spleen, blood, and bone marrow) significantly increases the throughput of patient samples (8). Specifically, metal-labelled cell barcoding (MCB), explored by Bodenmiller *et al.* (2012), uses a binary combination of seven different lanthanide ions to get 128 different combinations of lanthanide elements, increasing the combinations of lanthanide ions that can be used for barcoding (9). Thus, in this case, a barcode channel would be a combination of lanthanide metals conjugated to a functionalized antibody specific to a cellular marker. This high-content, high-throughput screening with MCB can be useful for clinical trials investigating novel therapeutics, pre-clinical testing of drugs, and *in vivo* and *in vitro* mechanistic investigation of human disease (8).

Limitations Of Mass Cytometry

Despite many advantages, a notable limitation with mass cytometry is the low throughput of cells, with roughly 30% of cells reaching the detector; this is significantly less than in flow cytometry, where 95% of cells reach the detector (5). The decreased throughput of mass cytometry poses severe constraints when detecting rare populations of cells, as low cellular recovery may lead to inaccurate quantitation. Another issue is the speed of analysis, where CyTOF processes 1000 cells per second, while flow cytometry processes 50 000 cells per second (5). However, this higher throughput results in a drift in signal intensity over time, increasing sampling bias when a large dataset is analysed (5). Using samples with a large number of cells can reduce signal drift, and using adequate quality controls within experimental systems can account for variations between samples. Finally, a limitation of mass cytometry is the inability to recover cells for further analysis, as cells are ionized into a "cloud" (2). However, this limitation can be circumvented by employing fluorescent-activated cell sorting (FACS) to isolate cells for functional assays on specific cell populations of interest (2).

The Future Of Mass Cytometry

The future of mass cytometry, particularly its clinical applications, has great promise in progressing scientific discovery (8). There is potential to develop computational methods for analyzing individual cells that could be used to predict alterations in cellular behaviour over time, and in different locations throughout the body. Mass cytometry could further enable time-dependent measurements at the single-cell level in complex heterogeneous tissue environments like malignant tumours. Specifically, the use of multiplexed ion-beam imaging (MIBI) has exciting potential with its use of secondary ion mass spectrometry, using antibodies tagged with elemental metal

reporters to visualize intact tissue slices (8). MIBI is similar to CyTOF, where both have the ability to measure surface and intracellular proteins on a single cell level; however, MIBI provides more specific information regarding cell interaction, cell morphology and localization within tissues (10). Specifically, MIBI uses secondary ion mass spectrometry (SIMS), which analyzes the secondary ejected ions from a solid surface, originating from a focused primary ion beam. MIBI allows clinicians to analyze up to 100 targets on tissue sections simultaneously, making it very effective within the heterogeneous cell populations often found in diseased tissues (e.g. malignant biopsies) (11). In addition to quantifying specific cells, MIBI provides high definition images showing cell morphology and localization (9). Overall, the implications of CyTOF and MIBI can be applied to visualize solid tissues from diseased states, including the central nervous system, bone marrow, spleen, and synovium, compartments relevant in inflammatory diseases (10).

Conclusion

Taken together, the utility of CyTOF is very promising, and scientists are merely at the infancy of clinical innovation and discovery by employing this technology. Although CyTOF does have a low throughput and slow speed of analysis, few experiments yield large quantities of arguably high quality data in clinical and research settings. Mass cytometry establishes a framework for time-dependent measurements at the single-cell level in complex tissue environments, with technological limitations being circumvented by both metal barcoding and the creation of computer analysis software. Overall, CyTOF technology will be crucial in elucidating the health status of patients through understanding the behaviour and distribution of individual cells, which will ultimately guide patient-specific treatment regimens of disease states based on cellular profiles (7). ■

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List of Abbreviations

CyTOF – cytometry by time of flight mass spectrometry

STAT3 – signal transducer/activator of transcription 3

CREB – cAMP response element binding protein

NF-kB – nuclear factor kappa-light-chain-enhancer of activated B cells

ICP – inductively coupled plasma

FCB – fluorescent cell barcoding

MCB – mass cell barcoding

MIBI – multiplexed ion-beam imaging

Matthew Boroditsky

Matthew is a 3rd year honours Bachelor of Health Sciences candidate at McMaster University, who has demonstrated a keen interest in the field of immunology. Joining McMaster's Cardio-Respiratory Research Lab in 2016, his work revolves around the investigation of therapeutic interventions in managing allergic asthma, as well as the general investigation behind allergen immunotherapy and its role in clinical practice.

Mobeen Mubasher

Mobeen is a 3rd year Bachelor of Health Sciences (honours) candidate at McMaster University. He has been involved with the Cardio-Respiratory Research Lab at McMaster University since January 2016. His primary research interests include asthma pathogenesis and the biology of eosinophils.

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John-Paul Oliveria

John-Paul is a 4th year Doctor of Philosophy candidate in the Department of Medical Sciences at McMaster University. He began his MSc in the McMaster Cardio-Respiratory Research Lab in 2011 and transferred to his PhD in 2013. His research focuses on the biology of B cells (IgE+ B cells and regulatory B cells) and the pathogenesis of allergic asthma. In the fall of 2017, he will be commencing a post-doctoral scholar appointment at Stanford University.