HHS Public Access

Author manuscript

Immunity. Author manuscript; available in PMC 2016 April 21.

Published in final edited form as:

Immunity. 2015 April 21; 42(4): 591–592. doi:10.1016/j.immuni.2015.04.006.

Thinking Outside the Gate: Single-Cell Assessments in Multiple Dimensions

Pia Kvistborg[1,19], **Cécile Gouttefangeas**[2,19], **Nima Aghaeepour**[3], **Angelica Cazaly**[4], **Pratip K. Chattopadhyay**[5], **Cliburn Chan**[6], **Judith Eckl**[7], **Greg Finak**[8], **Sine Reker Hadrup**[9], **Holden T. Maecker**[10], **Dominik Maurer**[11], **Tim Mosmann**[12], **Peng Qiu**[13], **Richard H. Scheuermann**[14,15], **Marij J.P. Welters**[16], **Guido Ferrari**[17], **Ryan R. Brinkman**[3,20,*], and **Cedrik M. Britten**[18,20,21,*]

[1]Netherlands Cancer Institute, 1006 BE Amsterdam, the Netherlands [2]Institute for Cell Biology, Department of Immunology, University of Tübingen, 72076 Tuebingen, Germany [3]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC V5Z 1L3, Canada [4]University of Southampton, Southampton SO17 1BJ, UK [5]ImmunoTechnology Section, Vaccine Research Center, NIH, Bethesda, MD 20892, USA [6]Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC 27710, USA [7]Helmholtz Zentrum München, 85764 Neuherberg, Germany [8]Vaccine and Infectious Disease Division, Department of Biostatistics, Bioinformatics, and Epidemiology, Fred Hutchinson Cancer Research Center, Seattle, WA 98155, USA [9]Center for Cancer Immune Therapy, Herlev Hospital, 2730 Herlev, Denmark [10]Institute for Immunity, Transplantation, and Infection, Stanford School of Medicine, Stanford University, Stanford, CA 94305, USA [11]Immatics Biotechnologies GmbH, 72076 Tübingen, Germany [12]David D. Smith Center for Vaccine Biology and Immunology, University of Rochester Medical Center, Rochester, NY 14642, USA [13]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA 30322, USA [14]J. Craig Venter Institute, La Jolla, CA 92037, USA [15]Department of Pathology, University of California, San Diego, San Diego, CA 92093, USA [16]Department of Clinical Oncology, Leiden University Medical Center, 2300 RA Leiden, the Netherlands [17]Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA [18]BioNTech RNA Pharmaceuticals GmbH, 55131 Mainz, Germany

Over the last two decades, our ability to interrogate the immune system on a single-cell level has increased dramatically (Chattopadhyay and Roederer, 2012; Bendall et al., 2011), allowing an opportunity to better understand the immunological mechanisms underlying disease. Complex flow cytometry (FCM) data are now surpassing our ability to fully analyze and interpret all information via current standard approaches, such as 2D dot plots and Boolean gates. Indeed, the number of potential cell subpopulations increases exponentially with the number of parameters assessed, making it difficult to decipher all possible

[*]Correspondence: rbrinkman@bccrc.ca (R.R.B.), cebritten@web.de (C.M.B.).
[19]Co-first author
[20]Co-senior author
[21]Present address: Immuno-Oncology & Combinations DPU, Oncology R&D, GSK, Stevenage SG1 2NY, UK

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Acknowledgments and one table and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2015.04.006.

combinations included in the raw data (e.g., 512 potential subsets with nine markers) via the traditional approaches (Bendall and Nolan, 2012). This could limit the translation of technical advances into new diagnostics or therapies. Newly developed bioinformatics tools that have the potential to bridge this gap are now available. The aim of this letter is to foster the implementation and adoption of these novel computational methodologies for unbiased analysis of complex cytometry data.

In recent years, a host of new data-analysis tools have emerged, creating workflows for processing and analyzing complex FCM datasets; however, these have gone mostly unnoticed by immunologists. Table S1 provides an overview of many of the currently available tools and their specific applications. They can be assigned to specific categories arranged in a “FCM data-analysis workflow” from compensated data as input to biologically interpretable results as output. The vast majority of the listed tools for FCM data processing, analysis, and visualization are made available by the bioinformaticians at no cost and include open source code and unrestrictive software licensing, opening up these computational approaches to broad use by the research community. Many of the tools have been developed to address similar analysis objectives via quite different approaches. They might provide optimal results for different datasets, such that there is no “right” or “best” tool, and using several algorithms in combination might yield even better results and exceed the possibilities offered by manual analysis. Comprehensive comparative studies by the Flow Cytometry: Critical Assessment of Population Identification Methods (FlowCAP) project have shown that many of these tools have reached a level of maturity that matches, or even surpasses, the results produced by human experts (Aghaeepour et al., 2013).

The development of computational approaches addresses many needs associated with high-dimensional datasets. However, for the immunology community, three main challenges have surfaced, and tackling them will facilitate a paradigm shift in the analysis of FCM data. First, despite the focused efforts by bioinformaticians to develop novel tools for analyzing FCM data, only a minority of immunologists are aware of the advantages offered to the field. These tools need to be presented in immunology forums rather than limited to bioinformatics journals and conferences. Second, even though the vast majority of the computational tools are open source and thus freely available, most do not have user-friendly interfaces, limiting their use to investigators with programming expertise. Third, as a consequence of the first two challenges, there is a lack of general understanding of how these novel tools work. This has two opposing effects. In some cases, skepticism increases because of a feeling that direct control of analysis has been lost and that results are unverifiable. In other cases, overconfidence occurs, and no real effort is made to validate results. This can lead to the reporting of “significant” cell populations that actually arise from experimental artifact (and quality-control issues).

These are challenges that must be addressed, both in terms of generalizable strategies and within each individual experiment, with the goals of broader adoption and more accurate results.

Inter-disciplinary collaborations between immunologists and bioinformaticians might help address these points, as demonstrated by pioneering collaborations that identified

immunological correlates of HIV protection in high-dimensional FCM data (Aghaeepour et al., 2012). Such collaborations could also be implemented within institutions and research groups by convening bioinformaticians and immunologists or by team members trained in both immunology and bioinformatics. Hence, inter-disciplinary collaboration should be encouraged as soon as a study is conceived and should continue through the entire study (from wet bench experimentation to final data analysis to publication).

Another, possibly game-changing solution is to develop user-friendly web or computer interfaces that would allow immunologists with little bioinformatics background to rationally combine the available tools and run datasets through different workflows to achieve optimal results. Work on making this model a reality is ongoing. The FLOCK algorithm (Qian et al., 2010) has been implemented in the Immunology Database and Analysis Portal (https://immport.niaid.nih.gov), which supports management of FCM data, cell-population identification, cross-sample comparison, and result visualization with a simple user interface. Also, a comprehensive suite of tools for processing and analyzing FCM data has been implemented within the GenePattern infrastructure (Spidlen et al., 2013). Finally, the OpenCyto framework provides open-source tools for analyzing FCM data within an extensible and flexible interface to simplify the construction of re-usable FCM workflows while facilitating comparative analysis against manually gated results in order to enhance user confidence (Finak et al., 2014).

Progress in cytometry technology generates complex datasets for which exhaustive analysis by existing practices is difficult. Solutions for deciphering multi-dimensional FCM and mass cytometry datasets exist but have not yet reached most immunologists. Here, we describe a list of available computational tools with the aim of enhancing awareness, access, and acceptance and discuss models to bridge the existing gap between immunology and bioinformatics. We predict that interdisciplinary efforts to address the current data-analysis bottlenecks will rapidly enhance our knowledge of the immune system, guide immunotherapy development, accelerate biomarker discovery, and ultimately benefit patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

Aghaeepour N, Jalali A, O'Neill K, Chattopadhyay PK, Roederer M, Hoos HH, Brinkman RR. Cytometry A. 2012; 81:1022–1030. [PubMed: 23044634]

Aghaeepour N, Finak G, Hoos H, Mosmann TR, Brinkman R, Gottardo R, Scheuermann RH, FlowCAP Consortium; DREAM Consortium. Nat Methods. 2013; 10:228–238. [PubMed: 23396282]

Bendall SC, Nolan GP. Nat Biotechnol. 2012; 30:639–647. [PubMed: 22781693]

Bendall SC, Simonds EF, Qiu P, Amir AD, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, et al. Science. 2011; 332:687–696. [PubMed: 21551058]

Chattopadhyay PK, Roederer M. Methods. 2012; 57:251–258. [PubMed: 22391486]

Finak G, McDavid A, Chattopadhyay P, Dominguez M, De Rosa S, Roederer M, Gottardo R. Biostatistics. 2014; 15:87–101. [PubMed: 23887981]

Qian Y, Wei C, Eun-Hyung Lee F, Campbell J, Halliley J, Lee JA, Cai J, Kong YM, Sadat E, Thomson E, et al. Cytometry B Clin Cytom. 2010; 78(1):S69–S82. [PubMed: 20839340]

Spidlen J, Barsky A, Breuer K, Carr P, Nazaire MD, Hill BA, Qian Y, Liefeld T, Reich M, Mesirov JP, et al. Source Code Biol Med. 2013; 8:14. [PubMed: 23822732]