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Mass Cytometry: The Time to Settle Down

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Mass cytometry (CyTOF) technology was first described by Bandura et al. in 2009 (1) boosting the number of measurable markers per single cell and revolutionizing the flow cytometry field toward a horizon of a theoretical 100 measurements This revolution also boosted the development of other single cell multi-parameter technologies such as spectral flow cytometry (2) and chip-based cytometry (3), together with conventional flow cytometry that can now reach 50 theoretical parameters (4). The aim of this special issue is to mark the point that mass cytometry is presently a well-established technology with a large community of scientists committed to its development. It represents also a “settling moment” as described in a previous editorial (5) to next bring the technology to full maturity.

This special *Cytometry Part A* issue includes the first OMIP describing a mass cytometry panel for the immune phenotype of human peripheral leukocytes together with a series of manuscripts introducing new reagents, protocols, quality controls and, also, a nice example of how the multidimensional nature of mass cytometry can address important biological question such as the status of a “challenged” immune system in comparison to a system kept in the clean environment of a pathogen-free laboratory.

For those accustomed to traditional flow cytometry, one of the main drawbacks of mass cytometry is the absence of forward and side light scatter measurements to appreciate cell size and internal complexity. In this issue Stern et al. (this issue, page 14) use two plasma membrane staining assays based on wheat germ agglutinin and osmium tetroxide to evaluate cell size in mass cytometry experiments. Resolution is not comparable to conventional flow cytometry light scatter measurements; nevertheless, the combined use of these new membrane specific moieties, combined with phenotypic markers and the use of algorithms able to simultaneously evaluate multiple measurements hold promise for an extended use of these two reagents. To increase the assortment of available labels, Schulz et al. (this issue, page 25) introduce streptavidin coupled silver nanoparticles that can be used to include biotinylated reagents in mass cytometry panels. Of note, silver isotopes are detected in

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channels where at the moment no other reagents are available and hence the new reagent can be easily integrated in existing antibody panels. Wheat germ agglutinin and osmium were previously used to stain plasma membranes whereas silver nanoparticles are already in use in a wide range of immunoassays. Hence, the manuscripts by Stern et al. and Schulz et al. are nice examples of innovation created by changing the domain of usage.

In the near future, mass cytometry will be used for clinical and longitudinal studies requiring an improvement of standardized protocols, methods to facilitate longitudinal analysis, and to accelerate the time needed for sample acquisition. Four manuscripts go in this direction. The OMIP-034 by Baumgart et al. (this issue, page 34) describes a basic 26 antibody panel able to identify neutrophils, eosinophils, basophils, monocytes, dendritic cells, T and B lymphocytes. Of note, the panel leaves several channels free to be completed with additional "drop-in" markers, and therefore a common backbone can be shared for multiple purposes. The panel described in OMIP-34 was designed keeping in account of the minimal but significant signal interference described by Takahashi et al. (this issue, page 39). Of interest for longitudinal studies and to overcome instrument breakdowns is the idea from Sumatoh et al., to cryopreserve stained samples (this issue, page 48). Finally, to accelerate mass cytometry acquisition, cell population enrichment is a valid option to decrease the time of acquisition as shown by Pelàk et al. that uses a CD81-targeted immunoaffinity matrix for this purpose (this issue, page 62).

The manuscript from Melchiotti et al. (this issue, page 73) introduces a neglected aspect in current mass and flow cytometric analysis, that is, the quality control of the analysis procedure. Mass cytometry opened a domain in between hypothesis driven biology and system biology, where the analyzed markers are still a choice of the scientist but the multidimensional nature of the dataset require the use of tools typically used in system biology. Several of these tools are not deterministic and hence results may show variations simply by repeating the same algorithm on the same dataset. Moreover, each algorithm requires the scientist to choose a series of parameters, such as the number of desired clusters, which may affect the final result. Therefore, we need to establish the robustness of our analysis procedure and the manuscript of Melchiotti et al. takes us in this direction proposing the "cluster stability" as an important quality control for results obtained from clustering algorithms.

The companion special issue of *Cytometry Part B* also contributed to the standardization of protocols and advancement in data analysis. The manuscript by Leelatian et al. (6) compares several tissue preparation protocols used before mass cytometry analysis, and the manuscript by Abraham et al. (7) shows how a tool previously used for stock exchange trends and microarray datasets (Radviz) can be successfully applied to the visualization of mass cytometry data.

Bringing together the concepts of standardization of protocols (OMIP) and quality control in data analysis procedures, it will be necessary to start a debate on how to standardize analysis methodology in order to avoid jeopardizing the field with a new pipeline for each new manuscript and refocus the content on science. The Editors of *Cytometry Part A* & *Part B*

hope to further this task by hosting manuscripts dedicated to the standardization of the analysis procedures.

Finally, Japp et al. (this issue, page 85) return to the basic science and take advantages from the multidimensional nature of mass cytometry to address similarity and differences between clean laboratory mice typically used in immunological studies and “wild” mice that probably are more representative of the real world and are probably a model of immune system more similar to the human “not clean” reality. Such a question couldn’t be comfortably addressed with the available dimensions of conventional flow cytometry and demonstrate how a wide vision of the immune system can grab information not visible in fewer dimensions.

Mass cytometry is becoming a known and established technology enlightening us with the power of multidimensionality within the field of single cell technology. These combined issues of *Cytometry Parts A* and *B* demonstrate that the mass cytometry field is moving forward and palpably contributing to the overall advancement of cytometry.

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