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Mass cytometry: a powerful tool for dissecting the immune landscape

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Advancement in methodologies for single cell analysis has historically been a major driver of progress in immunology. Currently, high dimensional flow cytometry, mass cytometry and various forms of single cell sequencing-based analysis methods are being widely adopted to expose the staggering heterogeneity of immune cells in many contexts. Here, we focus on mass cytometry, a form of flow cytometry that allows for simultaneous interrogation of more than 40 different marker molecules, including cytokines and transcription factors, without the need for spectral compensation. We argue that mass cytometry occupies an important niche within the landscape of single-cell analysis platforms that enables the efficient and in-depth study of diverse immune cell subsets with an ability to zoom-in on myeloid and lymphoid compartments in various tissues in health and disease. We further discuss the unique features of mass cytometry that are favorable for combining multiplex peptide-MHC multimer technology and phenotypic characterization of antigen specific T cells. By referring to recent studies revealing the complexities of tumor immune infiltrates, we highlight the particular importance of this technology for studying cancer in the context of cancer immunotherapy. Finally, we provide thoughts on current technical limitations and how we imagine these being overcome.

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Introduction

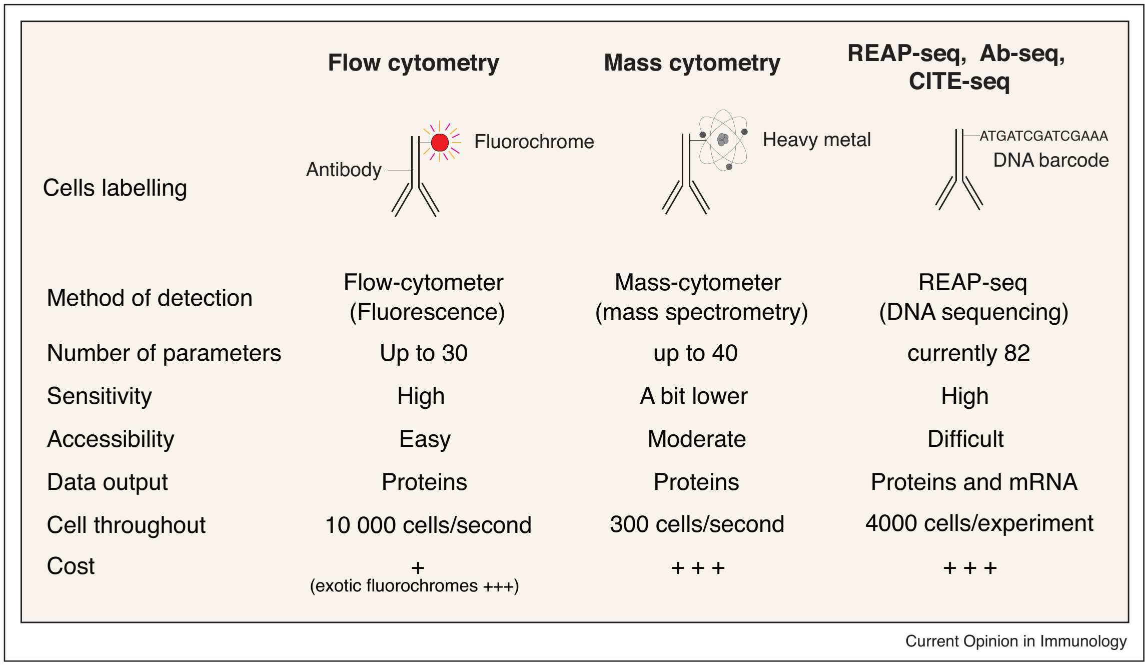
Exemplified by the ubiquitous use of flow cytometry in cellular immunology, single-cell analysis has long been an obsession of cellular immunologists. In recent years, with the development of high dimensional fluorescent flow cytometry, mass cytometry, single cell mRNA sequencing and other methods for high throughput single cell analysis, cellular subset analysis has entered a new high dimensional era that has exceeded the limits of traditional methods for defining, characterizing and quantifying immune cell subsets [1–3]. The development of mass cytometry (a.k.a. CyTOF — Cytometry by Time-Of-Flight) and the sudden increment in the number of parameters that can be reliably measured on individual cells was especially pertinent in exposing the limitations of traditional ways of defining immune cells [4–6]. With the development of many new visualizations, clustering and other high dimensional analysis methods, we are in the process of harnessing these challenging datasets [1,7]. Nonetheless, extracting the underlying meaning or functional utility of each of these countless cell ‘subsets’ remains a challenge. However, even without attempting to dissect and define, broader concepts and immunologically relevant hypotheses can be developed and tested from these complex datasets.

Here we examine the strengths and weaknesses of the various single cell analysis methodologies compared with mass cytometry. From there, we can comment on applications that we think best leverage mass cytometry and discuss strategies for the effective use of this method alone or in conjunction with other single-cell analysis methods. We then discuss progress in the use of mass cytometry to explore all types of lymphoid and myeloid cells across tissues in health and disease, in both mouse and humans. Next we describe the many benefits of mass cytometry as it is applied for the study of antigen-specific T cells through the use of peptide-MHC multimers. We highlight a number of studies that show the importance of mass cytometry as a tool for the study of cancer immunology that could lead to improvements in cancer immunotherapy. Lastly, we comment on how we envisage that the most important limitations of current single analysis methods might be overcome as sequencing-based methods continue to rapidly progress.

The place for mass cytometry among cutting edge single cell analysis methods

As alluded to above, progress in single cell analysis methodologies and the accompanying computational methods used to handle the resulting data have seen remarkable progress in recent years. One of the most

Figure 1



	Flow cytometry	Mass cytometry	REAP-seq, Ab-seq, CITE-seq
Cells labelling	Antibody Fluorochrome	Heavy metal	ATGATCGATCGAAA DNA barcode
Method of detection	Flow-cytometer (Fluorescence)	Mass-cytometer (mass spectrometry)	REAP-seq (DNA sequencing)
Number of parameters	Up to 30	up to 40	currently 82
Sensitivity	High	A bit lower	High
Accessibility	Easy	Moderate	Difficult
Data output	Proteins	Proteins	Proteins and mRNA
Cell throughout	10 000 cells/second	300 cells/second	4000 cells/experiment
Cost	+ (exotic fluorochromes +++)	+ + +	+ + +

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High dimensional single cell analysis at protein level. Comparison between flow-cytometry, mass cytometry and oligonucleotide-tagging based approaches (REAP-seq/At-seq/CITE-seq).

exciting phases of progress has been related to the development of single cell mRNA and other sequencing-based single cell genomics methods. For all single cell sequencing based methodologies, cellular throughput remains a significant limitation as compared with flow cytometry based methods. The development, applications and future of these have been exceptionally well described in recent reviews [8,9]. Therefore, we will focus our attention only on methods that allow for single cell analysis of protein expression. Along these lines, the past decade has also witnessed remarkable progress, going from 3 to 6 protein markers detected by flow cytometers (i.e. FACScan, BD Canto) all the way to oligo-tagging based methods, which have been used to measure the expression of as many as 82 proteins expression simultaneously at single cell level (with the theoretical maximum being much higher) [10••]. All these tools use antibodies for marker detection which are coupled either to a fluorochrome (flow-cytometry), a metal (mass cytometry) or a DNA-oligonucleotide (e.g. Ab-seq [11], REAP-seq [10••] or CITE-Seq [12•]) (Figure 1). Although mass spectrometry-based single cell proteomics are also being developed, these methodologies are still in their infancy and are severely limited in cellular throughput and sensitivity for low abundance proteins [13]. Data acquired using these different methods can be easily analyzed using standard bi-axial representation and gating strategies to identify different cell populations and marker expression profiles (using software such as FlowJo® or BD FACSDiva™). Recently, new tools that incorporate high dimensional analysis methods allow a deeper understanding of the data, by offering multiple new functions and features, such as dimensionality reduction for visualization, clustering for quantifying cellular subsets and cellular trajectory analysis to infer changes in marker expression associated with differentiation processes [2].

Here we focus our attention on comparing the strengths and weaknesses of three attractive state-of-the-art approaches. First is the most recent generation of fluorescent flow cytometers, such as spectral-analyzer based flow cytometers [14] or the BD FACSymphony, which uses 5 lasers, numerous photomultiplier tube detectors and currently offers the possibility to analyze up to 30 different antibody markers [15]. The second is the CyTOF™ mass cytometer that allows detection of up to 40 different antibody markers on each cell [4]. Lastly, we consider approaches that use oligonucleotides as tags for antibodies, which offer the potential for a much higher degree of multiplexing capacity. One early study demonstrated the utility of this approach and employed Nanostring™ technology as a means to quantify each tag [16]. More recently, several groups have used DNA sequencing of single cells to quantify the binding of antibodies on each cell (e.g. Abseq [11], REAP-seq [10••] or CITE-Seq [12•]). One such study impressively employed an antibody panel to probe 82 different markers simultaneously. These approaches have the additional advantage of